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IRIS Toxicological Review of Perfluorononanoic Acid (PFNA) and Related Salts

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Integrated Risk Information System
Center for Public Health and Environmental Assessment
Office of Research and Development
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ABBREVIATIONS

ABP	androgen binding protein
AC50	activity concentration at 50%
ACOG	American College of Obstetricians and Gynecologists
ACTH	adrenocorticotropic hormone
ADHD	attention-deficit/hyperactivity disorder
ADME	absorption, distribution, metabolism, and excretion
A/G	albumin/globulin
AGD	anogenital distance
AIC	Akaike's information criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMH	anti-Müllerian hormone
AOP	adverse outcome pathway
APD	anopenile distance
AR	androgen receptor
ASA	active systemic anaphylaxis
ASD	anoscrotal distance
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area-under-the-concentration-curve
BAF	bioaccumulation factor
BCF	bioconcentration factor
BCRP	breast cancer resistance protein
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMI	body mass index
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
$BW^{3/4}$	body weight raised to ¾ power
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service registry number
CDR	Chemical Reporting Data
CERAPP	Collaborative Estrogen Receptor Activity Prediction Project
CHO	Chinese hamster ovary (cell line cells)
CI	confidence interval
CL	confidence limit
CL	clearance
CL_H	human clearance
C_{max}	maximum concentration
CPAD	Chemical and Pollutant Assessment Division
CPHEA	Center for Public Health and Environmental Assessment
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DDEF	data-derived extrapolation factor
DEG	differentially expressed gene
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
E2	estradiol
EMEA	European Medicines Agency
EPA	Environmental Protection Agency
ER	estrogen receptor
ER	extra risk
Fa	fraction absorbed
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
FSH-R	follicle stimulating hormone receptor
FSIQ	full-scale intelligence quotient
FTOH	fluorotelomer alcohol
GD	gestation day
GDH	glutamate dehydrogenase
GDM	gestational diabetes mellitus
GF	glomerular filtration
GFR	glomerular filtration rate
GGT	γ-glutamyl transferase
GLDH	glutamate dehydrogenase
GLP	good laboratory practices
GM	geometric mean
GSH	glutathione
GST	glutathione-*S*-transferase
HAWC	Health Assessment Workplace Collaborative
HDL	high-density lipoprotein
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
HOMA	homeostatic model assessment
HTS	high-throughput screening
Ig	immunoglobulins
IGF-1	insulin like growth factor 1
i.p.	intraperitoneal
IQR	interquartile range
IRIS	Integrated Risk Information System
IPCS	International Programme on Chemical Safety
IUR	inhalation unit risk
i.v.	intravenous
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDL	low-density lipoprotein
LDS	lactate dehydrogenase
L-FABP	liver fatty acid binding protein
LH	luteinizing hormone

LOD	limit of detection
LOQ	limit of quantitation
LOAEL	lowest-observed-adverse-effect level
MIS	müllerian inhibiting substance
MOA	mode of action
MDR	multidrug resistance-associated protein
Na^+	sodium
NH_4	ammonium
NHANES	National Health and Nutrition Examination Survey
NCI	National Cancer Institute
NIS	sodium-iodide symporter
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NTP	National Toxicology Program
OATP	organic anion transporting polypeptide
OCTN2	organic cation/carnitine transporter 2
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
ORD	Office of Research and Development
OSF	oral slope factor
osRFD	organ-/system-specific RfD
PD	pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCOS	polycystic ovary syndrome
PECO	populations, exposures, comparators, and outcomes
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFCA	perfluoroalkyl carboxylic acids
PFDA	perfluorodecanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PFUnDA	perfluoroundecanoic acid
PIQ	performance IQ
PK	pharmacokinetic
PND	postnatal day
POD	point of departure
POI	primary ovarian insufficiency
PPARα	peroxisome proliferator-activated receptor alpha
PPARβ/δ	peroxisome proliferator-activated receptor beta/delta
PPARγ	peroxisome proliferator-activated receptor gamma
PVDF	polyvinylidene fluoride
PWS	public water system
PXR	pregnane X receptor
RBC	red blood cell
RD	relative deviation
RfC	inhalation reference concentration
RfD	oral reference dose
RNA	ribonucleic acid
RR	risk ratio
RT-PCR	reverse transcription polymerase chain reaction
RWT	relative wall thickness
RXR	retinoid X receptor
SD	Sprague-Dawley
SD	standard deviation
SDH	sorbitol dehydrogenase
SDQ	strengths and difficulties questionnaire
SE	standard error
SGA	small for gestational age
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SREBP	sterol regulatory element-binding protein
StAR	steroidogenic acute regulatory
T3	3,5,3′-triiodothyronine
T4	thyroxine
TBG	thyroid binding globulin
TH	thyroid hormone
TNFα	tumor necrosis factor alpha
TPO	thyroid peroxidase
TR	thyroid hormone receptor
TRH	thyrotropin releasing hormone
TSCA	Toxic Substances Control Act
TSH	thyroid stimulating hormone
TTR	transthyretin
TWA	time-weighted average
UA	uric acid
UCMR	Uncontaminated Monitoring Rule
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
V_d	volume of distribution
VIQ	verbal IQ
WBC	white blood cell
WOS	Web of Science
WRAVMA	Wide Range Assessment of Visual Motor Abilities
WHO	World Health Organization
WT1	Wilms tumor gene

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EXECUTIVE SUMMARY

ES.1 SUMMARY OF OCCURRENCE AND HEALTH EFFECTS

Perfluorononanoic acid (PFNA; CASRN 375-95-1),[1] and its related salts are members of the group of per- and polyfluoroalkyl substances (PFAS). This assessment applies to PFNA as well as non-metal and alkali metal salts of PFNA that would be expected to fully dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body). Thus, while this assessment would not necessarily apply to non-alkali metal salts of PFNA because of the possibility of PFNA-independent contributions of toxicity, it does apply to PFNA salts including sodium perfluorononanoic acid [Na^+PFNA; CASRN 21049-39-8] and ammonium perfluorononanoic acid [$NH4^+$PFNA; CASRN 4149-60-4]), and other non-metal or alkali metal salts of PFNA. The synthesis of evidence and toxicity value derivation presented in this assessment focuses on the free acid of PFNA given the currently available toxicity data.[2]

Concerns about PFNA and other PFAS stem from their high environmental persistence as they are resistant to hydrolysis, photolysis, and biodegradation. Given the legacy use of PFNA in polyvinylidene fluoride (PVDF) production in the United States, and increasing use in other regions, long-chain PFAS, such as PFNA, have been and continue to be released to the environment through various waste streams (NLM, 2013;ATSDR, 2021; NJDWQI, 2015). Moreover, products containing PFNA are still in use, and may continue to be sources of trace contamination with disposal and breakdown of PVDF fluoropolymers in the environment (Prevedouros et al., 2006). Industrial releases, discharges from wastewater treatment facilities that treat domestic and industrial waste, stormwater runoff, land applications of biosolids and industrial waste, release from aqueous film-forming foams, degradation of polymers containing PFNA and PFAS precursors (e.g., 8:2 fluorotelomer alcohol [FTOH]) are all potential sources of environmental contamination. PFNA also has been detected in household dust, soil, food products, and drinking water (ATSDR, 2021; NJDWQI, 2015).

[1]The CASRN given here is for n-PFNA (linear isomer). The source of PFNA used in the animal toxicity study by NTP (2018) was reported to be >99% pure, giving this CASRN. Other animal studies also provided test article purity, and these details can be found in HAWC. None of the studies referenced in this assessment explicitly state that only the linear form was used. Therefore, there is the possibility that some proportion of the PFNA 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]With exception of a small number of *low* confidence/*uninformative* studies, the currently available *high* and *medium* confidence studies for PFNA evaluate effects of the free acid form, and so normalization from a salt to free acid using a molecular weight conversion was unnecessary. However, formulas for converting candidate values of the free acid form to different PFNA salts would be calculated by multiplying the candidate value for the free acid of PFNA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{481}{464} = 1.037$ and for the sodium salts the ratio would be $\frac{MW\ sodium\ salt}{MW\ free\ acid} = \frac{486}{464} = 1.047$.

The Integrated Risk Information System (IRIS) program is developing a series of five PFAS assessments (perfluorobutanoic acid [PFBA], perfluorohexanoic acid [PFHxA], perfluorohexane sulfonate [PFHxS], PFNA, perfluorodecanoic acid [PFDA], and their associated salts) at the request of EPA National Programs (see December 2018 IRIS Program Outlook). These human health assessments represent one component of the broader PFAS strategic roadmap at EPA (PFAS strategic roadmap), the goal of which is to characterize and reduce PFAS exposure and effects. For example, the EPA Office of Water has proposed a National Drinking Water Regulation (NPDWR) to establish Maximum Contaminant Levels for individual PFAS (PFOS and PFOA) and a PFAS mixture (involving PFHxS, PFNA, PFBS, and GenX Chemicals) (Proposed PFAS National Primary Drinking Water Regulation). Additionally, EPA's Office of Water has proposed a framework for estimating noncancer health effects from PFAS mixtures (U.S. EPA, 2023g). The EPA Center for Computational Toxicology and Exposure (CCTE) has developed a tiered toxicity testing strategy for evaluating PFAS using new approach methods (NAMs) that will inform future category grouping and read-across efforts to fill data gaps for PFAS with limited or no toxicity data (PFAS Chemical Lists and Tiered Testing Methods Descriptions).

The systematic review protocol for the five assessments outlines scoping and problem formulation efforts, including a summary of other federal and state assessments of PFNA, as well as the systematic review and dose-response methods used to conduct the evaluation (see also Section 1.2 for further discussion of assessment methods). This 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, including a summary of the history of protocol revisions.

Human epidemiological studies have examined associations of PFNA exposure to health outcomes, specifically fetal growth restriction, liver serum biomarkers, antibody responses, infectious disease outcomes, sensitization and allergic responses, semen parameters, reproductive hormones, pubertal development, thyroid hormones, neurodevelopment, serum lipids, cardiovascular disease and associated risk factors, metabolic effects, time to pregnancy and other female reproductive responses, and urinary effects. With the exception of the developmental (i.e., reduced birth weight) and hepatic (i.e., increased serum ALT in adults) health outcomes, the ability to draw judgments concerning associations that are based on the human evidence is limited. Experimental animal studies with PFNA have focused on the oral exposure route. One acute, single-dose inhalation exposure study was identified that was *low* confidence and inadequate for reference value derivation, so an RfC was not estimated. The available animal studies of oral PFNA exposure examined a variety of noncancer endpoints, specifically those relevant to developmental, hepatic, immune, male, and female reproductive, endocrine, cardiometabolic, urinary and other health effects. There was insufficient evidence to make a judgment on whether PFNA exposure might affect the development of any specific cancers.

For noncancer endpoints, this assessment concludes that the ***evidence demonstrates*** that oral exposure to PFNA causes developmental effects in humans, and that the ***evidence indicates*** that oral exposure to PFNA is likely to cause hepatic and male reproductive effects in humans given sufficient exposure conditions.[3] There is *robust* epidemiological evidence that PFNA exposure is associated with deficits in birth weight, and this finding is supported by coherent findings of postnatal growth restriction and to a lesser degree decreased birth length. While there was residual uncertainty related to potential bias from exposure biomarker sample timing and regarding potential impact of PFAS co-exposures, these sources of uncertainty are not likely to fully explain the consistent results seen across the birth weight endpoints detailed in the synthesis or subsequent EPA meta-analyses (see Section 3.2.2, Wright et al. (2023), and Appendices C.1 and D.1 for further discussion of the fetal growth restriction evidence base and meta-analyses). In support of the findings in humans, there is also *moderate* evidence of developmental toxicity from animal studies showing coherent results in mice, including reduced survival, deficits in postnatal body weight gain, and delays in attainment of developmental landmarks (eye opening, vaginal opening, and preputial separation). The evidence integration judgment for hepatic health effects is based on *moderate* epidemiological evidence of increased serum markers (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and bilirubin) in humans. This judgment is supported by *robust* evidence from short-term (≤28 days) and developmental toxicity studies in rodents showing a consistent pattern of liver enlargement across species, sex, and lifestage, as well as coherent histopathological abnormalities including mild necrosis, intrahepatic cholestasis, triglycerides accumulation and clinical pathology in adult rats (predominantly males). Further support comes from the mechanistic evidence that supports PPARα-dependent and -independent mode of action (MOA) pathways that could contribute to the hepatotoxicity (see Section 3.2.3). For male reproductive effects, the hazard determination is based on *moderate* evidence of a consistent, dose-dependent, and coherent pattern of effects in adult, pubertal, and prepubertal rodents that includes decreased reproductive organ weights (testis, epididymis) and testosterone (serum and testicular), impaired spermatogenesis, delayed reproductive system development, and corresponding histopathological evidence of structural changes to reproductive tissues (see Section 3.2.4).

Given the certainty in the hazard evidence for developmental effects, the epidemiological endpoint of decreased birth weight in humans and the animal developmental toxicity endpoints of decreased postnatal survival, reduced postnatal body weight, and delayed developmental landmarks in mice were advanced for dose-response modeling. The point of departure (POD) based on decreased birth weight in humans was advanced as a candidate for lifetime and subchronic organ-/system-specific reference dose (osRfD) derivations. The endpoint of increased ALT in humans was also advanced for deriving a candidate reference value for lifetime effects. Quantitatively, the resulting chronic hepatic osRfD supported the selected overall oral reference

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

value based on developmental effects (decreased birth weight) as the osRfDs for both were nearly identical. The negligibly higher developmental osRfD of 7×10^{-9} mg/kg-day was selected over the hepatic osRfD of 6×10^{-9} mg/kg-day to be the overall lifetime oral reference dose (RfD) because of greater overall confidence in the value, including higher confidence in the precision of the POD (see Section 5.2.1, Table 5-19 for a summary of confidence in the lifetime osRfD).

Several hepatic and male reproductive endpoints from the short-term rodent studies were also advanced for dose-response modeling for the purpose of deriving subchronic reference values, given the *robust* and *moderate* evidence, respectively, for effects indicating a likely hazard. The liver endpoints advanced for modeling included increasing relative liver weights in rats and mice and hepatocyte lesions (hypertrophy) in rats. The male reproductive endpoints advanced for dose-response modeling included decreasing reproductive organ weights and histopathology (testis and epididymis), decreasing serum and testicular testosterone and sperm counts in rats. The PODs based on decreasing relative liver weight in adult female rats and decreasing absolute whole epididymis weight in adult mice were advanced for osRfDs derivations as they were the most sensitive PODs for these outcomes (see Section 5.2.2 for a summary of the subchronic osRfD derivation).

The available ***evidence suggests*** but is not sufficient to infer that oral exposure to PFNA may cause immune, thyroid, neurodevelopmental, and cardiometabolic effects given sufficient exposure conditions.[4] The evidence for immune effects is based on *medium* confidence epidemiological studies showing potential immunosuppression, primarily related to associations of prenatal and childhood exposures with decreased antibody responses following vaccinations (see Section 3.2.6). The hazard conclusion for thyroid effects is based on *moderate* evidence of large dose-dependent reductions in serum free and total T4 in adult female rats and in serum free T4 in adult male rats. While a dose-response assessment is typically not conducted for health effect judgments of "***evidence suggests,***" for this assessment, immunosuppression in children and reduced serum total T4 in adult female rats were advanced for dose-response modeling (but not for RfD derivation) to facilitate comparisons with other PFNA PODs and to inform UF selection given that these effects are observed with other PFAS (and have been used quantitatively in assessments of those other PFAS). Studies in humans also showed PFNA associations with increasing diagnoses of behavioral disorders (e.g., attention-deficit/hyperactivity disorder [ADHD]) but with overall high levels of uncertainty in the evidence base. The hazard conclusion for cardiometabolic effects is based on human studies showing positive associations of PFNA exposure with elevated serum cholesterol, as well as some coherent evidence of heightened cardiovascular risk factors (blood pressure, atherosclerosis, metabolic syndrome, adiposity). However, this evidence base also had

[4]With the exception of immune and thyroid endpoints, 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).

important uncertainties related to unexplained inconsistencies in results. There was little evidence of an association between PFNA exposure and diabetes and insulin resistance. The neurodevelopmental and cardiometabolic outcomes with suggestive evidence of hazard were not advanced for dose-response modeling.

For other health outcomes evaluated (adrenal, female reproductive, urinary, and other noncancer endpoints), the ***evidence is inadequate*** to evaluate the potential for effects, as the available epidemiological and animal evidence bases for these health outcomes are unclear and/or incoherent. These outcomes were likewise not advanced for modeling.

Table ES-1 summarizes health effects with sufficient evidence available to synthesize and draw hazard conclusions, and the toxicity values derived for these health effects.

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

Organ/system	Integration judgment	Toxicity value	Value (mg/kg-d)	Confidence	UF_C	Basis
Developmental	***Evidence demonstrates***	Lifetime osRfD; Subchronic osRfD	7×10^{-9}	*Medium-high*	30	Decreased birth weight, males and females combined, human, from a 10-study meta-analysis
Liver	***Evidence indicates (likely)***	Lifetime osRfD	6×10^{-9}	*Medium*	30	Increased ALT, adult female, human
		Subchronic osRfD[a]	7×10^{-7}	*Medium*	1,000	Increased relative liver weight, adult female, rat
Male reproductive	***Evidence indicates (likely)***	Lifetime osRfD	Not derived[b]			
		Subchronic osRfD	2×10^{-6}	*Medium-low*	1,000	Decreased absolute epididymis weight (whole), adult rat
Lifetime and Subchronic RfD			7×10^{-9}	*Medium-high*	30	Decreased birth weight, males and females combined, human, from a 10-study meta-analysis

osRfD = organ-/system-specific reference dose (in mg/kg-d); lifetime osRfD = reference dose (in mg/kg-d) for lifetime exposures; subchronic RfD = reference dose (in mg/kg-d) for less-than-lifetime exposures; UF_C = composite uncertainty factor. Refer to Tables 5-17 and 5-21 for descriptions of how uncertainty factors were derived for the lifetime and subchronic osRfDs, respectively.

[a]For derivation of the candidate subchronic liver osRfD, there was insufficient data on the mechanism and pharmacodynamics of the chronic liver effects in humans to extrapolate to a subchronic exposure. Therefore, the animal data were selected to derive the subchronic osRfD for liver effects.

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

ES.2 LIFETIME AND SUBCHRONIC ORAL REFERENCE DOSE (RfD) FOR NONCANCER EFFECTS

The derived RfD and lifetime osRfDs correspond to chronic, lifetime exposures, and are estimates of an exposure for a given duration to the human population (including susceptible subgroups and/or lifestages) that are likely to be without appreciable risk of adverse health effects. Additionally, a less-than-lifetime, subchronic RfD and several subchronic osRfDs were also derived for PFNA. These subchronic toxicity values are presented because they may be useful for certain decision purposes (e.g., site-specific risk assessments with less-than-lifetime exposures).

Developmental endpoints (i.e., decreased birth weight) were advanced for dose-response modeling based on EPA's meta-analyses results of different medium and high confidence study subsets, as well as three individual high confidence epidemiological studies (i.e., (Wikström et al., 2020; Sagiv et al., 2018; Manzano-Salgado et al., 2017a)). EPA considered using a POD from the meta-analyses or based on an individual study and ultimately selected the meta-analysis of a subset of 10 studies that evaluated associations using maternal PFNA sampling primarily early in pregnancy, alleviating concerns for potential hemodynamic effects (see Appendix C.1.4 for further discussion of pregnancy hemodynamics). Additionally, the meta-analysis was preferred as it captured greater data diversity that contributed increased heterogeneity in examined populations and PFNA exposure distributions. An uncertainty of the meta-analysis was that it required re-expression of two of the 10 effect estimates from the natural scale to the log scale of exposure, and re-expression from the log scale to the natural scale of exposure in performing the benchmark dose (BMD) modeling. Using a sensitivity analysis, EPA estimated that the average systemic bias on the effect estimates from re-expression applying an exposure distribution similar to that used in the POD derivation would be approximately 30% on the effect estimates (see Section 5.2.1, "Modeling results in humans (decreased birth weight)" for further discussion of the BMD modeling). While the magnitude of this bias on the BMD and benchmark dose lower confidence limit (BMDL) cannot be estimated with confidence, it is expected to result in a lower POD than the value that would be calculated without bias. Therefore, it is not justified to select a still lower value for the POD from single studies in order to avoid the limitation of bias from re-expression. Ultimately, the substantially larger amount of additional data supporting the 10-study meta-analysis was judged to outweigh any bias that may be introduced by re-expressing the regression coefficients.

The resulting PODs, whether based on the meta-analysis or on the individual epidemiological studies, were close in relative magnitude to each other (e.g., POD_{HED} values ranging from 1.0×10^{-7} to 2.3×10^{-7} mg/kg-day) providing confidence that either approach would be suitable for informing the RfD for this endpoint (see Section 5.2.1, Table 5-11 for a summary of values). Of the individual candidate human studies, the Sagiv et al. (2018) and Wikström et al. (2020) studies were preferred because they evaluated maternal PFNA serum concentrations primarily in the first trimester of pregnancy (median gestational age of 9 and 10 weeks, respectively), minimizing concerns for pregnancy-related hemodynamic effects, and reported

results on a natural (i.e., untransformed) scale, eliminating uncertainties in the BMD modeling with re-expression of regression coefficients from logarithmic scales.

Using the meta-analysis of 10 studies, a $BMDL_{ER5}$ of 1.81×10^{-3} mg/L[5] was determined and used as the POD, and the POD_{HED} of 2.2×10^{-7} mg/kg-day was calculated by multiplying the POD and human clearance (CL_H) estimate of 0.124 mL/kg-day for women of reproductive age. A CL_H estimate of 0.09 mL/kg-day for elimination by all routes was taken from Chiu et al. (2022) and is presumed to represent the population average for total excretion among human males of all ages and females outside of reproductive age. In accordance with analysis of human serum PFNA concentrations from NHANES, clearance in women between 12.4 and 40 years of age was estimated to be approximately 40% higher than their male counterparts (i.e., 0.124 mL/kg-day). This clearance rate is expected to provide a reasonable estimate of PFNA maternal serum concentrations, hence fetal exposure, throughout pregnancy. The developmental osRfD was determined by dividing the POD_{HED} by a composite uncertainty factor of 30 to account for interindividual pharmacokinetic and pharmacodynamic differences in human susceptibility ($UF_H = 10$), and deficiencies in the toxicity evidence base ($UF_D = 3$). This developmental osRfD based on reduced fetal birth weight was selected as the RfD because it was interpreted with *medium-high* confidence and was based on a sensitive POD. This RfD is assumed to be protective of all observed health effects associated with lifetime PFNA exposure.

Hepatic endpoints (i.e., increased serum ALT, a marker of hepatic injury) from two *medium* confidence epidemiological studies, Kim et al. (2023) and Nian et al. (2019), were also advanced for modeling to derive a chronic toxicity value. A lifetime osRfD of 6×10^{-9} for increased risk of liver effects was calculated based on epidemiological evidence from Kim et al. (2023), which was judged to be of *medium* confidence. Of the candidate human studies, this cross-sectional study was preferred because PFNA was considered to be the strongest driver of ALT increases (8.6%) in a Korean population and this study also included mixtures modeling to identify the independent effect of PFNA. A $BMDL_{ER10}$ of 2.02×10^{-3} mg/L was determined and used as the POD that was based on Kim et al. (2023). The POD_{HED} of 1.8×10^{-7} mg/kg-day was derived by multiplying the POD and human clearance (CL_H) estimate for human males and females outside of reproductive age (0.09 mL/kg-d), since the endpoint is considered relevant to both sexes of all lifestages. The hepatic effect osRfD was determined by dividing the POD_{HED} by a composite uncertainty factor of 30 to account for interindividual pharmacokinetic and pharmacodynamic differences in human susceptibility ($UF_H = 10$), and deficiencies in the toxicity evidence base ($UF_D = 3$). This hepatic effect osRfD based on increased serum ALT is considered supportive of the developmental lifetime reference value.

[5]The internal dose (blood plasma concentration) POD was based on a BMDL hybrid approach using an extra risk (ER) of 5%, and the range of the observed data and dosimetric adjustment based on PFNA-specific pharmacokinetic information.

For subchronic toxicity values, the same meta-analysis of 10 studies described for the overall lifetime RfD was also selected as the basis for the overall subchronic RfD of 7×10^{-9} mg/kg-day (see Table ES-1). Additional candidate subchronic osRfD values were also derived for liver and male reproductive effects in rats from the 28-day study by NTP (2018) (see Table ES-1). While it was determined there was too much uncertainty to extrapolate the 28-day study to a lifetime toxicity value, it was considered reasonable to extrapolate a 28-day exposure for purposes of deriving subchronic candidate values. The epidemiological studies considered in deriving lifetime toxicity values (Kim et al. (2023) and Nian et al. (2019)) were not considered for use in deriving candidate subchronic toxicity values because subjects were interpreted to have been exposed for longer than a subchronic duration. The POD_{HED} values for the animal endpoints were derived using a pharmacokinetic (PK) model developed by EPA to estimate internal doses in mice and male rats in the various bioassays, or by application of the measured PFNA concentrations in female rats at the end of the NTP bioassay. These internal doses were then converted to POD_{HED} values by multiplying by the lifestage appropriate values of CL_H, as was done for human birth weight and liver effects described above. A data-derived extrapolation factor (DDEF) approach was also evaluated for dose extrapolation but was considered inferior to the PK approach because animal bioassay PK data were not well predicted by the assumption of steady state implicit in the DDEF. Published physiologically based pharmacokinetic (PBPK) models were found to be inadequate in describing the PK data, in particular because the embedded assumption about distribution from the blood to body tissues is contradicted by available PK data. The candidate subchronic osRfDs for the liver and male reproductive health outcomes were then calculated by dividing the HED PODs by a UF_C of 1,000. This composite UF was based on residual uncertainty regarding pharmacokinetics and pharmacodynamics (UF_A = 3), interspecies differences (UF_H = 10), short-term study duration (UF_S = 10), and database deficiencies (UF_D = 3).

ES.3 CONFIDENCE IN THE ORAL REFERENCE DOSE (RfD)

The overall confidence in the selected lifetime and subchronic developmental osRfD is *medium-high* and is driven predominantly by the *medium-high* confidence in the overall *robust* epidemiological evidence for developmental effects and *medium-high* confidence in the quantitative estimate based on an EPA meta-analysis of well-conducted studies. Confidence in the evidence base for derivation of the lifetime and subchronic developmental osRfD is *medium-high* based on *robust* evidence of reduced birth weight reported in multiple epidemiological studies, coherence across other fetal and postnatal endpoints, and cross-stream coherence demonstrated by *moderate* animal evidence from two developmental toxicity studies in two strains of gestationally exposed mice reporting consistent and dose-dependent effects on postnatal growth metrics (reduced survival, postnatal body weight gain, and delayed developmental landmarks). EPA's meta-analysis of birth weight showed statistically *robust* results across analyses of different study confidence and sample timing strata indicative of associations even among the early biomarker group, addressing concerns about potential effects of pregnancy hemodynamics on these results. Although it remains a source

of some uncertainty, there was also no compelling evidence to suggest that confounding by other PFAS was primarily responsible for the inverse associations. This conclusion was based on analysis of the subset of PFNA studies evaluating single and multi-PFAS models in relation to birth weight deficits. Confidence in the quantification of the POD supporting the RfD is *medium-high*, given the POD was based on a 10-study meta-analysis using a BMD hybrid approach within the range of the observed data and dosimetric adjustment based on PFNA-specific pharmacokinetic information. Both the meta-analysis and PK approach introduced some uncertainty (see Section 5.2.1. Oral Reference Dose Derivation, "Modeling results in humans (decreased birth weight)" for discussion of meta-analysis data re-expression and "Consideration of uncertainty in the pharmacokinetic extrapolation for PFNA" for analysis of PK uncertainties). However, confidence was increased given that the selected POD based on 10 early sampling studies was consistent with PODs derived from the individual epidemiological studies and additional meta-analyses of varying sample time and study confidence. Confidence in the studies supporting the RfDs is *high*, considering the conduct of individual *high* and *medium* confidence studies used in the meta-analysis and the results of the meta-analysis itself, which further reduces uncertainties in individual studies. Considering these aspects, an overall confidence rating of *medium-high* was selected for both the chronic (lifetime) and subchronic RfD.

ES.4 NONCANCER EFFECTS OBSERVED FOLLOWING INHALATION EXPOSURE

The only available toxicological inhalation study was an acute (<24 hour) *low* confidence study in animals (Kinney et al., 1989) that was considered unsuitable for deriving a chronic or subchronic inhalation reference concentration (RfC). No studies were available in humans.

ES.5 EVIDENCE FOR CARCINOGENICITY

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), EPA concluded that there is ***inadequate information to assess carcinogenic potential*** for PFNA by either oral or inhalation routes of exposure. Exposure durations tested in the animal toxicology studies (≤28 days) were considered too short (insensitive) for evaluation of carcinogenic outcomes and no informative human studies were identified. Therefore, the lack of data on the carcinogenicity of PFNA 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 ASSESSMENT METHODS

The Integrated Risk Information System (IRIS) Program is developing a series of five PFAS assessments (i.e., perfluorobutanoic acid [PFBA], perfluorohexanoic acid [PFHxA], perfluorohexane sulfonate [PFHxS], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], and their associated salts) (see 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 EPA (PFAS strategic roadmap). The systematic review protocol (see 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 PFNA. 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 subsequently updated based on those public comments. Appendix A links to the updated version of the protocol, which summarizes the history of revisions. In addition to these ongoing IRIS PFAS toxicity assessments, EPA's Office of Research and Development is carrying out several other activities related to PFAS, including creation of PFAS systematic evidence maps (SEMs) (Shirke et al., 2024; Carlson et al., 2022; Radke et al., 2022) and consolidating and updating PFAS data on chemical and physical properties, human health toxicity, and pharmacokinetics, as well as on environmental fate and transport.

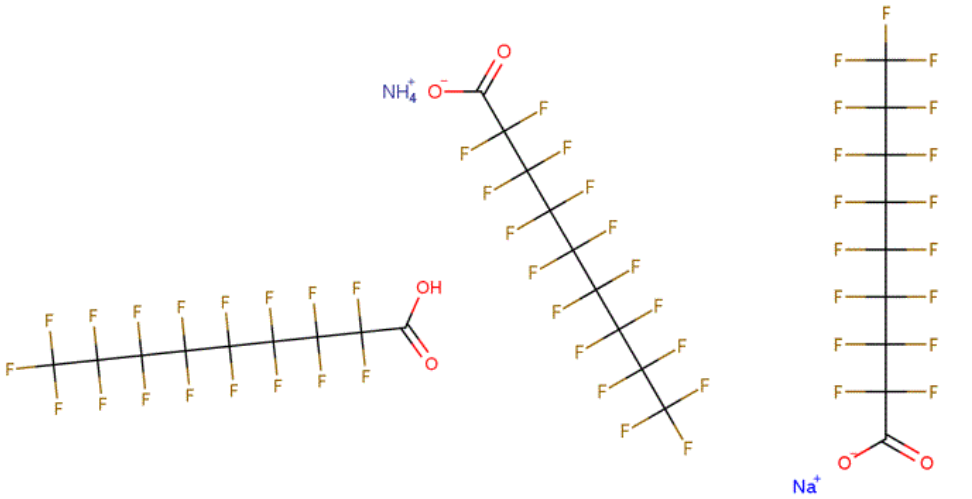
1.1. BACKGROUND INFORMATION ON PFNA

This section provides a brief overview of aspects of the physiochemical properties, sources, human exposure, and environmental fate characteristics of PFNA (CASRN 375-95-1), and its salts (sodium PFNA [Na^+PFNA; CASRN 21049-39-8] and ammonium PFNA [$NH4^+$PFNA; CASRN 4149-60-4]) that may provide useful context for this assessment. This summary and the longer overview in the assessment systematic review protocol (see Appendix A) are not intended to be a comprehensive description of the available information on these topics. Readers are encouraged to refer to source materials cited below and more recent publications on these topics.

1.1.1. Physical and Chemical Properties

PFNA and its salts (Na^+PFNA, NH_4^+PFNA) are members of the group of per- and polyfluoroalkyl substances (PFAS). The specific chemical formula of PFNA is $C_9HF_{17}O_2$, for Na^+PFNA is $C_9F_{17}NaO_2$, and for NH_4^+PFNA is $C_9H_4F_{17}NO_2$. More specifically, PFNA is classified as a member of a subset of PFAS called perfluoroalkyl carboxylic acids (PFCAs) that have structural features

consisting of a carbon backbone that is fully fluorinated and bonded to a carboxylic acid functional group (OECD, 2018; Lau et al., 2007). Because PFNA and its salts are PFCAs containing more than seven perfluorinated carbon groups, they are considered long-chain PFCAs (ATSDR, 2021; Buck et al., 2011). Consistent with the protocol, to simplify the terminology used throughout this assessment, PFNA and its salts are generally referred to using the broad and more recognizable term, PFAS, rather than the more specific term PFCA. The chemical structures of PFNA, Na^+PFNA, and NH_4^+PFNA are presented in Figure 1-1[6], and select physicochemical properties are provided in Table 1-1. When available, experimental values are provided in the table but predicted values that may be less reliable are included in the absence of experimental data.



	PFNA	PFNA Ammonium salt	PFNA Sodium salt
CASRN	375-95-1	4149-60-4	21049-39-8
DTXSID	8031863	20880205	50896632

Figure 1-1. Linear chemical structures (from left to right) of perfluorononanoic acid (PFNA), sodium perfluorononanoate (Na^+PFNA), and ammonium perfluorononanoate ($NH4^+$PFNA).

[6]While this figure shows the linear structures, the assessment may also apply to other nonlinear isomers of PFNA and related salts as described in the Executive Summary.

Table 1-1. Predicted or experimental physicochemical properties of PFNA

Property (unit)	Value		
	PFNA (free acid)[a]	Na^+PFNA[a]	NH_4^+PFNA[a]
Molecular formula	$C_9HF_{17}O_2$	$C_9F_{17}NaO_2$	$C_9H_4F_{17}NO_2$
Molecular weight (g/mol)	464	486	481
Melting point (°C)	68	81*	78*
Boiling point (°C)	213	193*	193*
Density (g/cm^3)	1.78*	1.75*	1.75*
Vapor pressure (mmHg)	8.72×10^{-3}	8.98×10^{-2}*	8.98×10^{-2}*
Henry's law constant (atm-m^3/mole)	1.17×10^{-9}*	1.17×10^{-9}*	1.17×10^{-9}*
Water solubility (mol/L)	2.80×10^{-3}	1.64×10^{-3}*	1.64×10^{-3}*
pKa	−0.23*	−0.23*	−0.23*
Log Koa: octanol-air	4.20*	4.20*	4.20*
Log Kow: octanol-water	3.54*	5.78*	6.62*
Soil adsorption coefficient (Koc)	$2.82 \times 10^{+3}$*	$2.82 \times 10^{+3}$*	$2.83 \times 10^{+3}$*
Bioconcentration factor	165*	4.90*	4.90*

[a]EPA Comptox Chemicals Dashboard (U.S. EPA, 2018a); experimental average values indicated where available; otherwise, predicted average values denoted by (*). Accessed 9/6/2023 and 2/28/2024.

1.1.2. Sources, Production, and Use

PFAS are man-made compounds that have been used widely over the past several decades in consumer products and industrial applications because of their resistance to heat, oil, stains, grease, and water. PFNA has been used primarily as a processing aid in the emulsion process used in the production of polyvinylidene fluoride (PVDF), which is a fluoropolymer designed to be both temperature resistant and chemically nonreactive. PVDF uses include as insulation for wire and circuit boards, as well as valves, pipes, and other components that come into contact with reactive chemicals (Lohmann et al., 2020; NJDWQI, 2015; Wang et al., 2014b; Prevedouros et al., 2006). PFNA is not an intended constituent of PVDF but has rather been released during manufacture of PVDF and is a residual byproduct contained in PVDF-finished products for industrial and consumer uses (Kotthoff et al., 2015; NJDWQI, 2015; Prevedouros et al., 2006). Most PFNA in the global environment is posited to be linked to its historical use as a processing aide in PVDF (Lohmann et al., 2020; Wang et al., 2014b; Prevedouros et al., 2006). Prevedouros et al. (2006) estimates that 60% of PFNA used in PVDF production was released to the environment, resulting in 0.4–1.4 million kg of global emissions from 1975 to 2004. Contamination reported by the New Jersey Department of Environmental Protection states that over 80% of ~125,000 kg of Sulflon S-111 (primarily PFNA) used from 1991 to 2010 was released to the air and water. PFNA has also been detected at low levels in aqueous film-forming foams for fire suppression (Laitinen et al., 2014).

EPA has worked with companies in the fluorochemical industry to phase out the production and use of long-chain PFAS such as PFNA (2010/2015 PFOA Stewardship Program). However, in addition to its high environmental persistence (see below), products containing PFNA are still in use and may be imported into the United States. Thus, products containing PFNA may continue to be a source of contamination due to their disposal and breakdown in the environment (Kim and Kannan, 2007). Sources of PFNA may also occur from the abiotic and biotic degradation of 8:2 fluorotelomer alcohol (FTOH), which is used in the production of fluorotelomer products added to finished products, such as paints, coatings, and textiles (NJDWQI, 2015; Butt et al., 2014; Ellis et al., 2004). Additionally, polyfluoroalkyl phosphoric acid diesters (e.g., 8:2 diPAPs) used and/or detected in some food packaging, wastewater sludge, and paper mill fibers may release FTOH that can breakdown to degrade to PFNA (NJDWQI, 2015; D'Eon et al., 2009).

1.1.3. Environmental Fate and Transport

Concerns about PFNA and other PFAS stem from the resistance of these compounds to environmental hydrolysis, photolysis, and biodegradation (Sundström et al., 2012). Because of the strength of the carbon-flourine bond, PFAS, including PFNA, are highly stable and persistent in the environment (ATSDR, 2021) and are found worldwide in the air, soil, groundwater, surface water, and in tissues of plants, wildlife, and humans (2010/2015 PFOA Stewardship Program). PFNA and other PFAS may migrate to drinking water wells and surface water sources via plumes associated with spills and/or discharges. Deposition from air emissions to soil and subsequent migration to groundwater is another potential transport pathway. PFNA released to the air will exist in the vapor phase given its vapor pressure (NLM, 2017, 2016, 2013; Kim and Kannan, 2007), although particle-bound concentrations of PFNA have also been measured, which have different properties (Kim and Kannan, 2007). Wet and dry deposition to surfaces are potential removal processes for particle-bound PFAS in air (e.g., to surface water or soils) (ATSDR, 2021). Vapor-phase PFNA is not expected to be susceptible to direct photolysis by sunlight but can be degraded in the atmosphere by reacting with photochemically produced hydroxyl radicals (ATSDR, 2021; NLM, 2017, 2016, 2013). The atmospheric half-life for these reactions is estimated to be 31 days for PFNA (NLM, 2013). Long-range transport of PFNA and other long-chain PFAS to remote polar regions of the Arctic and Antarctica has also been shown, and the mechanisms for this circulation are a source of ongoing study (Garnett et al., 2022; ITRC, 2020; Joerss et al., 2020).

PFNA would be expected to have limited mobility in soil given its soil adsorption coefficient (see Table 1-1). Because PFNA exists as an anion, volatilization from moist soil is not expected to be an important transport process as PFNA is expected to adsorb to suspended particles in water, and soil and sediment fractions (NLM, 2013). Uptake of PFAS in soil by plants can occur (ATSDR, 2021). There is a general increase in bioaccumulation potential in aquatic biota (evaluated as bioconcentration factors [BCFs] and bioaccumulation factors [BAFs]) for PFAS with increasing chain length (e.g., PFNA>PFOA>PFHxA) (Burkhard, 2021).

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

The general population may be exposed to long-chain PFAS such as PFNA by inhalation of indoor or outdoor air, dietary ingestion of contaminated drinking water and foods, accidental ingestion of dust, and dermal contact with PFAS-containing products (ATSDR, 2021; Poothong et al., 2020; NLM, 2013). The oral route of exposure is considered the most important among the general population, including hand-to-mouth transfer from handling materials containing these compounds (ATSDR, 2021). Contaminated drinking water and foods are considered important sources of exposure to long-chain PFAS such as PFNA. Due to the high water solubility and mobility of these compounds in groundwater (and lack of remediation technology at water treatment facilities), it is possible for populations consuming drinking water from any contaminated watershed to receive PFAS exposure (ATSDR, 2021). In addition to oral exposure, inhalation and dermal routes of exposure also appear to be relevant exposure pathways for PFNA but these routes are not well quantified (ATSDR, 2021).

Water

PFNA is among the 30 contaminants EPA monitors in drinking water as part of the fifth Uncontaminated Monitoring Rule (UCMR5; October 2023 update) (U.S. EPA, 2023b). Under the UCMR, public water systems (PWSs) serving more than 10,000 people and a representative sample of PWSs serving 10,000 or fewer people are required to be monitored for contaminants that are not yet subject to regulatory standards under the Safe Drinking Water Act. PFNA was detected at or above the minimum reporting level (MRL) of 0.004 µg/L in 20 of the 3,073 PWS sites monitored and in 31 of 10,024 samples collected (detections ≥MRL ranging from 0.004 to 0.024 µg/L) (U.S. EPA, 2023b). A systematic evidence map by Holder et al. (2023) reported that 9 of 24 studies indicated PFNA detections in 50% or more of samples.

Food

Data for potential PFNA exposure from dietary sources in the United States are limited. PFNA was not detected in food items collected from five grocery stores in Texas or in food packaging collected from U.S. fast food restaurants (Schaider et al., 2017; Schecter et al., 2012). However, Stahl et al. (2014) characterized PFAS in fish tissue at 157 Great Lakes sites and found PFNA in 69% of the samples, with a maximum concentration of 9.7 ng/g. PFNA was also found in lettuce, with a bioaccumulation factor of 0.77 for lettuce grown in municipal soil and 2.85 for industrially impacted soil (Blaine et al., 2013). Data from other countries (e.g., South Korea, Sweden, Spain, Serbia, Saudi Arabia) report that PFNA is detectable in samples of fish and shellfish, meat, processed foods, and beverages and water (both tap and bottled) (Heo et al., 2014; Pérez et al., 2014; Vestergren et al., 2012). In a Norwegian population, the mean dietary intake was estimated at 38 pg/bw-day (Poothong et al., 2020). A systematic evidence map reported 13 of 46 and one of five references observed detectable PFNA in 50% or more of food samples or food

packaging, respectively (Holder et al., 2023). The relevance of these detects (and the associated PFAS levels) to U.S. products is unknown.

Air and Dust

PFAS 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, 2021). For example, Kato et al. (2009) analyzed dust samples collected from 39 homes in the United States, United Kingdom, Germany, and Australia and detected PFNA in 25.6% of the samples. Likewise, Strynar and Lindstrom (2008) analyzed dust samples from 110 homes and 10 day-care centers in North Carolina and Ohio and detected PFNA in 42.9% of the samples. Poothong et al. (2020) found a significant correlation between PFNA in serum and intake from floor dust. Indoor air samples (n = 4) from a town in Norway had mean PFNA concentrations of 2.7 pg/m^3 (Barber et al., 2007). Air concentrations of PFNA precursors, specifically 8:2FTOH, have also been correlated with serum PFNA (Poothong et al., 2020). PFNA also has been detected with high frequency (96% samples) in dust from fire stations across the United States and Canada at median concentrations of 7.2 ng/g and maximum concentrations of 203 ng/g, which was significantly higher than median concentrations (0.15 ng/g) detected in dust from residential homes in this study (Ji et al., 2020). A systematic evidence map reported 13 of 17 and three of five references observed detectable PFNA in 50% or more of samples from dust or indoor air, respectively (Holder et al., 2023).

PFNA has not been evaluated under the Air Toxics Screening Assessment (EPA AirToxScreen). However, PFNA was measured at concentrations ranging from less than the limit of detection (LOD) to 0.4 pg/m^3 in the vapor and particle phases of air samples collected from an urban area of Albany, New York, in 2006 (Kim and Kannan, 2007).

Military and Industrial Sites

PFNA has been detected at 10 U.S. military sites in 71.4% of the surface soil samples, 12.1% of the sediment samples, 36% of surface water samples, and 46.4% of groundwater samples (see Table 1-2) (ATSDR, 2021; Baduel et al., 2015). Limited testing at two National Priorities List (NPL) hazardous waste sites reported median PFNA levels in soil of 27.2 ppb (ATSDR, 2021). PFNA was also detected above the MRL (0.096 µg/L) in groundwater near an industrial site in New Jersey (Post et al., 2013). Kim and Kannan (2007) analyzed lake water, rainwater, snow, and surface water runoff from Albany, New York, and reported concentrations of PFNA ranging from less than the LOD to 5.9 ng/L. PFNA has also been detected downstream of industrial discharge locations in the Delaware River at concentrations up to 0.976 µg/L from 2007 to 2009 (NJDWQI, 2015).

Table 1-2. Perfluorononanoic acid levels in soil, sediment, and water at 10 military installations

Media	Measurement	Value
Surface soil	Frequency of detection (%) Reporting limit (µg/kg) Median (µg/kg) Maximum (µg/kg) Number of samples (all sites)	71.43 0.23 1.30 23.0 100
Subsurface soil	Frequency of detection (%) Reporting limit (µg/kg) Median (µg/kg) Maximum (µg/kg) Number of samples (all sites)	14.42 0.24 1.50 6.49 112
Sediment	Frequency of detection (%) Reporting limit (µg/kg) Median (µg/kg) Maximum (µg/kg) Number of samples (all sites)	12.12 0.38 1.10 59.0 40
Surface water	Frequency of detection (%) Reporting limit (µg/L) Median (µg/L) Maximum (µg/L) Number of samples (all sites)	36.00 0.017 0.096 10.0 36
Groundwater	Frequency of detection (%) Reporting limit (µg/L) Median (µg/L) Maximum (µg/L) Number of samples (all sites)	46.38 0.018 0.105 3.00 149

Source: ATSDR (2021) and Anderson et al. (2016). Samples collected in 2014.

Biomonitoring and Susceptible Populations

The presence of PFNA in human blood provides evidence of exposure among the general population. PFNA was monitored in serum samples collected from 1999 to 2018 as part of the National Health and Nutrition Examination Survey (NHANES). Approximately 2,000 survey participants were included in each sampling set, with an additional group of 639 children aged 3–11 years tested in 2013–2014 (see Table 1-3). Results of this monitoring over time indicate a current downward trend in serum concentrations of PFNA. According to these data, median values in human sera declined from 0.6 µg/L between 1999 and 2000 to 0.4 µg/L between 2017 and 2018 (25th–75th percentiles were 0.4, 0.9 and 0.3,0.7 respectively).

Table 1-3. Serum concentrations of PFNA reported by the National Health and Nutrition Examination Survey (NHANES) 1999–2010, 2011–2012, 2013–2014, 2015–2016, and 2017–2018 (µg/L)

Population group	Measurement	Serum concentration 1999–2000	Serum concentration 2003–2004	Serum concentration 2005–2006	Serum concentration 2007–2008	Serum concentration 2009–2010	Serum concentration 2011–2012	Serum concentration 2013–2014	Serum concentration 2015–2016	Serum concentration 2017–2018
Total population (includes ages 12 and older)	Geometric mean	0.548 (*N* = 1432)	0.966 (*N* = 2094)	1.09 (*N* = 2120)	1.22 (*N* = 2100)	1.26 (*N* = 2233)	0.881 (*N* = 1,904)	0.675 (*N* = 2,168)	0.577 (*N* = 1,993)	0.411 (*N* = 1,929)
	50th percentile	0.600	1.00	1.10	1.23	1.23	0.860	0.700	0.600	0.400
	95th percentile	1.80	3.20	3.60	3.28	3.77	2.54	2.00	1.90	1.40
12 to 19 yr	Geometric mean	0.470 (*N* = 497)	0.852 (*N* = 640)	0.929 (*N* = 640)	1.16 (*N* = 357)	1.10 (*N* = 364)	0.741 (*N* = 344)	0.599 (*N* = 402)	0.481 (*N* = 353)	0.348 (*N* = 313)
	50th percentile	0.500	0.800	0.900	1.15	1.07	0.680	0.500	0.500	0.400
	95th percentile	1.20	2.80	2.70	2.54	2.62	2.06	2.00	1.20	1.20
20 yr and older	Geometric mean	0.563 (*N* = 935)	0.984 (*N* = 1,454)	1.11 (*N* = 1,480)	1.23 (*N* = 1,743)	1.29 (*N* = 1,869)	0.903 (*N* = 1,560)	0.685 (*N* = 1,766)	0.591 (*N* = 1,640)	0.419 (*N* = 1,616)
	50th percentile	0.600	1.00	1.10	1.23	1.23	0.890	0.700	0.600	0.400
	95th percentile	1.80	3.40	3.90	3.36	3.94	2.64	2.00	1.90	1.40

Source: NHANES, 2022: https://www.cdc.gov/exposurereport/data_tables.html (accessed 7/25/2022).

Data for 3- to 5- and 6- to 11-year-olds were only reported separately in 2013–2014; 3- to 5-year-olds (N = 181) had geometric mean of 0.764 (50th percentile 0.620, 95th percentile 3.49), while 6- to 11-year-olds (N = 458) had geometric mean of 0.809 (50th percentile 0.750, 95th percentile 3.19).

NHANES data have been used to identify subpopulations that may have higher PFNA exposure, such as those living in communities with contaminated water supplies or people with certain dietary patterns. Graber et al. (2019) found that people living in a New Jersey community with elevated levels of PFNA in their water supply had serum concentrations that were nearly threefold higher on average than the general population surveyed by NHANES. Christensen et al. (2017) reported an association between self-reported fish and shellfish consumption and higher levels of PFNA in serum. Christensen et al. (2017) and Haug et al. (2010) used data on serum PFAS levels and 30-day, self-reported fish and shellfish ingestion rates from NHANES 2007–2014 to explore potential relationships between PFAS exposures and fish consumption. PFNA (as well as PFDA and PFHxS) were among the PFAS detected in the serum of at least 30% of the NHANES participants, and after adjusting for demographic characteristics, total fish and shellfish consumption was associated with elevated serum PFNA. Populations that rely on seafood and/or subsistence diets, possibly including some Native American tribes, may also be disproportionately exposed (Caron-Beaudoin et al., 2020; Byrne et al., 2017). For example, biomonitoring in pregnant Inuit women in the Nunavik region of Canada, for whom fish is a dietary staple, were found to have 6.3-fold higher serum levels of PFNA compared with results for women of childbearing age participating in the Canadian Health Measure Survey (CHMS) (Caron-Beaudoin et al., 2020). The same study found a significant correlation ($\beta = 0.332$; $p < 0.05$) between serum concentrations of PFNA and omega-3/omega-6 polyunsaturated fatty acid ratios, indicating fish consumption as a key source of PFNA exposure. The authors suggested that higher ecological levels of 8:2 FTOH, which biodegrades to PFNA, was a likely contributor to exposure (Caron-Beaudoin et al., 2020).

Occupational exposure among people with frequent contact with PFAS/PFNA may have higher exposures compared with the general population. For example, PFNA also has been detected in the serum of firefighters (Graber et al., 2021; Trowbridge et al., 2020), with significantly higher levels of PFNA in the serum of female firefighters in San Francisco than in a cohort of office workers (Trowbridge et al., 2020). Because these chemicals can be found in ski wax, individuals who engage in professional ski waxing may be more highly exposed to PFNA through inhalation of dust or fumes (Nilsson et al., 2010a; Nilsson et al., 2010b).

PFNA exposure during susceptible early lifestages has been demonstrated from studies reporting detectable levels of PFNA in placental tissue, amniotic fluid, cord blood, and human milk (ATSDR, 2021; Lu et al., 2021; Macheka-Tendenguwo et al., 2018; Zhang et al., 2013b). The human placental:maternal blood serum ratio increases with gestation suggesting bioaccumulation in the placenta (Mamsen et al., 2019; Mamsen et al., 2017). PFNA has been detected in human breast milk samples from various countries including the United States (Macheka-Tendenguwo et al., 2018). Mondal et al. (2014) reported PFNA levels in maternal serum decreased with breastfeeding duration, with a nonsignificant increase in infant serum PFNA concentrations with breastfeeding duration. Other studies reported PFNA increases significantly in infant serum with breastfeeding (Gyllenhammar et al., 2018a; Rosen et al., 2018; Mogensen et al., 2015), and nulliparous women

have been reported to have 62% higher PFNA concentrations than parous women (Brantsæter et al., 2013), suggesting both placental and lactational transfer. Together, these and other epidemiological studies (e.g., (Mamsen et al., 2019; Gyllenhammar et al., 2018a; Liew et al., 2018a; Macheka-Tendenguwo et al., 2018; Winkens et al., 2017; Mondal et al., 2014; Zhang et al., 2013b; Fei et al., 2007)) suggest that PFAS, including PFNA, cross the blood-placental barrier and may accumulate in the placenta. Human placental and breast milk transfer efficiencies may depend on PFAS chain length and binding affinity to serum- and breast milk-protein complexes.

1.2. SUMMARY OF ASSESSMENT METHODS

This section summarizes the systematic review and dose-response methods used for developing this assessment. A more detailed description of the methods is provided in the systematic review protocol (see Appendix A).

1.2.1. Literature Search and Screening

The detailed search approach, including the query strings and populations, exposures, comparators, and outcomes (PECO) criteria, are provided in Table 1-4 and Appendix B, respectively. Results of the current literature search and screening efforts are documented below. Briefly, a literature search was first conducted in 2017, and regular yearly updates are performed. 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, until 2019)[7]
- TSCATS (Toxic Substances Control Act Test Submissions)

Additionally, relevant literature not found through database searching have been identified by:

- Review of studies cited in studies meeting the PFNA PECO criteria or published reviews of PFNA; finalized or publicly available U.S. federal and international assessments (e.g., the Agency for Toxic Substances and Disease Registry [ATSDR] assessment (ATSDR, 2021)).
- Searches of published PFAS SEMs (Carlson et al., 2022; Radke et al., 2022; Dessingou et al., 2012), starting in 2021.

[7]In December 2019 TOXLINE content was migrated to PubMed (https://www.nlm.nih.gov/databases/download/toxlinesubset.html).

- 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 EPA PFAS assessments. For example, epidemiology studies relevant to PFNA were sometimes identified by searches focused on one of the other four PFAS currently being assessed by the IRIS Program.

- Other gray literature (i.e., primary studies not indexed in typical databases, such as technical reports from government agencies or scientific research groups; unpublished laboratory studies conducted by industry; or working reports/white papers from research groups or committees) brought to the attention of EPA. Specific sources of gray literature are described in the protocol (see Appendix A).

All literature is tracked in EPA Health and Environmental Research Online (HERO) database (HERO Page). The PECO criteria (see Table 1-4) identify the evidence that addresses the specific aims of the assessment and to focus the literature screening, including study inclusion/exclusion.

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 non-mammalian models of genotoxicity. (Note: Other in vitro, in silico, or non-mammalian models will be tracked as potential supplemental material.)
Exposures	Human: Studies providing quantitative estimates of PFNA 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 PFNA based on administered dose, dietary level, or concentration. (Note: Non-oral and non-inhalation studies will be tracked as potential supplemental material.) PFNA mixture studies are included if they employ an experimental arm that involves exposure to PFNA alone. (Note: Other PFNA mixture studies are tracked as potential supplemental material.) Studies must address exposure to following: PFNA (CASRN 375-95-1), PFNA sodium salt (CASRN 21049-39-8), or PFNA ammonium salt (CASRN 4149-60-4).

PECO element	Evidence
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 PFNA 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 non-phenotypic endpoints addressing the potential biological or chemical progression of events contributing toward toxic effects will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol].)
PBPK models	Studies describing physiologically based pharmacokinetic (PBPK) and other pharmacokinetic (PK) models for PFNA (CASRN 375-95-1), PFNA sodium salt (CASRN 21049-39-8), or PFNA ammonium salt (CASRN 4149-60-4).

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

- In vivo mechanistic or MOA studies
- Non-PECO routes of exposure (e.g., intraperitoneal [i.p.] injection) and populations (e.g., non-mammalian 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

[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 of the protocol for details).

- PFAS mixture studies (no individual PFNA comparisons)
- Other assessments or records with no original data (e.g., reviews, editorials, commentaries; abstract-only)

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

1.2.2. Evaluation of Individual Studies

The detailed approaches used for the evaluation of epidemiological and animal toxicological studies used in the PFNA assessment are provided in the systematic review protocol (see Appendix A). The general approach for evaluating studies is the same for epidemiological and animal toxicological studies, although the specifics of applying the approach differ; they are described in detail in Appendices A, Sections 6.2 and 6.3 of the protocol, respectively.

The key concerns for the review of epidemiology and animal toxicological studies are potential bias (systematic errors or deviations from the truth related to internal validity that affect the magnitude or direction of an effect in either direction) and insensitivity (factors that limit the ability of a study to detect a true effect; low sensitivity is a bias toward the null when an effect exists). In evaluating individual studies, two or more reviewers independently arrived at judgments regarding the reliability of 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 and endpoints within the same study. The results of these reviews were tracked within EPA's version of the Health Assessment Workplace Collaboration (HAWC). To develop these judgments, each reviewer assigned a category of good, adequate, deficient (or not reported, which generally carried the same functional interpretation as deficient unless otherwise specified), or critically deficient (listed from best to worst methodological conduct) related to each evaluation domain representing the different characteristics of the study methods that were evaluated on the basis of criteria outlined in HAWC.

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

1) *High* confidence: No notable deficiencies or concerns were identified; the potential for bias is unlikely or minimal, and the study used sensitive methodology.

2) *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.

3) *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).

4) *Uninformative*: Serious flaw(s) were identified that make the study results unusable. *Uninformative* studies were not considered further, except to highlight possible research gaps.

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

Additional Epidemiology Considerations

Detailed methods for epidemiological study evaluation are described in the systematic review protocol (see Appendix A, Section 6.2 of the protocol). A few considerations for addressing potential confounding in the PFNA literature are briefly described below.

Confounding and effect measure modification across PFAS is a potential source of uncertainty when interpreting the results of epidemiological studies of individual PFAS (e.g., quantifying effects of an individual PFAS can potentially be confounded by other PFAS). 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 between co-occurring PFAS. In a preliminary analysis of six studies in the inventory examining mutually adjusted PFAS models, correlations differed across the PFAS pairs (see Appendix C, Table C-1). While some pairs have correlation coefficients consistently at or above 0.6 (e.g., PFNA and PFDA), the correlations for most vary from 0.2 to >0.8 depending on the study and exposure sources. 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 evaluations (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 when high correlations are observed. This challenge 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 in which concern is minimal for substantial confounding across PFAS as well as other sources of confounding. Examples 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 as described in the systematic review protocol (see Appendix A), 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 in Section 1.2.4, below). Approaches used in this assessment compared results across studies in populations with different PFAS exposure mixture profiles, considered results of multipollutant models when available, and examined the strength of associations for other correlated PFAS. In addition, overall certainty in the epidemiological evidence is decreased when there is residual confounding across PFAS (see Appendix A, Section 10 of the protocol, and Section 1.2.4, below).

1.2.3. Data Extraction

The detailed data extraction approach is provided Appendix A, Section 8 of the protocol. Briefly, data extraction and content management were carried out using HAWC for all health effects for animal studies and some health effects (i.e., where data visualizations were necessary to understanding the evidence synthesis judgments) for epidemiological studies. Data extraction elements that were collected from epidemiological, controlled human exposure, and animal toxicological studies are described in HAWC (HAWC). Not all studies that meet the PECO criteria went through data extraction: studies evaluated as being *uninformative* were not considered further and therefore did not undergo data extraction. All findings are considered for extraction, regardless of the statistical significance of their findings. The level of extraction for specific outcomes within a study may differ (i.e., ranging from a narrative to full extraction of dose-response effect size information). For quality control, data extraction was performed by one member of the evaluation team and independently verified by at least one other member. Discrepancies in data extraction were resolved by discussion or consultation within the evaluation team.

1.2.4. Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes (see Appendix A, Sections 9 and 10 of the protocol for full details). As described below in Section 2, for each assessed health effect, the evidence syntheses provide a summary discussion of each body of evidence considered in the review that directly informs the integration across evidence to draw an overall judgment for each health effect. The available human and animal evidence pertaining to the potential health effects are synthesized separately, with each

synthesis providing a summary discussion of the available evidence that addresses considerations regarding causation that are adapted from (Hill, 1965). Mechanistic evidence is also synthesized as necessary to help inform key decisions regarding the human and animal evidence; processes for synthesizing mechanistic information are covered in detail in Appendix A (see Section 9.2 of the protocol).

The syntheses of the human and animal evidence on each health effect focus on describing aspects of the data that best inform causal interpretations, including the exposure context examined in the sets of studies. 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). The synthesis of mechanistic information typically differs from evaluations of phenotypic evidence (e.g., from routine toxicological studies). This difference 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 [MOA]); consistency with known biological processes), rather than evaluations of individual apical endpoints considered in relative isolation.

Following the syntheses of human and animal evidence and mechanistic data, integrated judgments are drawn across all lines of evidence for each assessed health effect. During evidence integration, a structured and documented process is used, as discussed below.

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

The animal, human, and mechanistic evidence judgments are then combined to draw an overall judgment that incorporates inferences across evidence streams. Specifically, the inferences considered during this integration include the human relevance of the animal and mechanistic evidence, coherence across the separate bodies of evidence, and other important information (e.g., judgments regarding susceptibility). Note that without evidence to the contrary, the human

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

As discussed in the protocol (see Appendix A), the methods for evaluating the potential carcinogenicity of the five PFAS being assessed within the IRIS Program follow processes laid out in the EPA cancer guidelines (U.S. EPA, 2005); however, for PFNA, data relevant to cancer were sparse and did not allow for such an evaluation (see Section 3.3).

1.2.5. Dose-Response Analysis

The details for the dose-response analysis employed in this assessment can be found in Appendix A (see Section 11 of the protocol). Briefly, although procedures for dose-response assessments were developed for both noncancer and cancer health hazards, and for both oral and inhalation routes of exposure following exposure to PFNA, the existing data only supported derivation of an oral reference dose (RfD) for noncancer hazards (see Appendix A for the health hazard conclusions necessary for deriving other values). An RfD is an estimate, with uncertainty that may span 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, 2002b). The derivation of reference values, like an RfD, depends on the nature of the health hazard conclusions drawn during evidence integration. For noncancer endpoints, 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 of the protocol for exceptions for performing quantitative analysis for ***evidence suggests*** determinations). Consistent with EPA practice, the 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.

- The derivation of cancer risk estimates and reference values may involve extrapolation to exposures lower than the POD; the approaches to be applied in these assessments are described in more detail in Appendix A, Section 11.2 of the protocol.

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 it eliminates the need to perform interspecies extrapolations. For reference values, this assessment derives a candidate value from each suitable data set. Evaluation of these candidate values yields a single organ/system-specific value (for oral exposure, an organ/system-specific reference dose [osRfD]) for each organ/system under consideration from which a single overall reference value (for oral exposure, a reference dose [RfD]) 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 (https://www.epa.gov/bmds) that can be applied to typical data sets, including those that are nonlinear. In situations in which 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 a guideline on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results [see the EPA *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a)]. Additional judgment or alternative analyses are used if the procedure fails to yield reliable results; for example, if the fit is poor, modeling may be restricted to the lower doses, especially if there is competing toxicity at higher doses. For each modeled response, a POD from the observed data was estimated to mark the beginning of extrapolation to lower doses. The POD is an estimated dose (expressed in human equivalent terms) near the lower end of the observed range without significant extrapolation to lower doses. The POD is used as the starting point for subsequent extrapolations and analyses. For noncancer effects, the POD is used in calculating the RfD.

For those outcomes in which an osRfD is derived, the level of confidence in the primary study used to derive the reference value, evidence base associated with the reference value, the quantification of the POD, and the overall confidence designation are provided, along with the rationales for selecting these confidence levels. An overview on characterizing confidence is provided in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Briefly, the confidence ranking reflects the degree of belief that the reference value will change (in either direction) with the acquisition of new data. It is not a statement about confidence in the degree of health protection provided by the reference value. In addition, the confidence ranking is intended to reflect considerations not already covered by the UFs and is not linked directly to the UF values. Confidence rankings are not discrete entities and for any given parameter, the level of confidence may fall along the continuum between *low* to *high*. For example, a designation of *high* confidence in the study/studies used in dose-response analysis may

not translate to the assessment reporting a *high* level of confidence in the completeness of the evidence base of available studies or the overall confidence in the derived toxicity value. Additionally, different components of the overall confidence in the derived toxicity value may factor more heavily in that final determination given assessment- or endpoint-specific situations. In other words, confidence in the evidence base may be the predominating factor in the overall confidence in one toxicity value, whereas the quantification of the POD may be the most important factor in the confidence for another toxicity value.

2. LITERATURE SEARCH AND SCREENING RESULTS

The database search (through April 2023) yielded 3,316 unique records for perfluorononanoic acid (PFNA; see Figure 2-1). Of the 3,316 identified records, 1,381 were excluded during title and abstract screening, and 989 were advanced and reviewed at the full-text level. Of the 989 full-text screens, 585 were considered to meet the populations, exposures, comparators, and outcomes (PECO) eligibility criteria (see Table 1-4). The studies meeting PECO criteria at the full-text level included 534 epidemiologic studies, 36 animal studies, 10 genotoxicity studies, and 9 physiologically based pharmacokinetic (PBPK) modeling studies. The assessment synthesis, integration, and dose-response reflect studies identified through the 2022 literature search update, but studies from the 2023 update were reviewed for impact on the draft synthesis, integration, and dose-response conclusions. The decisions on inclusion for these studies are summarized in Appendix B, Table B-2.

The evidence base of epidemiological and animal studies includes developmental (including neurodevelopmental), immune, liver, endocrine (thyroid and adrenal glands), reproductive, cardiometabolic, and renal effects, among other potential adverse health outcomes (see Figure 2-2). The evidence for these potential health effects is presented and assessed in Sections 3.2.1–3.2.11. There are currently no chronic or carcinogenicity studies in animals exposed to PFNA, and the small number of other available genotoxicity and mechanistic studies in humans and animals informing potential carcinogenic activity with PFNA exposure are summarized in Section 3.3. Of the 3,316 studies screened, a total of 946 were found to contain potentially relevant supplemental material (e.g., mechanistic, non-PECO route of exposure, ecotoxicity). This corpus of studies included potentially relevant in vivo mechanistic studies, such as i.p. injections, in vitro bioassays, and in silico modeling to inform an understanding of mode of action and cross-stream coherence of results from the available human and animal evidence. These supplemental data are summarized in the *Mechanistic and Supplement Information* sections for each health outcome in Section 3. Additionally, high-throughput screening data on PFNA are currently available from EPA's CompTox Chemicals Dashboard (U.S. EPA, 2019) and relevant information from this resource is presented in Section 3 and Appendix C.

Graphical representations of outcome-specific study evaluation results are presented in each hazard section (see Sections 3.2.1–3.2.11). Detailed rationales for domain and overall confidence ratings for the study evaluations are available in HAWC.

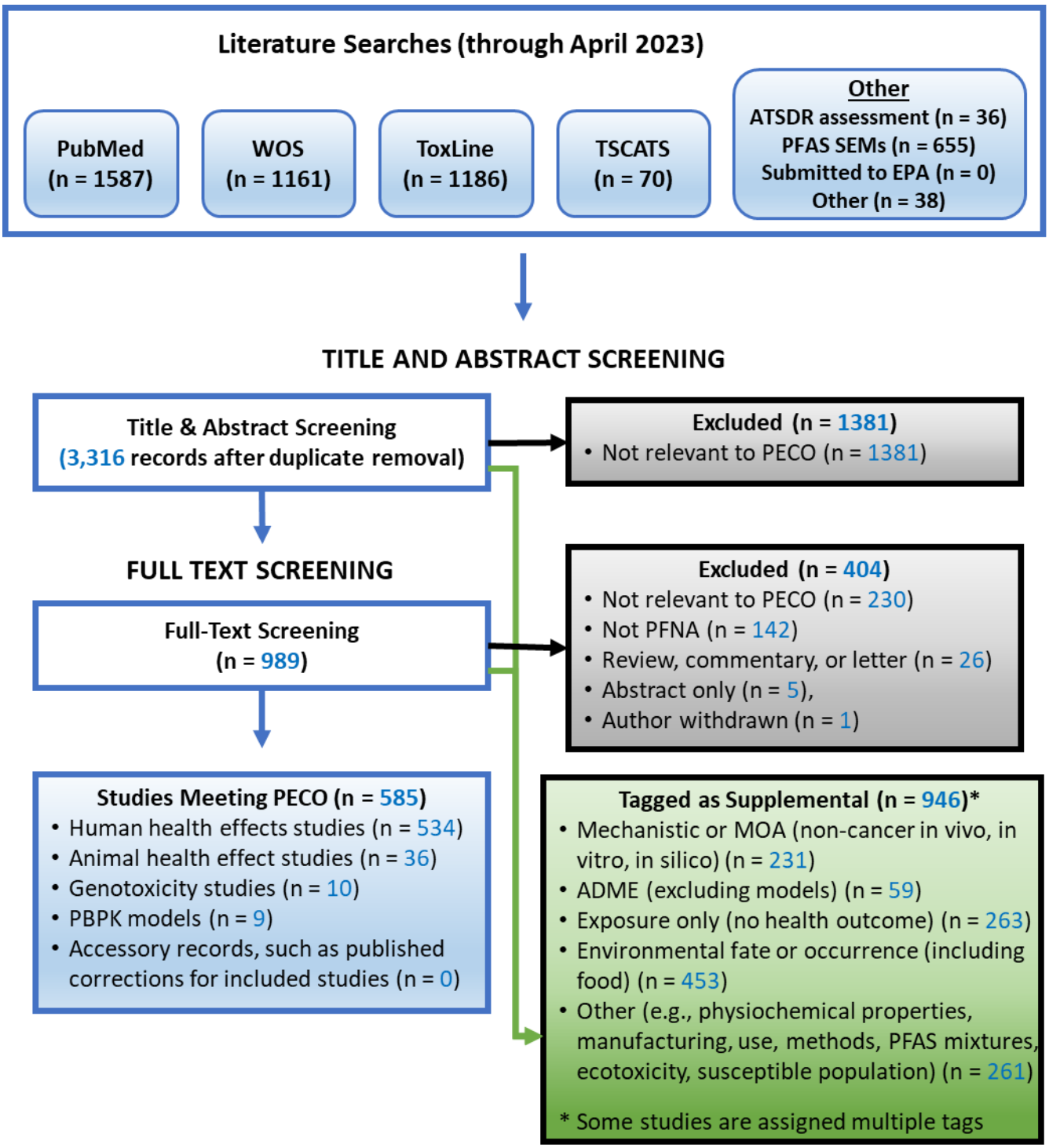


Figure 2-1. Literature search and screening flow diagram for PFNA.

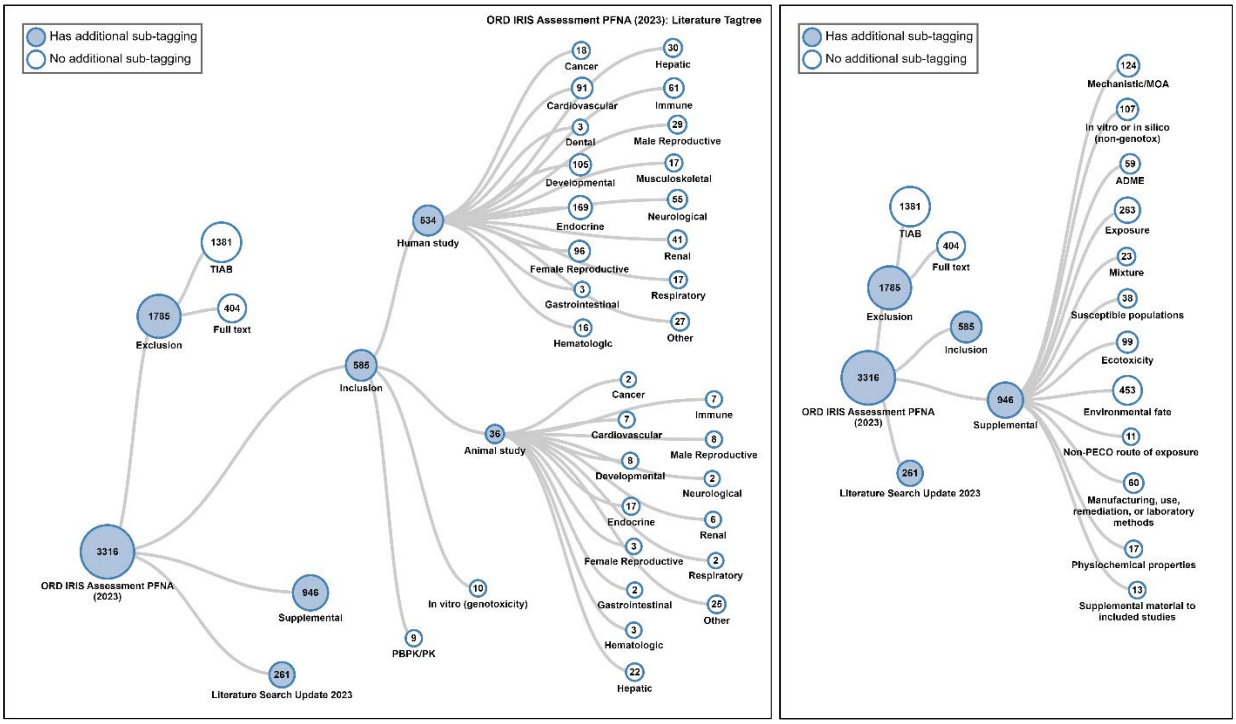


Figure 2-2. Literature tagtree of the PFNA evidence base by health outcome for epidemiologic and animal studies and by non-PECO category for supplemental studies.[a] See interactive HAWC link: PFNA Literature Tagtree.[b]

[a]Studies can be assigned multiple tags for outcomes and mechanistic categories.
[b]The Literature Search Update 2023 study counts are also included in the ORD IRIS Assessment PFNA 2023 study counts. See Appendix B, Table B-2 for a complete list of studies included in the 2023 update.

3. PHARMACOKINETICS, EVIDENCE SYNTHESIS, AND EVIDENCE INTEGRATION

3.1. PHARMACOKINETICS

Perfluorononanoic acid (PFNA) and its salts have absorption, distribution, metabolism, and excretion (ADME) properties similar to other perfluoroalkyl acids. However, many studies do not distinguish between isomers of PFNA, which differ in the branching of the perfluoroalkyl chain. When evaluated separately, the linear isomer (n-PFNA) was found to have a 1.5- to 2-fold longer half-life in rats than a branched isomer (iso-PFNA), which is terminated in an isopropyl group (Benskin et al., 2009). Since most of the available studies did not distinguish the isomers, EPA presumes that at least some of the variability in results between studies is due to different proportions of isomer analyzed, but that differences in ADME properties between isomers are less than the factor of 2 observed for half-life. The remainder of Section 3.1 addresses the reported empirical results under the assumption that the ADME results are average values for the different isomers.

The ADME characteristics of PFNA include fairly efficient if somewhat slow absorption from the gastrointestinal tract following oral exposure, high affinity for protein binding, and efficient renal reuptake.

The results of Benskin et al. (2009) demonstrate quantitative pharmacokinetic (PK) differences in rats between the two isomers. In particular, 1 day after dosing of a 49%/51% mixture the iso-/n- ratio in blood was 61%/39%, indicating 50% more efficient absorption of iso-PFNA, with reported elimination half-lives of 21 and 41 days for iso- and n-PFNA, respectively. The remaining PK studies appear to have evaluated only n-PFNA and do not specifically address the kinetics of different isomers. Therefore, the following PK analysis does not attempt to distinguish between the isomers, and quantitative results are presumed to primarily reflect the PK of n-PFNA.

The rat NTP bioassay does not specifically mention the isomeric purity of the test material (NTP, 2018), but the CAS number is for the linear form, and it is presumed that the observed toxicity represents this form. De Silva et al. (2009b) evaluated the presence of branched PFNA (and other PFAS) isomers in environmental and human blood samples from North America and only reported a small fraction of the PFNA present as branched isomers, e.g., less than 2% in blood samples. Therefore, the estimated clearance of PFNA in humans, obtained from epidemiological biomonitoring data, is likewise presumed to primarily represent n-PFNA and the presence of a small fraction of branched isomer is not likely to significantly impact this evaluation. If iso-PFNA has a similar toxicological potency as n-PFNA (i.e., for a given tissue concentration) and clearance of iso-PFNA is faster than of n-PFNA in humans,

as was seen in rats, an oral reference dose (RfD) obtained from analysis of n-PFNA data will be protective of iso-PFNA as well.

The PK characteristics of PFNA may be in part due to the similarity between PFAS and medium-chain fatty acids, which are tightly controlled in the body and maintained as an energy source (Papamandjaris et al., 1998). However, unlike its fatty acid analog, PFNA is considered impervious to metabolism in mammals due to the perfluoro substitution of the alkyl chain. Due to the slow clearance mediated through interaction with binding proteins and transporters (Sheng et al., 2016; Bischel et al., 2010; Woodcroft et al., 2010), PFNA tends to accumulate in the plasma, liver, and kidney (Kim et al., 2019; Iwabuchi et al., 2017; Gao et al., 2015; Benskin et al., 2009). PFNA binds to albumin in the plasma, which is maintained in relatively large concentrations (Bischel et al., 2011; Bischel et al., 2010). In the liver, PFNA has been shown to interact with liver fatty acid binding protein (L-FABP) (Sheng et al., 2016; Woodcroft et al., 2010). Enterohepatic recirculation of PFNA may also occur, which could increase levels of PFNA in the liver (Ruggiero et al., 2021; Genuis et al., 2010). Distribution to the kidney is likely due to protein interactions as well, as transporters have been identified that resorb PFNA from the filtrate (Zhao et al., 2017b). Uptake transporters have been identified on the basolateral surface of the proximal tubule as well, which transport PFNA from the serum to the kidney tissue (Weaver et al., 2010).

PFNA is primarily excreted in urine and feces. Female rats and mice excrete PFNA much faster than male rats and mice. It has also been proposed that PFNA is excreted in blood (and other fluids) lost due to menstruation and childbirth, but direct evidence of excretion in menstrual fluid is not available and evaluation of serum PFNA levels in men versus women from NHANES for multiple PFAS does not indicate that menstrual fluid loss acts consistently across PFAS (see Section 3.1.4, "Menstrual clearance in humans," below). The NHANES data do indicate that PFNA clearance is higher in women of childbearing age than in men over the same age range, although the mechanism for this difference is unclear. This additional excretion in women appears to occur between 12.4 and 40 years of age and results in a lower half-life, estimated at 2.9 years, compared with 4.0 years for men and younger and older women (analysis below). Measured concentrations of PFNA in breastmilk (see Section 3.1.2, "Human distribution during gestation and childhood") and the correlation of PFNA concentration in young children with length of breastfeeding (Koponen et al., 2018) clearly show that lactation is a route of excretion for the mother and exposure for the infant.

Given the available PFNA studies considered for use in dose-response analyses (see Section 5), to perform animal-to-human extrapolation of toxicity points of departure (PODs) and to interpret human epidemiological analyses using measured blood concentrations of PFNA, PK parameters need to be identified for male and female rats, mice, and humans. These parameters can either be used with a PK model that relates exposure to blood concentration over time in each species and sex or to estimate species- and sex-dependent differences in the average internal dose. The two key parameters to be determined are the clearance (CL; mL/kg-day) and volume of distribution (Vd; mL/kg). The ratio of maternal and cord serum and the ratio of maternal serum to

breast milk are also model parameters required for application of a PK model to developmental exposure scenarios and are helpful when interpreting corresponding toxicological data. While several physiologically based pharmacokinetic (PBPK) models have been described for PFNA (or that include predictions for PFNA), EPA found that a key assumption in these models for PFNA distribution to tissues was contradicted by the PK data and hence that a PBPK model that could adequately predict PFNA PK data was not available. A classical one- or two-compartment PK model was developed and evaluated. While the classical PK model did not predict measured concentrations from toxicity studies (validation data) in rats and mice as well as desired, EPA concluded that it was the best option for estimating internal doses in male rats and in mice; results shown in Appendix E.4.1. For female rats, direct interpolation of the measured concentrations from the NTP 28-day bioassay was determined to be the best option for estimating internal doses for PODs identified from that toxicity study. The available PBPK models and the PK modeling approach evaluated are discussed briefly in the PK modeling section (see Section 3.1.6 and Appendix E.4), while the discussion of the literature used to estimate the PK parameters is discussed in the relevant ADME sections below.

3.1.1. Absorption

In a rat oral exposure study, peak tissue concentrations were essentially reached at 12 hours, although the concentration in whole blood (but not serum) appeared to trend upward until 24 hours and that in brain until 72 hours (Iwabuchi et al., 2017). This pattern indicates that absorption was complete by 12 hours but distribution into red blood cells and brain was not yet complete at that time. Maximum serum concentrations in another study were reached prior to the first observation at 24 hours in rats after oral exposure (Tatum-Gibbs et al., 2011). Kim et al. (2019) measured blood concentrations at shorter timepoints up to 24 hours and although they did not report the time of maximum concentration (T_{max}), their plotted data also appear to show a maximum at 24 hours in both male and female rats (i.e., neither have a T_{max} < 24 hours).

Kim et al. (2019) presented pharmacokinetic data using both intravenous and oral gavage dosing in rats, and their analysis showed similar bioavailability between sexes in rats with a bioavailability of 79 ± 15% in female rats and 77 ± 6% in male rats (mean ± SD). Another study presented by Fujii et al. (2015) reported both intravenous and oral PK data in mice, from which the estimated bioavailability appears to be 99% in males and 100% in females of that species. Fujii et al. (2015) only measured blood concentrations for 24 hours after dosing, making their estimates of AUC in blood, used to evaluate bioavailability, more uncertain. However, examination of the plotted data indicates that while T_{max} may have been 12 hours, there was no significant difference in concentration between 12 hours and 3 hours in female mice and 6 hours in male mice, so absorption was essentially complete by those earlier timepoints (Fujii et al., 2015).

Oral bioavailability, parameterized as F_{abs}, is typically estimated by comparing blood concentrations, in particular the AUC, observed after oral versus IV dosing. If the distribution, metabolism, and excretion of a compound are identical after absorption, AUC(oral)/AUC(IV) is

interpreted as the fraction of an oral dose absorbed. However, in several PK studies for PFNA and other PFAS, the empirical AUC obtained after oral dosing exceeded that after IV dosing. Specifically, for PFNA, the dose-normalized 24-hour AUCs reported by Fujii et al. (2015) were equal or higher after gavage than IV doses, which would seem to indicate F_{abs} at or above 100%. But absorption greater than 100% is physically impossible. In part the nominal result may be due to the use of an oral dose in the study 10 times higher than the IV dose together with some nonlinearity in absorption or clearance. Data from an oral developmental study in mice by Das et al. (2015) indicate saturation of liver concentrations in pregnant dams and postnatal pups with increasing dose, which would allow for a greater serum concentration relative to dose at higher exposures. Also, the fact that the serum concentration was only tracked for 24 hours would result in a very uncertain estimation of long-term clearance. Properly, the fraction absorbed should be based on the area-under-the-curve (AUC) of serum concentration from time zero to infinity, which depends on the long-term clearance.

From EPA's PK analysis of the rat data (see Appendix E.1) the mean (90% CI) fraction absorbed estimated in male and female rats was 0.86 (0.66–1) and 0.94 (0.89–1), respectively. A fraction absorbed could not be estimated by EPA for female mice since the only PK data available for analysis were from intravenous exposure. Given the results of the Fujii et al. (2015) mouse PK study, the fraction absorption in the mouse is assumed to be 1.0. Since quantitative data on oral bioavailability are not available for humans, a health-protective assumption of 100% (F_{abs} = 1) will be made. Given that the reported bioavailability for rats was 77%–79% (Kim et al., 2019), the range estimated by EPA for rats was 66%–100%, and bioavailability appears to be near 100% in mice (Fujii et al., 2015), absorption in humans is expected to be at least 66%. Therefore, the assumption of 100% bioavailability in humans is not anticipated to overestimate human uptake by more than 50% (i.e., 100%/66%).

As noted above, in a study that compared two common PFNA isomers, n-PFNA and iso-propyl PFNA, the iso-PFNA showed greater uptake than n-PFNA, potentially due to greater bioavailability (Benskin et al., 2009). After a single dose with an approximately equal mixture of the two isomers, the day 1 blood composition was 61.2% iso-PFNA and 38.8% n-PFNA. After 72 hours of continuous exposure, the isomers were approximately equal in blood despite faster excretion of the iso-PFNA (Benskin et al., 2009).

Kinney et al. (1989) evaluated the toxicity of PFNA after inhalation exposure of rats to particulate PFNA. While the dosimetry of PFNA was not evaluated as a part of the study, the fact that statistically significant changes in liver and body weight were observed at 67 and 500 mg/m^3 PFNA air concentration versus controls clearly shows that absorption occurs by the inhalation route. Unfortunately, quantitative ADME data from inhalation exposure that would enable interpretation of these response data are not available.

3.1.2. Distribution – General Considerations

Animals (Rats and Mice)

PK data from Kim et al. (2019), Iwabuchi et al. (2017), Ohmori et al. (2003), and Tatum-Gibbs et al. (2011) were used to evaluate the volume of distribution in rats, while PK data from Tatum-Gibbs et al. (2011) and Fujii et al. (2015) were used to evaluate the Vd in mice (see Table 3-1). The Vd values in both rats and mice support a high relative amount of the chemical located in plasma compared with other tissues. For example, approximately 12% of PFNA is in the plasma of male and female rats as a proportion of the internal dose of PFNA in the animal. This distribution to the plasma compartment is notably high because the volume fraction of plasma is 34–35 mL/kg in rats (Everett et al., 1956) so only 3.4%–3.5% of a chemical that distributed evenly between plasma and the rest of the body would be found in the plasma.

Some of the calculations presented in Kim et al. (2019) appear to have either been reported inconsistently with each other and the PK parameter units given or perhaps are in error. Details of EPA's analysis of these results are provided in Appendix E.2. In brief, by using the standard relationship Vd = dose/C_{max}, EPA estimated the female rat Vd as 183.4 mL/kg using the reported Vd of 45.86 mL while assuming 0.25 kg BW, which is identical to dose/C_{max} for the female rats and in the range of values reported for female rats by Ohmori et al. (2003) and Tatum-Gibbs et al. (2011). Therefore, the mL values for female rats from Kim et al. (2019) were normalized by 0.25 kg for reporting in Table 3-1. On the other hand, if one assumes that the reported value of "363.09 mL" for male rats is actually mL/kg, the value is similar to that reported by Iwabuchi et al. (2017) (next paragraph), so that value is assumed to apply given units of mL/kg.

Iwabuchi et al. (2017) evaluated PFNA PK in male rats but reported Vd as "kg tissue volume/kg BW," which is unusual. While the text does not indicate that a correction for oral absorption was used, only that Vd = dose/(AUC × ke), the value of 0.36 kg tissue/kg BW listed for PFNA appears to have been calculated using a fraction absorbed of ~ 80%, which is appropriate. Therefore, the Vd for serum of Iwabuchi et al. (2017) was corrected for the density of serum (1.024 kg/L; (Sniegoski and Moody, 1979)), yielding 350 mL/kg, which is close to the value reported for male rats by Kim et al. (2019) (interpreted as mL/kg). Hence, the Vd value listed in Table 3-1 for male rats for Iwabuchi et al. (2017) is 350 mL/kg.

Ohmori et al. (2003) evaluated PFNA PK in Wistar rats while the other rat PK studies used Sprague-Dawley. Since the values of Vd reported by Ohmori et al. (2003) are in the range of the other studies, there does not appear to be a significant difference between the rat strains for this parameter and the evaluation of overall PK parameters assumes they all represent the same population.

In mice, Vd values were recalculated as dose/C_{max} for males from the results of Tatum-Gibbs et al. (2011) because the reported value had a very high uncertainty range while the C_{max} values did not (see Appendix E.2 for further details), yielding 503 mL/kg for the 1 mg/kg dose and 348 mL/kg for the 10 mg/kg dose. Because of the variation in reported and calculated Vd estimates in mice, Vd

was also calculated as dose/C_{max} for the female mice in Tatum-Gibbs et al. (2011), yielding 262 and 207 mL/kg at 1 and 10 mg/kg, respectively. For Fujii et al. (2015), the reported mean Vd values are almost identical to dose/C_{max} for the fitted curve, e.g., 140 versus 150 mL kg for female mice, so the reported values are used. While the Vd values for mice calculated from Tatum-Gibbs et al. (2011) are much larger than those reported by Fujii et al. (2015), the 95% confidence interval in the C_{max} reported by Tatum-Gibbs et al. (2011) was approximately a factor of 2 (upper/lower bound), so the C_{max} values are considered a *robust* measure of distribution in that study. The difference between the two studies may result from the difference in mouse strain used, CD-1 versus FVB/NJc1.

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 E.1. A similar Bayesian analysis for mice including only the data from Tatum-Gibbs et al. (2011) is also presented. A combination of that data with the data of Fujii et al. (2015) was not possible because individual timepoint data, which are needed for the analysis, were not available for Fujii et al. (2015). It is notable that the range of Vd estimated from the CD-1 mouse data of Tatum-Gibbs et al. (2011) appears to be significantly higher than obtained with FVB/NJc1 (Fujii et al., 2015). However, the difference could be due to the PK analysis method used. Therefore, the Vd estimated from the data of Tatum-Gibbs et al. (2011) will be assumed to apply to all mouse strains for dosimetric extrapolation.

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				
Iwabuchi et al. (2017)	Wistar	Oral	0.05	350[b] *239.6 (140.6–338.9)*
Tatum-Gibbs et al. (2011)	SD	Oral	1	113 (67–158) *184.4 (139.9–225.6)*
			3	139 (82–196) *137.8 (106.4–170.1)*
			10	110 (65–154) *125.6 (98.57–151.6)*
Kim et al. (2019)	SD	i.v.	3	363[c] *467.5 (403.8–528.1)*
		Oral	3	NR *331.0 (160.4–490.2)*
Ohmori et al. (2003)	Wistar	i.v.	22.6	286.8 ± 12.5 *239.6 (140.6–338.9)*
Population-level summary				*234.8 (147.3–318)*
Female rats				
Tatum-Gibbs et al. (2011)	SD	Oral	1	125 (86–164) *249.5 (186.8–317)*
			3	171 (104–238) *247.6 (181.3–316.3)*
			10	146 (90–201)[d] *260.6 (194.3–321.6)*
Kim et al. (2019)	SD	i.v.	0.5	NR *310.3 (271.2–349.8)*
		Oral	0.5	NR *267.4 (221.5–312.7)*
		i.v.	1	NR *319.9 (286.1–354)*
		Oral	1	NR *290.9 (245.5–337.1)*
		i.v.	3	183.4 ± 14.6[e] *303.4 (260.2–345.9)*
		Oral	3	NR *326.4 (267.3–388.8)*
		Oral	10	NR *336.3 (279.9–396.2)*
Ohmori et al. (2003)	Wistar	i.v.	22.6	243.1 ± 48.9 *235.6 (162.5–309.6)*

Study	Strain	Route	Dose (mg/kg)	Volume of distribution (mL/kg)[a]
Population-level summary				*283.2 (243.9–322.3)*
Male mice				
Tatum-Gibbs et al. (2011)	CD-1	Oral	1	503[f]
			10	348[f]
Bayesian analysis of pooled Tatum-Gibbs et al. (2011) *male mouse data*				*576.1 (524.3–631.8)*
Fujii et al. (2015)[g]	FVB/NJc1	i.v.	0.145	220 ± 60
Female mice				
Tatum-Gibbs et al. (2011)	CD-1	Oral	1	262[h]
			10	207[h]
Bayesian analysis of pooled Tatum-Gibbs et al. (2011) *female mouse data*				*319.6 (280.8–358.9)*
Fujii et al. (2015)[g]	FVB/NJc1	i.v.	0.145	150 ± 40

[a]Values in plain text are as reported for each study unless otherwise noted. NR = not reported. Values in italics are the mean (90% credible interval) from the Bayesian analysis described in Appendix E.1.

[b]Values reported as "kg tissue volume/kg BW" were converted to mL/kg using a density of 1.024 kg/L for serum (Sniegoski and Moody, 1979); see text for other details.

[c]Kim et al. (2019) reported Vd as 363.1 ± 182.7 mL, which is in the range of values from other studies if interpreted as mL/kg.

[d]Tatum-Gibbs et al. (2011) lists 0.0146 (0.090–0.201) L/kg, but this seem like a clear typographical error since dose/C_{max} = 0.146 L/kg, while 0.0146 is outside the confidence interval, and the text states that the Vd for females is within the range of 0.125–0.171 L/kg.

[e]Kim et al. (2019) reported Vd as 45.86 ± 3.65 mL. Calculating dose/C_{max} = 183.4 mL/kg. If a body weight of 0.25 kg is assumed, 183.4 mL/kg × 0.25 kg = 45.85 mL, almost identical to the reported mean Vd (mL). Therefore, the value of Vd reported was normalized by 0.25 kg.

[f]Tatum-Gibbs et al. (2011) provide a single value (range) of 328 (0–1,060) for male mice, indicating high uncertainty in the estimation method. Therefore, separate values of Vd were calculated for each dose level as dose/C_{max}.

[g]Fujii et al. (2015) does not provide the time-course data necessary for the Bayesian PK analysis.

[h]Tatum-Gibbs et al. (2011) provide a single value (range) of 192 (165–220) for female mice. However, for consistency with the analysis of male mice, dose-specific values were calculated as dose/C_{max}.

In addition to distribution to plasma, studies in rats have shown additional distribution of PFNA to the liver and kidney, as well as minor distribution to other tissues (Kim et al., 2019; Iwabuchi et al., 2017; Gao et al., 2015; Benskin et al., 2009). More specifically, Benskin et al. (2009) showed the highest distribution in rats to liver, followed by blood > kidney > lung > heart > spleen > testes > muscle > fat > intestines > brain. While the tissue distribution observed in rats was fairly consistent among the various tissues for most of the studies, there was a very wide range in the liver/blood ratio, from ~ 0.2 in female rats reported by Gao et al. (2015) to 12 reported by Iwabuchi et al. (2017) (both from exposure in drinking water). More detailed information is provided in Appendix E.2.

Summary of volume of distribution in rats: The analyses of Vd (along with other PK parameters) by various authors was complicated by differences in method of analysis and some apparent reporting errors, as described above. Also, the original data were not available for all studies, limiting EPA's reanalysis. Nevertheless, the interpreted and re-estimated values indicate a reasonable range of uncertainty and that there is not a significant difference between the two rat strains for which there are data (Sprague-Dawley and Wistar), supporting the use of the pooled population analysis. The rat population mean summary Vd values of 235 and 283 mL/kg for male and female rats, respectively (Tabel 3-1, Appendix E), will therefore be taken as appropriate values for dosimetric analysis and extrapolation of rat dose-response data.

Summary of volume of distribution in mice: With the fewer data available the results for mice are less robust, but the estimated mean values of 579 and 320 mL/kg obtained with the CD-1 mouse data of Tatum-Gibbs et al. (2011) for males and females, respectively, will be applied for dosimetric analysis and extrapolation for dose-response data from that species.

Humans

Chiu et al. (2022) estimated the Vd and other PK parameters in humans by fitting a one-compartment PK model to individual and population blood concentration data from communities with high PFAS levels in the drinking water, while using general U.S. population blood concentration data to estimate background exposures, via Bayesian analysis. The resulting geometric mean (95% CI) was 0.19 (0.11–0.30) L/kg, which is a bit lower than mean values EPA estimated for rats and mice, but in the range of reported values (see Table 3-1). The value is assumed to apply to humans of all ages since estimates of PFNA distribution specifically in children are not available.

Pérez et al. (2013) describes human tissue PFNA levels from a small sample of human cadavers between 28 and 83 years of age. Of the five tissues examined (liver, bone, brain, lung, and kidney), PFNA levels were highest in the brain, with a median level of 13.5 ng/g, followed by kidney, which was slightly lower at 10.9 ng/g, then lung (3.5 ng/g) and liver (1.0 ng/g). Levels in the bone were below the limit of detection (LOD) (4.18 ng/g). The high amount of PFNA in brain, shared by PFHxA and PFDA, is unusual compared with most of the PFAS in the study and is discrepant with the low brain distribution seen in rats (see Appendix E.2). Wang et al. (2018a)

measured concentrations in matched blood and cerebrospinal fluid (CSF) and found the median (GM) of CSF/serum was 1.3 (1.37)%, indicating very low penetration, similar to that of other PFAS. Further research is needed to understand the mechanism by which PFNA appears to be preferentially accumulating in the human brain.

Another window into human distribution has been provided by liver biopsies and matched serum samples (Yeung et al., 2013). These samples are often taken from people suffering from liver disease, which may affect the applicability of the results to the general population. The paired samples revealed lower relative levels in liver tissue compared with serum than reported in most of the animal studies, with mean liver:serum concentration ratios of 0.33 in hepatocellular carcinoma patients, 0.18 in patients with cirrhosis due to chronic hepatitis C infection, and 0.63 in patients with both conditions (Yeung et al., 2013). These values were based on a relatively small sample of 55 patients but are in the range reported for rats by Gao et al. (2015). Distribution between components of human blood, discussed in detail in Appendix E.2.1, indicates that most PFNA in the blood is in the plasma with minimal distribution to red blood cells.

Summary of human volume of distribution outside of gestation: The volume of distribution in humans estimated by Chiu et al. (2022) (0.19 L/kg) is assumed valid. Since specific data on distribution in children are not available, this value is assumed to apply to all ages.

Human distribution during gestation and childhood

Serial samples of maternal serum during pregnancy showed a significant mean 33% decrease in PFNA concentration between the first and third trimester (Glynn et al., 2012). Similarly, Oh et al. (2022) showed a similar decline in the median PFNA maternal serum concentration from 0.6 to 0.4 ng/mL, while Chen et al. (2021) observed a decline from a median blood concentration of 2.33 to 1.24 ng/mL and Pan et al. (2017) observed a decline in the median concentration from 0.83 to 0.65 ng/mL between the first and third trimester. Declines in maternal serum levels can be attributed to the distribution of the maternal body burden into growing maternal tissues, placenta, and fetus, as the rate of this growth is quite rapid compared with the timescale over which PFNA and other long-chain PFAS are likely to accumulate (i.e., their half-lives in the body). Note that while blood plasma volume/kg increases in women during pregnancy, albumin levels decline, so the total amount of PFNA found in blood will depend on the product of blood volume and albumin concentration, which may not vary significantly because of these opposing trends. However, the increase in pregnancy-specific tissues and other maternal tissues results in an unambiguous increase in overall distribution volume.

In contrast to these previous results, Taibl et al. (2023) report higher serum levels of PFNA in first (GM = 0.37 ng/mL) and third trimester (GM = 0.41 ng/mL) women than first trimester women (GM = 0.26 ng/mL), with the difference between the third and first trimester indicated as statistically significant ($p < 0.05$). A detailed discussion of Taibl et al. (2023) is provided in Appendix E.2.3. Briefly, if one assumes that the first trimester concentration is the result of chronic exposure, an increase of 60% (from 0.26 to 0.41 ng/mL) over only 6 months (first to third

trimester) can be explained only as the result of a significant increase in exposure. If PFNA excretion were reduced to zero (which is highly unlikely), given a chronic exposure rate consistent with the average first trimester 0.26 ng/mL serum concentration, the increase in serum concentration between the first and second trimester would be no more than one-seventh of that reported. While it may also be possible that a biological mechanism unique to the African American women evaluated by Taibl and colleagues explains the discrepancy between their results and those of other longitudinal studies discussed above, EPA is not aware of such a mechanism. Hence, it appears that the observed change resulted from a significant increase in exposure to the population during the perinatal period, so the results are not considered relevant to the estimation of developmental dosimetry under conditions of constant exposure.

Many studies have evaluated distribution of PFNA between the mother and fetus by measuring levels in cord blood or serum and related tissues, compared with maternal serum. Liu et al. (2011) found a median ratio of cord serum to maternal serum of 0.57 for PFNA. Male and female infants had similar cord serum to maternal serum ratios. Glynn et al. (2012) also found that the mean maternal PFNA level during the third trimester was higher than the level in cord blood even after accounting for the decreased levels in blood compared with serum. Data digitized by EPA from Figure 3 of Glynn et al. (2012) showed a mean maternal serum level of 0.55 ng/g serum at the third trimester and a cord blood level of 0.13 ng/g whole blood. Pearson's correlations between PFNA levels in cord blood and maternal serum were not statistically significant at the first trimester but were significant at the third trimester. Pan et al. (2017) evaluated the correlation between the cord/maternal serum ratio and several factors and in particular found the ratio had a negative correlation with maternal glomerular filtration and serum albumin and a positive correlation with fetal albumin, which confirms the roles of excretion and serum binding in determining overall body burden and distribution, respectively.

Many other studies evaluating gestational distribution obtained similar results, although estimates of the ratio between cord and maternal serum concentrations varied among them (Li et al., 2020a; Han et al., 2018; Manzano-Salgado et al., 2017a; Yang et al., 2016b; Yang et al., 2016a; Cariou et al., 2015; Kato et al., 2014; Hanssen et al., 2013; Ode et al., 2013; Zhang et al., 2013b; Gutzkow et al., 2012; Kim et al., 2011b; Needham et al., 2011; Monroy et al., 2008). The results from these are described in greater detail in Appendix E.2.2.

Mamsen and colleagues reported PFNA concentrations in specific fetal tissues (Mamsen et al., 2019; Mamsen et al., 2017). A more extensive discussion of the results is provided in Appendix E.2.2. While these data are limited, they indicate distribution from maternal serum to fetal tissue in the first trimester is similar to that of maternal serum to maternal tissue. Distribution to fetal tissues in the second and third trimesters appears to be greater than in the first trimester, but this result is based on maternal blood samples collected in the first trimester and the fetuses involved suffered intrauterine death, hence may have different characteristics than healthy fetuses. The data for the second and third trimester fetuses suggest that distribution to them is greater but within a

factor of two of that estimated using the Vd estimated for human adults by Chiu et al. (2022), 0.19 L/kg. Because the mass of the fetus is a small fraction of maternal body weight and this difference in distribution is modest, EPA concluded that the overall volume of distribution in the pregnant woman including the placenta and fetus should be almost the same as estimated in nonpregnant adults, i.e., 0.19 L/kg (see Appendix E.2.2). The exact level of distribution to the fetus is uncertain, but quantification, in particular calculation of human equivalent doses (HEDs), will be based on maternal exposure or serum concentrations and hence does not require a specific extent of fetal distribution. The HED calculation does implicitly assume that fetal distribution in the general population is similar to that in the population for which the corresponding POD was estimated. For estimates from human epidemiological data this is simply assuming that distribution in the human children evaluated for dose-response is similar to distribution in the general population of children. For estimates from developmental studies in mice the HED calculations assume that distribution to fetal mice is no less than distribution to fetal humans from their respective mothers. The data from Mamsen et al. (2019); Mamsen et al. (2017) clearly demonstrate distribution to human fetuses and the available data in mice likewise demonstrate distribution to the mouse fetal liver, where concentrations ranged from 17% below to 79% above the concentrations in maternal liver on GD 17 (for maternal doses of 1, 3 and 5 mg/kg-day) (Das et al., 2015). Hence, the relative fetal distribution in humans versus mice appears to be within a factor of 2 of each other.

For the purpose of pharmacokinetic modeling (see details in Section 3.1.6 and Appendix E.4), the volume of distribution in pregnant and juvenile mice will be assumed equal to that of adults, primarily because data on distribution in the mouse fetus and pup is limited to liver and serum concentrations. However, based on the data from Mamsen and colleagues, the Vd (L/kg) in the pregnant mother (with placenta, fetus, amniotic fluid, etc.) was assumed to remain constant through the first trimester, but then to increase linearly by 7% between the end of the first trimester and the end of pregnancy. (The value of 7% comes from assuming that 35% of total gestational weight gain (Pitkin, 1976), or 5.5 kg, represents the fetus, placenta, and amniotic fluid. If the Vd for this mass is twice that of the rest of the maternal tissues, the effective increase in distribution volume is 5.5 kg/78.4 kg = 0.07, where 78.4 kg is the total maternal weight at the end of pregnancy in the human PK model of Kapraun et al. (2022).) Note that the total effective volume of the mother and fetus V_{total} = Vd*BW_{total}, is then assumed to increase due to both the growth in the body weight (BW) of the mother and fetus and the increase in Vd. The change in Vd accounts for the relative contribution of the products of conception to the remaining maternal tissues. Hence, even though the distribution in the fetus and placenta may be double that of other maternal tissues early in the second trimester, because the fetus is growing with time the impact on Vd is expected to increase more gradually during the latter two trimesters.

After birth, Vd in the child is assumed to decline to adult levels in parallel with the age-related change in extracellular water, which was estimated to be 2.4 times the adult value in

newborns and to decline to adult values by age 10 years (Friis-Hansen, 1961). While the distribution of PFAS is expected to be determined in large part by their binding to serum albumin and other proteins, the age-dependence of extracellular water is used for convenience, since it varies from a value close to two in newborns and is assumed to reflect other changes in body composition.

Summary of distribution during gestation: An overall mean ratio of umbilical cord to maternal serum PFNA concentration of 0.575 was calculated from the set of available data, indicating a lower degree of serum binding in fetal versus maternal blood. The Vd in the infant at birth is assumed to be double that of the adult and then to decline to adult levels at age 10 in parallel with the quantity of extracellular water. Because the tissue distribution in the placenta and fetus in the second and third trimesters appear to be approximately twice that estimated for human adults (0.19 L/kg), for the purpose of PK simulation the corresponding Vd in the pregnant mother, together with the fetus and all pregnancy-related tissues and fluids, is likewise assumed to increase 7% between the end of the first trimester and the end of pregnancy for the purpose of PK modeling (see Appendix E.2.2).

3.1.3. Metabolism

PFNA is thought to not be metabolized in mammals, although no studies have specifically attempted to identify potential metabolites using labeled PFNA. Studies have examined similar compounds, including perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA) and identified only the parent compound in excreta (Vanden Heuvel et al., 1991a, b). These compounds have 8-carbon and 10-carbons chains respectively, compared with a 9-carbon chain for PFNA. The fluorocarbon bonds are very stable and resistant to chemical modification. Furthermore, the terminal carboxylic acids were not conjugated in the studies examining radiolabeled PFOA and PFDA (Vanden Heuvel et al., 1991a, b). Thus, it is reasonable to infer that PFNA would likewise not be metabolized in mammals.

PFNA has been shown to be produced by metabolism of some other PFAS. This includes generation by 8-2 fluorotelomer alcohol in mouse, rat, and human hepatocytes (Henderson and Smith, 2007; Nabb et al., 2007; Martin et al., 2005) and observation of dose- and time-dependent accumulation of PFNA in the liver of mice given 8-2 fluorotelomer alcohol in the diet (Kudo et al., 2005). However, production of PFNA was not observed in an incubation of 8-2 fluorotelomer alcohol with human microsomes, which more narrowly assess metabolism by cytochrome P450 specifically (Nabb et al., 2007).

3.1.4. Excretion

Excretion in Animals (Rats and Mice)

Fecal and urinary excretion are the main routes by which PFNA is excreted. In a study of SD rats, Kim et al. (2019) reported urinary excretion approximately 10-fold greater than fecal

excretion, with a cumulative urinary excretion of 14.33% ± 9.30% and 34.56% ± 2.21% of the applied dose in males and females, respectively, and cumulative fecal excretion of 1.28% ± 0.45% and 3.13% ± 2.18% of the applied dose in males and females, respectively, 60 days after an IV dose of 3 mg/kg. The reported ratio of fecal to urinary excretion was then 1.28/14.33 = 0.089 (8.9%) in males and 3.12/34.56 = 0.090 (9.0%) in females. Since this is a non-oral exposure route, the fecal excretion is sourced from PFNA in systemic circulation. In contrast, after a single gavage dose of 0.39 mg/kg to male rats Benskin et al. (2009) found greater excretion in feces, with only 32% n-PFNA and 35% iso-PFNA of the average total daily excretion in urine and the remainder (68 and 65%, respectively) in feces after 38 days, hence the ratio of fecal to urinary excretion reported by Benskin et al. (2009) was 68/32 or 213% for n-PFNA and 65/35 or 186% for iso-PFNA. The greater excretion in feces relative to urine observed by Benskin et al. (2009) compared with Kim et al. (2019) may be partly explained by fecal excretion of PFNA that has not been absorbed into systemic circulation. But given the estimated F_{abs} of 86% in male rats, if only 9% of systemic PFNA was excreted in feces, the total fecal excretion should be about 22% after oral exposure, one-third of the amount reported by Benskin et al. (2009). Both studies used Sprague-Dawley rats but Kim et al. (2019) used a dose almost 8 times higher than Benskin et al. (2009). So, a dose-dependent effect could also exist, where the higher dose administered by Kim et al. (2019) resulted in a greater extent of urinary excretion. A large fraction of the urinary excretion by female rats in the Kim et al. (2019) study occurred in the first few days after dosing, when plasma concentrations were highest. Urinary excretion in male rats occurred at a more constant rate but then the plasma concentrations only declined slowly in the males, with a half-life of over 40 days.

In a shorter study in Wistar rats, with i.p. injection of 25 mg/kg PFNA, male rats excreted 2% of the total dose in the urine and 4.5% of the total dose in feces after 120 hours (Kudo et al., 2001), a ratio similar to that obtained by Benskin et al. (2009) for n-PFNA. In this case, the greater relative excretion in feces cannot be attributed to unabsorbed PFNA, as in the study of Benskin et al. (2009), which was also performed in male rats. Kudo et al. (2001) also examined female Wistar rats, who excreted 51% of the total dose in urine and 1.5% of the total dose in feces after 120 hours. This observation shows a greater prevalence of urinary excretion in female versus male rats, which agrees qualitatively with the result of Kim et al. (2019), although the urinary clearance appears to be much faster in the i.p. study. Kudo et al. (2001) also observed that the biliary excretion rates for female rats were greater than for male rats, which opposes the trend seen in fecal excretion and suggests there are sex-specific differences in resorption of PFNA from bile in the rat gut. For studies that distinguished between n-PFNA and iso-PFNA, iso-PFNA was excreted more readily than n-PFNA. Both isomers were cleared more quickly in female rats than in males. The mean reported half-life was 44 days for n-PFNA in male rats and 26 days for iso-PFNA in male rats (Benskin et al., 2009; De Silva et al., 2009a). Only one study reported isomer-specific half-lives in female rats with 2.1 days for n-PFNA and 0.82 for iso-PFNA (De Silva et al., 2009a).

In male mice, Tatum-Gibbs et al. (2011) suggest that the half-lives for the 10 mg/kg dose is skewed toward large values because of the low elimination rate. They further suggest the mean value may not reflect the most likely value and the lower confidence interval of 69.5 days may be more reliable. However, Tatum-Gibbs et al. (2011) noted that the half-life in female rats they calculated was uncertain because the chemical levels decreased below detection limits 15 days after chemical administration.

Half-lives can be computed several different ways, a source of potential inconsistency when comparing results from different studies. Since EPA considers clearance (CL) to be the crucial metric for animal-human extrapolation, EPA calculated average half-life values (see Table 3-2) from the Vd (see Table 3-1) and the CL. CL was calculated using dose/AUC if not reported by a study. This estimate of half-life is more consistent than other methods because AUC is less sensitive to the specific method of PK analysis or random measurement errors in particular timepoints.

Clearance in animals (rats and mice)

A key pharmacokinetic parameter for extrapolation from animals to humans is the clearance (CL), which is interpreted as the volume of blood from which a compound is eliminated in a unit of time, scaled to body weight. Units of CL are typically L/kg BW/hour, but for convenience they are expressed here as mL/kg-day since values are in that range. Unfortunately, Benskin et al. (2009) and De Silva et al. (2009a) only report estimates of elimination half-life for PFNA, and the information provided in those papers does not allow calculation of clearance. But the half-lives estimated from other pharmacokinetic studies can be compared with these results to evaluate overall consistency. Pharmacokinetic parameters reported by or calculated from the results of Ohmori et al. (2003), Tatum-Gibbs et al. (2011), Fujii et al. (2015), Iwabuchi et al. (2017), and Kim et al. (2019) are shown Table 3-2, along with results for rats from EPA's hierarchical Bayesian analysis from partial pooling of the data, described in Appendix E.1.

Table 3-2. Pharmacokinetic parameters of PFNA in rats and mice[a]

Study [Strain]	Exposure route	Dose (mg/kg)	Fraction absorbed (F_{abs})	Clearance (CL) (mL/kg-d)	Volume of distribution (Vd) (mL/kg)	$t_{1/2}$ (d)
Male rats						
De Silva et al. (2009a) [Sprague-Dawley]	Dietary	0.010–0.014 (mg/d)	NR	NR	NR	47 (n-) 31 (iso-)
Benskin et al. (2009) [Sprague-Dawley]	Gavage	0.2	NR	NR	NR	40.6 (n-) 20.7 (iso-)
Tatum-Gibbs et al. (2011) [Sprague-Dawley]	Gavage	1	NR	3.89[b] *2.23 (1.53–2.92)*	113 *184.4 (139.9–225.6)*	42.1 (33.0–55.6)[c] *58.57 (43.19–73.45)*[c]
		3	NR	4.80[b] *2.65 (1.96–3.34)*	139 *137.8 (106.4–170.1)*	23.6 (20.2–27.8)[c] *36.34 (29.08–43.41)*[c]
		10	NR	3.80[b] *2.52 (1.94–3.09)*	110 *125.6 (98.57–151.6)*	28.0 (25.0–31.5)[c] *34.77 (28.83–40.37)*[c]
Kim et al. (2019) [Sprague-Dawley]	i.v.	3	NA	7.43 ± 1.44 *4.95 (3.15–6.62)*	282[d] *467.5 (403.8–528.1)*	40.20 ± 18.68[c] *69.74 (39.9–98.36)*[c]
	Gavage	3	0.77 ± 0.06	7.37[b] *4.26 (2.09–6.58)*	NR *331 (160.4–490.2)*	54.57 ± 2.54[c] *55.68 (35.2–75.25)*[c]
Iwabuchi et al. (2017) [Wistar]	Gavage	0.04[e]	NR	10.3[e] *4.00 (1.50–6.47)*	277.7[e] *239.6 (140.6–338.9)*	19 (13–26)[c] *48.68 (18.21–80.22)*
Ohmori et al. (2003) [Wistar]	i.v.	22.6	NA	6.9 ± 0.06 *6.77 (4.99–8.69)*	286.8 ± 12.5 *275.6 (236.6–315.8)*	29.6 ± 2.3 *29.84 (20.03–39.16)*[c]
Population summary (Bayesian analysis)	–	–	*0.86 (0.66–1)*	*3.68 (2.29–5.01)*	*234.8 (147.3–318)*	*46.5 (23.4–67.9)*[c]
Female rats						
De Silva et al. (2009a) [Sprague-Dawley]	Gavage	0.008–0.011 (mg/d)	NR	NR	NR	2.1 (n-) 0.82 (iso-)
Tatum-Gibbs et al. (2011) [Sprague-Dawley]	Gavage	1	NR	49.8[b] *75.93 (63.03–89.83)*	125 *249.5 (186.8–317)*	32.0 (3.2–119.1)[c] *2.274 (1.859–2.668)*[c]

Study [Strain]	Exposure route	Dose (mg/kg)	Fraction absorbed (F_{abs})	Clearance (CL) (mL/kg-d)	Volume of distribution (Vd) (mL/kg)	$t_{1/2}$ (d)
		3	NR	66.4[b] *71.73 (58.93–85.39)*	171 *247.6 (181.3–316.3)*	32.0 (3.2–119.1)[c] *2.388 (1.936–2.812)*[c]
		10	NR	56.5[b] *69.69 (57.12–82.04)*	146 *260.6 (194.3–321.6)*	32.0 (3.2–119.1)[c] *2.589 (2.181–3.021)*[c]
Kim et al. (2019) [Sprague-Dawley]	i.v.	0.5	NA	NR *69.03 (62.82–74.99)*	NR *310.3 (271.2–349.8)*	NR *3.114 (2.892–3.328)*[c]
	Gavage	0.5	NR	NR *69.34 (60.19–78.51)*	NR *267.4 (221.5—312.7)*	NR *2.671 (2.427–2.896)*[c]
	i.v.	1	NA	NR *73.62 (67.33–79.34)*	NR *319.9 (286.1–354)*	NR *3.011 (2.829–3.189)*[c]
	Gavage	1	NR	NR *70.48 (61.01–79.79)*	NR *290.9 (245.5–337.1)*	NR *2.86 (2.658–3.066)*[c]
	i.v.	3	NA	66.2[b] *70.83 (64.03–77.86)*	183[d] *303.4 (260.2–345.9)*	4.44 ± 0.16[c] *2.967 (2.751–3.18)*[c]
	Gavage	3	0.79 ± 0.15 (1.17)[f]	44.8[b] *60.17 (51.57—69.98)*	NR *326.4 (267.3–388.8)*	6.40 ± 1.11 *3.757 (3.488–4.047)*[c]
	Gavage	10	NR	NR *64.94 (55.07–74.45)*	NR *336.3 (279.9—396.2)*	NR *3.588 (3.333–3.841)*[c]
Ohmori et al. (2003) [Wistar]	i.v.	22.6	NA	105.7 *91.56 (68.27–111.6)*	243.1 *235.6 (162.5–309.6)*	2.44 *1.780 (1.468–2.042)*[c]
Population summary (Bayesian analysis)	–	–	*0.94 (0.89–1)*	*71.1 (63.8–79.6)*	*283.2 (243.9–322.3)*	*2.77 (2.39–3.15)*[c]
Male mice						
Tatum-Gibbs et al. (2011) [CD-1]	i.v.	1	NA	10.2[b]	328 (0–1,060)	34.4 (29.1–41.1)[c]
		10	NA	3.26[b]	328 (0–1,060)	228 (69.5–796)[c]
Bayesian analysis of pooled Tatum-Gibbs et al. (2011) male mouse data	–	–	*(1.0)*	*4.51 (2.86–6.18)*	*579.1 (524.3–631.8)*	*101.9 (54.75–135.9)*[c]

Study [Strain]	Exposure route	Dose (mg/kg)	Fraction absorbed (F_{abs})	Clearance (CL) (mL/kg-d)	Volume of distribution (Vd) (mL/kg)	$t_{1/2}$ (d)
Fujii et al. (2015)[g,h] [FVB/NJcl]	i.v.	0.145	NA	2.66[b]	220 ± 60	628[c]
	Gavage	1.45	0.991 (1.01)[i]	55[b]	NR	2.06[c]
Female mice						
Tatum-Gibbs et al. (2011) [CD-1]	i.v.	1	NA	7.08[b]	192 (165–220)	25.7 (22.7–29.3)[c]
		10	NA	4.85[b]	192 (165–220)	68.8 (44.2–120)[c]
Bayesian analysis of pooled Tatum-Gibbs et al. (2011) *female mouse data*	–	–	*(1.0)*	*4.89 (3.92–5.76)*	*319.6 (280.8–358.9)*	*46.5 (33.5–60.7)*[c]
Fujii et al. (2015)[g,h] [FVB/NJcl]	i.v.	0.145	NA	1.97[b]	150 ± 40	68.8[c]
	Gavage	1.45	1 (1.13)[i]	6.16[b]	NR	13.1

[a]Including parameters for ln-PFNA and unspecified isomers of PFNA, where the half-life is assumed to be mostly due to contribution from n-PFNA. Values in plain text are as reported from each study unless otherwise indicated, except conversion to the indicated units. Values in italics are the mean (90% credible interval) from the Bayesian analysis described in Appendix E.1. NR: not reported. Volumes of distribution (Vd) are mean values from Table 3-1; see Section 3.1.2 for details.

[b]Clearance calculated as dose (times F_{abs} for oral exposure) divided by the area-under-the-concentration-curve (AUC) from time = 0 to infinity.

[c]Beta-phase half-life from two-compartment model as reported or calculated as ln(2)/(k_e*24 h/d) when a one-compartment PK model was selected or ln(2)/(β*24 hr/d) for a two-compartment model from EPA analysis.

[d]Vd was calculated as dose/Cmax, given that Cmax is the initial concentration for i.v. dosing.

[e]The reported Vd for serum (0.36 "kg tissue volume/kg BW") was corrected using the absorption fraction of 0.79 for male rats from Kim et al. (2019) and converted to mL/kg BW using a serum density of 1.024 kg/L (Sniegoski and Moody, 1979). CL was then calculated as the resulting Vd multiplied by the reported k_e (0.037 d^{-1}).

[f]For female rats given a dose of 3 mg/kg, Kim et al. (2019) reported mean AUCs of 45.31 and 52.88 (µg-d/mL) for i.v. and oral administration, respectively, which corresponds to a mean F_{abs} = 52.88/45.31 = 1.17. The reported value of F_{abs} (0.79 ± 0.15) appears to have been estimated from the relative value of C_{max} after oral vs. i.v. dosing, which is technically incorrect.

[g]Fujii et al. (2015) lacks the detailed time-course data needed to be included in the Bayesian PK analysis.

[h]AUC was calculated using the empirical PK curve and parameters reported by the authors; because Fujii et al. (2015) only observed blood concentrations for 24 h, the AUC and $t_{1/2}$ are considered highly unreliable but are included for comparison.

[i]Fujii et al. (2015) reported a fraction absorbed based on the fecal CL after gavage vs. i.v. dosing, i.e., assuming the higher fraction found in feces after gavage dosing was the fraction not absorbed. The number in parentheses is the reported mean [AUC(oral)/(oral dose)] / [AUC(IV)/(IV dose)].

Lactation in animals

Lactation is presumed to act as both a route of excretion for the nursing mother and route of PFNA exposure for the offspring. Fujii et al. (2020) measured PFNA concentrations in the milk and plasma of lactating mice 24 hours after i.v. administration on PND 8–13 and observed a mean milk/plasma concentration ratio of 0.30. Fujii et al. (2020) then estimated an equivalent daily dose of the dams as the product of the dam plasma concentration and an estimate of clearance in mice. This estimated maternal daily dose is what one would predict would lead to the measured plasma concentration. The authors then estimated the daily dose to the pups based on the milk concentration, pup body weight, and estimated breast milk ingestion volume per day at the time of milk sampling (24 hours after dosing, PND 9–14). Dose to the pups was calculated as the ratio of the amount ingested in milk (concentration in milk × daily milk ingestion volume) divided by the pup BW on that day. Using these estimated maternal and pup doses, they estimated the relative dose to the pup versus the dam (ratio of mg/kg-day ingested by the pup to the equivalent maternal dose). The mean estimated dose ratio for PFNA was 9, suggesting that given a chronic exposure to a fixed dose to the dams (such that they reach steady state), the nursing offspring would be exposed to a dose 9 times higher (Fujii et al., 2020).

Das et al. (2015) observed PFNA plasma concentrations in pregnant and lactating mice as well as their pups, during and after gestational exposure. Their data are more difficult to interpret in terms of lactation since the pups are born with a body burden from gestational transfer and dosing was stopped a day before parturition. But the liver concentration in the pups at all dose levels and the serum concentration in the pups of the highest dose group (5 mg/kg) increased or stayed constant between PND 1 and PND 10. These data strongly indicate that lactational transfer of PFNA occurred from the PFNA accumulated in the dams during gestational exposure. In contrast the serum concentrations in the pups of dams exposed to 1 and 3 mg/kg PFNA declined between PND 1 and PND 10. It appears that the lactational transfer is sufficient to maintain the liver concentration in the growing pups, but not enough to maintain serum concentrations over 10 days at these lower doses.

As detailed in Appendix E, when simulations were performed with a one-compartment PK model parameterized for the mouse using the milk/maternal serum ratio of 0.3 reported by Fujii et al. (2020), the PFNA concentrations predicted in the pups significantly overpredicted those observed by Das et al. (2015). Model simulations matched the data of Das et al. (2015) much better when the milk/maternal plasma ratio was decreased about an order of magnitude from that reported by Fujii et al. (2020), i.e., from 0.3 to 0.03. A ratio of 0.03 is in the range measured in human mothers, discussed in "Lactation in humans," below. A difference between the study of Das et al. (2015) and that of Fujii et al. (2020) is that Fujii et al. (2020) dosed the lactating mouse dams and measured the milk concentrations 24 hours later, while Das et al. (2015) dosed the mouse dams during gestation, stopping 1 day before parturition and measured the concentration in the pups at PND 1, 10, 24, 42, and 70. PFNA serum concentrations in the pups declined after PND1 at

the lowest two doses and after PND10 at the highest dose (Das et al., 2015), but based on model predictions this decline was slower than would have occurred in the absence of lactational transfer. Thus, while both Fujii et al. (2020) and Das et al. (2015) demonstrate lactational transfer, it appears that when the maternal PFNA body burden is accumulated over time, as occurs in humans and occurred in the experiments of Das et al. (2015), the clearance from maternal serum to breast milk is significantly lower than shortly after a bolus exposure.

Lactational transfer was not evaluated in rats but given these observations in mice and measured PFNA concentrations in human breast milk (see Lactation in Humans), it is expected to be a relevant route of maternal excretion and developmental exposure in all mammals.

Excretion in Humans

Studies of PFNA excretion in humans have been primarily focused on quantifying clearance from serum or blood, based on correlations between concentrations in serum and urine. Excretion in menstrual fluid has also been estimated to be a significant route for women because urinary excretion is extremely low. If urinary clearance were an order of magnitude higher, menstrual fluid loss would not be a significant contributor to excretion. However, empirical data on serum concentrations of multiple PFAS in women of reproductive age versus men do not indicate a consistent difference that would be expected for menstrual clearance, which should be independent of the specific PFAS. Fecal excretion in humans has not been directly measured but might be assumed similar to that observed in rats. However, some rat data indicate that fecal clearance is a large fraction while other results report it as a small fraction of total clearance. Lactational transfer can also be a significant route of excretion for the mother, so is also reviewed here.

Clearance in humans

Urinary clearance in humans

Calafat et al. (2019) evaluated paired serum and urine measurements, but the focus of the analysis was on the frequency of detection (>98% of the individuals had detectible serum levels of PFNA) and the results were not useful for quantitative analysis of clearance. Worley et al. (2017) also report on the analysis of serum and urine samples from a community with drinking water contaminated with PFAS, but only 30.4% of participants samples had PFNA levels greater than the LOD (0.01 ng/L). The authors estimated half-life values for other PFAS based on their data and an estimation of ongoing exposure from drinking water levels of PFAS in that community, but the half-life of PFNA was not calculated. Hence, results from Worley et al. (2017) and Calafat et al. (2019) are not evaluated further here.

Zhang et al. (2013c) reported an analysis of PFNA concentrations in paired serum and urine samples and used those data to estimate urinary clearance in two subpopulations: (1) all males (22–88 years of age) and older females (age > 50 years) (n = 50, mean CL = 0.15 mL/kg-day, median CL = 0.094 mL/kg-day); and (2) younger women (age ≤ 50 years) (n = 16, mean CL = 0.25 mL/kg-

day, median CL = 0.20 mL/day/kg). That urinary clearance was observed to be much greater in the younger females than in the male and older female group may be due to the older average age of the latter group, since renal filtration tends to decrease with increasing age (Rule et al., 2004). Jain and Ducatman (2022) compared serum levels of several PFAS, including PFNA, in U.S. females and males as a function of age from 12 to 75 years. While Jain and Ducatman (2022) observed lower PFNA levels in women versus men 20–48 years of age, the difference was only 25%–30%, much less than would be predicted based on these urinary clearance values. However, the results of Jain and Ducatman (2022) would reflect total clearance in men versus women and if fecal (and other pathway) clearance is the same in women and men, the ratio of total clearance in women versus men would be lower than the ratio of urinary clearance in women versus men. For example, if fecal CL is 0.15 mL/kg-day and one adds the mean urinary CL values from Zhang et al. (2013c), the ratio of total CL in men versus women would be (0.15 + 0.15)/(0.25 + 0.15) = 0.3/0.4 = 0.7, i.e., in the range indicated by the results of Jain and Ducatman (2022). Further stratification of the population evaluated by Zhang et al. (2013c) could reveal if there are age- or sex-related differences in the renal clearance, but that type of examination may be limited by the sample size of only 50 individuals in the group of men and older women. To compare the renal clearance results of Zhang et al. (2013c) and possibly combine them with those of other studies that did not divide their study by age or sex, an overall population-weighted mean daily renal clearance was calculated by EPA from the group-specific mean CL values for Zhang et al. (2013c) to obtain 0.174 mL/kg-day for all males and females.

Zhang et al. (2013c) used a Vd of 170 mL/kg previously estimated by Thompson et al. (2010) for PFOA dosimetry in humans, as the Vd for PFNA. The clearance values estimated by Zhang et al. (2013c) for PFNA using this Vd correspond with median half-lives of 3.5 years in males and older females and 1.5 years in younger females.

Fujii et al. (2015) used paired serum and 24-hour urine samples from five male and five female healthy volunteers and calculated an overall rate of urinary clearance of 0.038 ± 0.01 (mean ± SD) mL/kg-day. While the number of subjects is less than Zhang et al. (2013c), the collection of 24-hour urine samples by Fujii et al. (2015) provides an estimate of clearance that is much less subject to intraday variability than the spot urine samples used by Zhang et al. (2013c). Hence there is no clear reason for ranking one study above the other in terms of data quality. The weighted mean urinary clearance for the entire study population of Zhang et al. (2013c) (0.174 mL/kg-day) is 4.6 times higher than the value of Fujii et al. (2015). As there is no obvious explanation for this discrepancy, its magnitude is considered a measure of uncertainty in the human PK.

Yao et al. (2023) estimated urinary clearance of PFNA and other PFAS in infants based on the ratio of the estimated urinary excretion rate to estimated cord serum concentration. Cord blood was collected at delivery and the concentration multiplied by two to account for the serum-to-whole-blood ratio. Urine was collected in disposable diapers collected over the first postnatal week

and later extracted for measurements. The methods do not specify how a daily average urine concentration was then determined from the set of samples for each infant, but it is presumed that the extracted urine from all diapers collected during the week was mixed prior to analysis, resulting in a "mixing cup" average concentration for the week. The resulting concentration was then multiplied by a reported average urine elimination rate in infants of 48 mL/kg-day, rather than using the actual urine volume collected. Serum concentrations and resulting urinary elimination of breastfed infants are expected to increase significantly after childbirth based on reported breast milk:maternal serum distribution and breast milk ingestion rates. While the cord blood concentration might match the infant blood concentration at the moment of birth, the resulting estimate of infant clearance is likely to be an overprediction of the true clearance rate. From a population of 60 infants the median (25th, 75th percentiles) urinary clearance was 0.047 (0.028, 0.100) mL/kg-day, with a mean value 0.082 mL/kg-day. The median is slightly higher than obtained for adults by Fujii et al. (2015) although well below the adult values of Zhang et al. (2013c), with the mean near the average of those two studies. The sample distribution is skewed, with a maximum estimated value of 0.543 mL/kg-day, perhaps due to the urine sample timing issue discussed here. While glomerular filtration is still developing in neonates, the expression of renal OAT1 and OAT3 is also below adult levels (Bueters et al., 2020), and urinary excretion of PFNA will depend on both of these opposing factors in a manner that cannot be quantitatively predicted. Given these uncertainties, the results of this study will not be used quantitatively, although they indicate that neonates will have similar or somewhat lower PFNA clearance than adults.

Fecal clearance in humans

Fujii et al. (2015) also collected 24-hour biliary samples from two women and three men. One of the women was being treated for pancreatic cancer while the other four subjects were being treated for choledocholithiasis (gallstones). Using a comparison with total clearance estimated from the empirical half-life of PFOA in humans, and a Vd value from mice, a resorption fraction of 98% was estimated. This resorption fraction was applied to the measured rate of biliary excretion from the five patients, resulting in an estimated fecal clearance of 0.024 mL/kg-day. Significant resorption of PFNA excreted in bile is also indicated by a case study where an asymptomatic 51-year-old male researcher, who had high levels of PFNA and other PFAS in his serum, was administered a bile acid sequestrant, cholestyramine. The treatment resulted in an increase of over 10-fold in fecal PFNA concentration and a corresponding decline in serum levels (Genuis et al., 2010). However, the small number of subjects evaluated by Fujii et al. (2015), disease status, and need to then estimate the fraction reabsorbed based on data from PFOA and mice make the quantitative value (98% resorption) highly uncertain. The observations by Genuis et al. (2010) were between the concentrations above and below the LOD, also confounding quantitation. The ratio of estimated fecal/urinary clearance of Fujii et al. (2015) was 0.63 and the ratio of fecal clearance estimated by Fujii et al. (2015), 0.024 mL/kg-day, to the overall population mean urinary clearance from Zhang et al. (2013c), 0.174 mL/kg-day, is 0.14. For comparison, the fecal/urinary

ratios reported for rats (see "Excretion in Animals (Rats and Mice)," above) are 0.089 in male rats and 0.091 in female rats (Kim et al., 2019), 2.13 in male rats (n-PFNA) (Benskin et al., 2009), and 2.25 (4.5%/2%) in male rats and 0.029 (1.5%/51%) in female rats (Kudo et al., 2001). Thus, there is a very wide range of variability or uncertainty in the ratio of fecal to urinary excretion of PFNA. While the ratio estimated from the results of Fujii et al. (2015) for both feces and urine (0.63), or combining the fecal excretion of Fujii et al. (2015) with urinary excretion of Zhang et al. (2013c) (0.14) are both in the range of values estimated from rat PK studies, that range is so large that both of these seem equally plausible. Hence, the true ratio in humans is considered a matter of uncertainty that can only be addressed by collection of additional data.

Menstrual clearance in humans

After estimating urinary clearance, Zhang et al. (2013c) went on to calculate total clearance values including a term for menstrual clearance but under the assumption that other clearance pathways are negligible. The menstrual clearance value was based on a study of PFOA and PFOS (Harada et al., 2005), which in turn used a volume of menstrual blood loss measured by Hallberg et al. (1966). Hallberg et al. (1966) was a general population study of menstrual blood loss in healthy women, which did not evaluate or control for exposure to exogenous chemical exposure, but it is presumed that their menstruation rate was not significantly affected by any such exposure. Hence this estimated clearance rate was not specific to PFOA or PFOS, nor any exogenous chemical. If menstrual fluid loss carries chemicals from systemic circulation at a concentration equal to that in blood, i.e., there is no resorption from the fluid and all chemicals present in blood are eliminated with the lost blood volume, the resulting clearance rate should be equally applicable to PFNA.

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 rather than a median. On the other hand, Verner and Longnecker (2015) reviewed Hallberg et al. (1966) and 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 an average human female body weight of 72 kg (mean value for women 21–30 years of age from Table 8-5 of U.S. EPA (2011a)), the corresponding average rate of clearance is 868 mL/(365 days)/(72 kg) = 0.033 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. Although the trend of lower PFNA serum concentration in patients compared with the general public was consistent, there was

no clear trend in relation to the number of recent blood draws or to 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 0.0081 mL/kg-day in a 72 kg woman, 25% of the rate estimated by Verner and Longnecker (2015).

Jain and Ducatman (2022) compared serum levels of several PFAS, including PFNA, in U.S. females and males as a function of age from 12 to 75 years and found that for all PFAS examined serum levels were lower in women between 13 and 50 years of age. Serum levels in females fell compared with males starting around age 13 and began to rise again between age 40 and 45, corresponding to the age range of menstrual elimination. Hence, these results might indicate that menstrual fluid loss contributes to PFAS clearance in females of childbearing age. However, the extent of the difference between females and male serum levels differs among the PFAS evaluated by Jain and Ducatman (2022). While one would expect the route to have a greater effect on PFAS with lower clearance by other pathways, it is not clear that the differences correlate in this way and not all women menstruate regularly due to use of birth control pills and other biological factors.

Glynn et al. (2020) evaluated PFAS serum concentrations in fifth-grade school children (aged 12 years) and found that PFNA concentrations in girls (n = 92) were significantly lower than boys (n = 108) (median 0.41 versus 0.47 ng/mL) but that PFNA levels in girls who had started to menstruate (n = 5) was not lower than those who had not, although concentrations of PFHxS and PFOA in girls who had started to menstruate was significantly lower than those who had not.

EPA evaluated data from the National Health and Nutrition Examination Survey (NHANES), the same source as Jain and Ducatman (2022), for PFDA and PFHxS as well as PFNA to evaluate the consistency of the effect. PFHxS and PFDA have low overall clearance similar to PFNA and Jain and Ducatman (2022) showed much larger differences between men and women for PFHxS than PFNA. Specifically, EPA analyzed the collection of NHANES waves from 2003–2004 through 2017–2018. Participants were included if they were 12 years of age and above and if they had measured PFAS levels but were excluded if they were pregnant or if they were currently breastfeeding. For all waves except 2003–2004, this information on reproductive status was available only for women aged 20–44 and resulted in a total of 16,162 measurements. In the case where a serum concentration was below the limit of detection (LOD), the value was imputed with the LOD/$\sqrt{2}$. Overall, PFNA was determined to be below the LOD for only 1.7% of the measurements, although 26.4% of the PFDA measurements were below the LOD. This analysis was carried out in R (R Core Team, 2022) and the R package "survey" was used to incorporate the NHANES survey strategy into the analysis and generate results applicable to the U.S. population (Lumley, 2023, 2004).

A consistent, meaningful difference in serum levels in men versus women was not found for PFDA although the differences reported by Jain and Ducatman (2022) for PFHxS (not shown) and for PFNA (see Figure 3-1) were effectively replicated. If menstrual fluid loss were the mechanism

giving rise to the observed differences between men and women for some PFAS, it should have an equal effect on all PFAS, but this is not the case. Hence, while the differences between men and women reported by Jain and Ducatman (2022) for some PFAS indicates a sex- and age-dependent factor, it appears unlikely that menstrual clearance is the mechanism. The observed concentration difference could also be the result of a difference in exposure of some PFAS to men versus women of reproductive age. However, the difference in urinary clearance observed by Zhang et al. (2013c) suggests a different mechanism that would result in higher clearance in women than in men that would not apply equally across PFAS. Renal transporters involved in the resorption of PFAS are known to be under hormonal control and the affinity of PFAS of varying chain-lengths differs for given transporters (Weaver et al., 2010). So, hormonal regulation of urinary resorption could explain differences in total clearance and observed serum levels between men and women for some PFAS and not others.

Given these observations, menstrual clearance as a specific mechanism will not be evaluated further as a clearance pathway for PFNA.

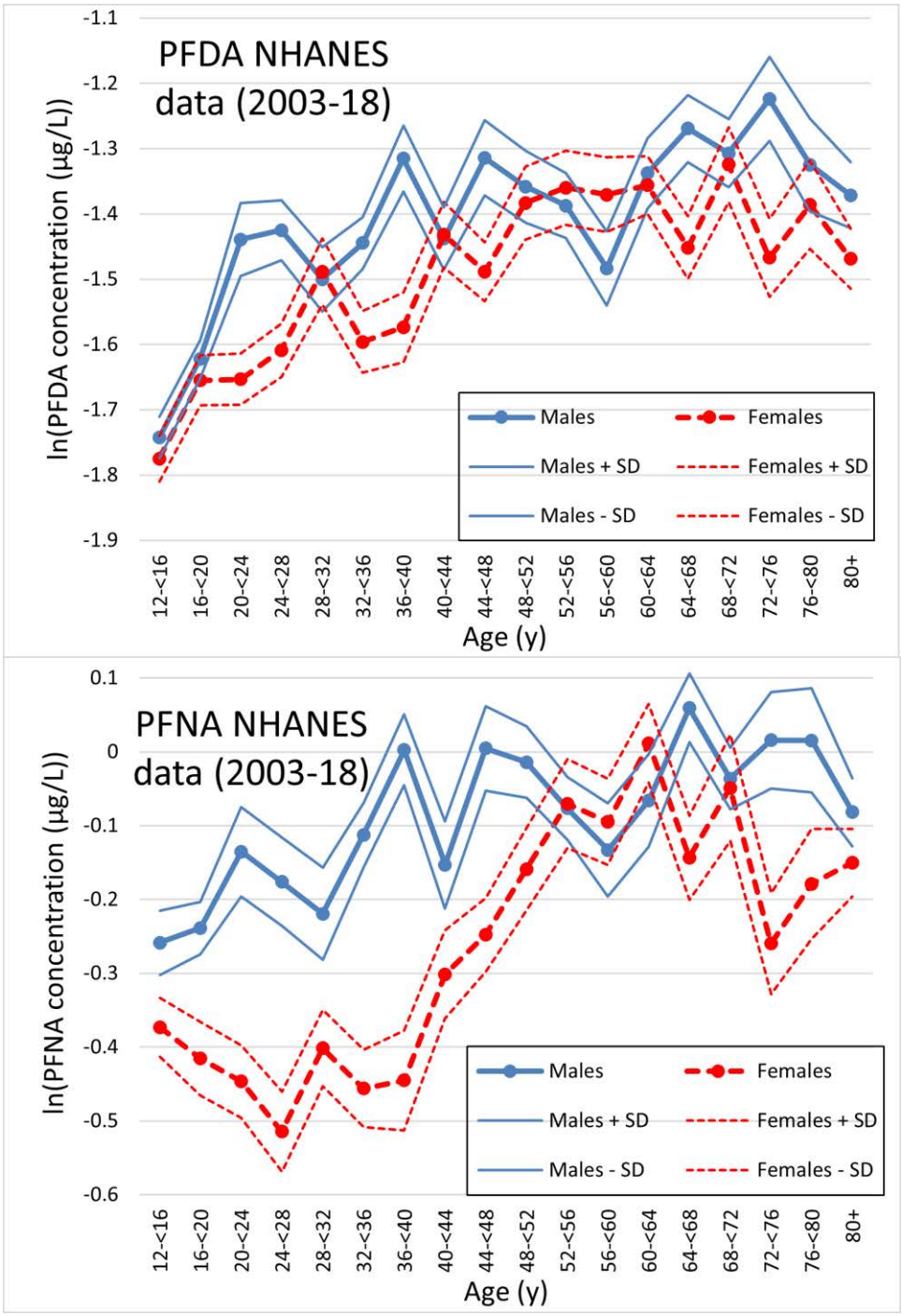


Figure 3-1. Serum concentrations of PFDA and PFNA in U.S. males versus females as a function of age. Data are from NHANES cycle years 2003–2018. Mean and standard deviation (SD) were calculated for each age range and sex after log-transforming the data.

While the results shown in Figure 3-1 indicate a difference in PFNA serum concentrations between males and females during the reproductive age range for women (~ 12–50 years of age), they are suggestive in showing that difference appearing in young women at the start of this age

range. Koponen et al. (2018) performed a longitudinal study in children between 1 and 10 years of age and estimated the body burden of PFNA and other PFAS based on the measured serum concentration and body mass at 1, 6, and 10–11 years of age. A noteworthy finding is that the body burden of females over this age range appeared to remain fairly constant while that in males increased steadily, although the body burden of the males and females at 1 year of age were quite similar. This result indicates that a sex-related factor other than menstruation, which applies even before menarche, leads to differences in the body burden of males and females. However, the median body burden in girls at age 6 years was only slightly lower than in boys (Koponen et al., 2018), so for the purpose of EPA's analysis, clearance in all children will be assumed equal.

Total clearance in humans

As described above, Chiu et al. (2022) estimated PK parameters in humans by fitting a one-compartment PK model to individual and population blood concentration data from communities with high PFAS levels in the drinking water, while using general U.S. population blood concentration data to estimate background exposures, via Bayesian analysis. The estimated geometric mean (GM) (95% CI) CL was 0.056 (0.033, 0.093) L/kg-year = 0.153 (0.090–0.255) mL/kg-day. However, since these data were estimated based on exposure level and serum data, they represent total clearance, not urinary clearance, which would include fecal and any other routes of clearance for the various study participants. While the analysis was restricted to data from adults, of whom roughly 50% are women, the female participants were primarily older, past menopause. Therefore, it is assumed the estimated CL represents that in men and older women and not women of childbearing age.

The clearance values estimated from the three human studies in adults with quantitative results are then as follows:

1) A total population-weighted mean urinary CL for Zhang et al. (2013c), estimated from spot samples from a group of 50 men and older women and 16 younger women (66 total) was calculated from the reported means and number of subjects for each group: 0.174 mL/kg-day.

2) The mean urinary CL from Fujii et al. (2015), estimated from 24-hour urine samples from five men and five women was 0.038 mL/kg-day, while the mean fecal CL, estimated from 24-hour bile samples from three men and two women was 0.024 mL/kg-day, resulting an estimated mean total CL of 0.062 mL/kg-day.

3) The estimated population GM for total CL of Chiu et al. (2022), based on data from 257 individuals and three population-level measures of PFNA exposure and serum concentration, was 0.153 (0.090–0.255) mL/kg-day (median, 95% CI).

Chiu et al. (2022) estimated CL using data for a reasonably large study population and the result includes all routes of excretion since it is based on estimates of exposure and observed serum concentrations rather than concentrations in specific excreta. As noted above, the estimate of fecal CL by Fujii et al. (2015) is based on biliary excretion data from only five individuals being treated for disease and relies on extrapolation of total CL from other PFAS. Also, Fujii et al. (2015) used a volume of distribution estimated for PFOA in mice. Hence, there is considerable uncertainty in the fecal CL of Fujii et al. (2015). Likewise, there is uncertainty in the option of extrapolating fecal CL from that reported for rats given the wide range reported for that species. While the mean urinary CL estimated from the results of Zhang et al. (2013c) was based on a population of 66 adults, it relied on urine spot samples which creates some uncertainty in the subsequent estimate of total daily excretion in urine, and they did not measure fecal excretion.

When estimating biological half-lives Zhang et al. (2013c) included a rate of menstrual CL = 0.029 mL/kg-day in younger women, which is only qualitatively supported by observations of slightly lower serum PFNA levels in women of childbearing age versus men (Jain and Ducatman, 2022). However, EPA's analysis did not find a meaningful, consistent difference in serum levels of PFDA in men versus women, although a difference was confirmed for PFNA (see Figure 3-1), which one would predict if menstrual clearance were a nonspecific mechanism. Hence EPA does not consider that specific route of excretion to be supported by the overall empirical data for PFAS. Therefore, the qualitatively best estimate of total CL in humans appears to be the result of Chiu et al. (2022), as it does not rely on uncertain estimates of fecal excretion that in turn rely to some degree on extrapolation from laboratory animals (and from other PFAS). While the mean urinary CL estimated from the results of Zhang et al. (2013c) was 14% higher than the (Bayesian sample median) GM total CL estimated by Chiu et al. (2022), it was below the 95% upper CI for the GM from Chiu et al. (2022). Further, that Zhang et al. (2013c) relied on urine spot samples creates some uncertainty in their result, which could explain the apparent discrepancy between total and urinary CL from these two studies.

Given the overall range of and uncertainty in human CL, EPA evaluated the specific value and range of CL reported. Chiu et al. (2022) also reported a lower bound individual CL from their analysis, i.e., the "1st percentile random individual," as being 0.0165 L/kg-year = 0.045 mL/kg-day, which is 3.4-fold lower than their median CL estimate (0.153 mL/kg-day). The uncertainty factor for intrahuman variability, UF_H = 10, is presumed to account for a variability of threefold in PK, the rest being variability in pharmacodynamics. Given that the analysis of Chiu et al. (2022) did not include children, use of the median CL (in conjunction with UF_H = 10) then may not be sufficiently health protective for the population as a whole. Therefore, EPA believes the lower 95% CI (of the GM clearance) estimated by Chiu et al. (2022), 0.090 mL/kg-day, should be used to represent the total CL in males of all ages, older females, and female children. EPA thereby assumes that total CL is unlikely to fall below 0.03 mL/kg-day for any individual, i.e., a factor of 3 below this population average.

EPA concluded based on concentrations of multiple PFAS in men versus women as a function of age that menstrual elimination was unlikely to be a specific mechanism of PFAS elimination (see section "Menstrual clearance in humans," above for details). However, a comparison of PFNA serum concentration in males versus females as a function of age using data from NHANES (cycle years 2003 to 2018) shown in Figure 3-1 does indicate a difference in clearance related to both sex and age. The average difference between mean (ln(PFNA concentration)) in women versus men from ages 20–40 years was calculated, then exponentiated, to estimate an average ratio of PFNA concentration in women of childbearing age versus their male counterparts. (Results shown in Figure 3-1 for ages 12–16 and 16–20 years were not used because they appear to show that the effect of adult hormonal regulation of transporters and adolescent growth are not fully realized during this age range.) The resulting average ratio of PFNA concentration in women versus men was 0.7229, indicating that women of reproductive age have clearance that is 1/0.7229 = 1.383 times that of their male counterparts. This adjustment will be used for PK model simulation and otherwise when estimating HEDs in women aged 12.4 (average age of menarche) to 40 years. That PFNA levels are observed to slowly decline in females between age 12 and 20 years is presumed to result from the long half-life, i.e., because it takes approximately 8 years for the impact of higher clearance in young women to be realized. And while menopause typically occurs around age 50, the NHANES data show increasing PFNA concentrations beginning with the 40–44 year cohort, so it is assumed that transporter levels switch to those similar to males at that age. The subsequent gradual increase in PFNA concentration is again assumed to be the result of its long half-life. It is recognized that changes in exposure and body weight are also likely factors in the age-related PFNA concentrations, but EPA assumes that the average difference between ages 20 and 40 years is due to hormonal regulation.

In conclusion:

1) For males of all ages and females below 12.4 and above 40 years of age, the population average total clearance of PFNA is assumed to be the lower 95% CI estimate of the GM from Chiu et al. (2022), **0.09 mL/kg-day**.

2) For women of age 12.4–40 years, clearance is assumed to be 1.383 times faster than in the rest of the population (0.09 mL/kg-day) based on EPA's analysis of serum PFNA concentration data from NHANES, resulting in a mean total clearance of **0.124 mL/kg-day**.

Pregnancy in humans

As discussed in Section 3.1.2, "Human distribution during gestation and childhood," Oh et al. (2022) observed a 4.3% decline in PFNA serum concentrations, Chen et al. (2021) observed an almost 50% decline, and Pan et al. (2017) observed a 23% decline during gestation. While hormonal and physiological changes during pregnancy might lead to a reduction in clearance, the increase in maternal tissue mass, including growth of the placenta, and growing fetus are expected

to increase the total volume of distribution for PFNA, effectively diluting the body burden accumulated prior to pregnancy. These data (Oh et al., 2022; Chen et al., 2021; Pan et al., 2017) indicate that this dilution more than offsets any concurrent reduction in clearance. Therefore, it is considered appropriate to apply the higher clearance estimated for women of reproductive age (0.124 mL/kg-day) when calculating HEDs for gestation-only exposure. For PK modeling, however, clearance will be assumed to decrease to the value estimated by Chiu et al. (2022) since the hormonal regulation that is assumed to result in the overall difference between women of reproductive age and men (see Figure 3-1) may change during pregnancy.

Brantsæter et al. (2013) evaluated the impact of previous pregnancy and total months of previous breastfeeding on serum concentrations in pregnant women and found that the current concentration was negatively correlated with both of those factors while there was a positive correlation with the time (years) since the most recent pregnancy. These results indicate that both pregnancy and breastfeeding reduced maternal body burdens, but that continued exposure results in increasing body burden with time after a pregnancy (and breastfeeding). Similarly, Tsai et al. (2018) measured PFAS levels in third trimester women and found decreasing mean levels with the number of previous pregnancies (1.5 ng/mL mean for women with zero previous pregnancies versus 1.1 ng/mL in women with two or more) but a modest increase with age at delivery (1.26 ng/mL for <24 years and 1.37 ng/mL for ≥35 years at delivery).

Lactation in humans

As with lactation in animals, breastfeeding is presumed to act as both a source of PFNA for the nursing infant as well as a route of excretion for the mother. In a study of a community with high levels of PFOA in drinking water, there was no significant trend in the PFNA serum levels of children (average age 2.5 years, range 1.7–3.4 years) versus months of breastfeeding as infants, although maternal PFNA levels did decrease significantly versus breastfeeding duration (Mondal et al., 2014). More specifically, the GM PFNA serum concentration in children breastfed >12 months was 17% higher than in children who were not breastfed, but the difference was not statistically significant. Given that the measurements were made in the children as much as 3 years after cessation of breastfeeding, at which point the intervening dietary exposure would have a significant influence and contribute to intersubject variability, and the analysis was only based on a total of 49 children among all the groups, the lack of statistical significance in the children is not surprising. While a separate set of serial measurements in a small sample of 19 mothers 3 weeks and 3 months after delivery showed significant correlation (r = 0.79) of PFNA levels between the two timepoints with no decrease over this time Glynn et al. (2012) and Oh et al. (2022) estimated a 1.2% decline in the first 6 months postpartum and a further decline of 0.8% between 6 and 24 months among their subjects (n = 42).

Fromme et al. (2010) observed PFNA concentrations in breastfeeding mothers, cord blood at birth, and their children. Of the 50 participants, 37 infants were exclusively breastfed, 6 predominantly, and 6 only partially during the sampling window of 5–7 months after birth (treated

as 6 months in the results). Mean (median) maternal blood concentrations declined somewhat from 0.8 (0.6) µg/L at birth to 0.7 (0.5) µg/L 6 months after delivery. Meanwhile mean (median) concentrations in cord blood were 0.4 (<0.4) µg/L but then concentrations in the infant blood were 1.1 (1.0) µg/L 6 months after birth, declining to 0.7 (0.6) µg/L at 19 months after birth. These patterns indicate significant lactational transfer to the infants in the first 6 months of life followed by a decline consistent with cessation of that exposure and growth of the child.

Very strong evidence for the contribution of lactational uptake to a child's body burden is provided by Koponen et al. (2018) who evaluated PFAS concentrations in 1-year-old children and found a significant correlation between the child's serum PFAS concentration and the number of months of breastfeeding of the child, including for PFNA. The regression shown in Figure 3 of Koponen et al. (2018) indicates that in a child breastfed for 12 months 70% of the PFNA is due to that source and hence that PFNA concentrations in a 1-year-old who is not breastfed is 70% lower than one who is breastfed for the full year.

Mogensen et al. (2015) also evaluated PFNA concentrations in children from birth to 5 years of age. The median (interquartile range, IQR) length of exclusive breastfeeding for 73 children with complete information was 4.5 (3.5, 6.0) months, followed by 4.0 (2.0, 7.0) months of partial breastfeeding. Serum concentrations were collected at birth (cord blood), 11, 18 and 60 months of age. Using a piecewise linear model applied to the log-transformed serum concentration between 0 and 18 months of age, PFNA concentrations were estimated to increase 20.8 (15.7, 26.1)%/month (mean, 95% CI) during periods of exclusive breastfeeding, only 5.2 (1.0, 9.6)%/month during periods of partial breastfeeding, and to decline 1.5 (2.8, 0.2)%/month during periods of no breastfeeding. The rate of increase for exclusively breastfed children is about one-half that estimated using the PK model described in Appendix E.4.2 if mean rates of milk ingestion and an average milk/maternal serum concentration ratio of 0.05 (see below) are assumed. In this aspect the results of Mogensen et al. (2015), like those of Fromme et al. (2010), appear reasonably consistent with predictions of the PK model, in particular that breastfeeding is expected to lead to a large exposure to infants.

The PK model predicts a 30% decline in serum concentrations between 1 and 5 years of age if it is assumed that the child is exposed to the same daily dose (mg/kg-day) as its mother after weaning and has the same clearance as an adult male or older woman. This decline is consistent with the observations of Fromme et al. (2010) and is predicted by the model because the child is expected to approximately double in mass between ages 1 and 5 years, diluting the body burden accumulated in the first year of life. However, Mogensen et al. (2015) reported a slight increase in median serum levels of children from 0.8 to 0.9 µg/L between 11 and 60 months of age. That serum levels increased slightly during this period of rapid growth suggests that this Faroe Island population had higher childhood exposures during that period than did their mothers in the preceding years, unlike other populations.

In apparent contrast to the studies just described, Papadopoulou et al. (2016) compared serum levels in 3-year-old children with those measured in the mother near parturition and did not find a significant correlation between the two for PFNA, although they did for several other PFAS. Likewise, there was no significant correlation between PFNA concentration in the mother or child with (length of) breastfeeding, although they trended as expected (positive correlation coefficient for the child, negative for the mother) (Papadopoulou et al., 2016).

By conservation of mass any PFNA delivered to an infant via breastmilk must come from the maternal body burden. Since any change in maternal serum levels will depend on the total amount of milk expressed during breastfeeding and ongoing exposure of the mother, the apparent discrepancy between these studies may be the result of differences in these quantities.

Clearance rate during breastfeeding

There are two options for estimating HEDs in the breastfeeding mother from estimated (POD) serum PFNA concentrations: direct application of an appropriate value for human clearance (i.e., HED = CL × C_{serum}) or by simulating the serum concentration over time for a given exposure using the PK model. For the first option, since the empirical studies discussed here indicate that PFNA concentrations postpartum are at or below serum levels in the woman prior to pregnancy, which are in turn determined by total CL in the woman prior to pregnancy, it is considered reasonable to apply the higher clearance estimated for women of reproductive age (0.124 mL/kg-day) when estimating HEDs. However, for the purpose of PK modeling, the mother's clearance (other than lactational transfer) will be assumed to decrease to 0.090 mL/kg-day (from Chiu et al. (2022)) at the onset of pregnancy and to remain there until the child is 12 months of age, when breastfeeding is assumed to cease, since breastfeeding affects hormone levels and there may not be a return to nonpregnancy levels of renal transporters until that time. (Average total clearance in children, however, is assumed to equal the lower 95% CI GM value estimated by Chiu et al. (2022), 0,090 mL/kg-day). EPA concluded that use of the PK model to simulate serum concentrations in human children involved too much uncertainty due to the limited data for PFNA PK in children (see Section 3.1.6) and used the clearance approach for HED calculations in this review (see Section 5.2). However, the distribution of PFNA from maternal blood to breast milk is quantitatively evaluated below since the distribution coefficient was used in evaluating the human lactational PK model and use of the model may be reconsidered.

Distribution from maternal serum to breastmilk

Liu et al. (2011) collected matched samples of maternal serum, cord serum, and breast milk from women within 1 week of delivery. These values can inform the rate of transfer from the mother to the infant via lactation. PFNA was above the LOD (LOD = 0.005 ng/mL) in 100% of the samples and the median ratio of milk to maternal serum was 0.04:1 while the mean value was 0.05:1 (Liu et al., 2011). Blomberg et al. (2023) found PFNA above their limit of quantification (LOQ = 0.01 ng/mL) in 45% of colostrum and 75% of breast milk samples, and measured ratios of

PFNA in colostrum (median 0.032:1, IQR 0.023–0.039) and breast milk (median 0.025:1, IQR 0.018–0.034) versus maternal serum in their subjects. Since the Liu et al. (2011) data were collected in 2009 from women in the Jiang Su province of China while Blomberg et al. (2023) sampled women from two municipalities in Sweden, one with previously high water contamination (Ronneby) and one with minimal contamination (Karlshamn), from 2015 to 2020, the difference in reporting frequency may well reflect differences in exposure of the respective study populations but may also depend on the use of a LOD cutoff by Liu that is 50% of the LOQ cutoff used by Blomberg.

Cariou et al. (2015) also measured PFNA in breast milk versus matched maternal serum samples. While the levels observed in breast milk were all below the LOQ, they appear to be consistent with a mean milk:serum ratio in the range of 0.01–0.05. Kärrman et al. (2007) reported a breast milk:maternal serum ratio of 0.01 but based on only two milk samples above the LOD.

While the data described above indicate considerable variability in breast milk:maternal serum concentration ratio, it is all within the range of 0.01–0.05 and hence the mean value of 0.05 reported by Liu et al. (2011) appears to be a reasonable upper bound on the population mean for PK modeling, i.e., the population mean is unlikely to be greater than 0.05 although some individual values may have higher ratios.

In summary, the observed pattern of PFNA decline during and after pregnancy is considered likely to result from the relatively rapid increase in maternal and fetal body weight during pregnancy, so the PFNA body burden distributes into a larger volume, and lactational transfer from the breastfeeding mother. Hemodynamic changes during pregnancy, not discussed at length here, may also increase the volume of distribution, but to a lesser extent than the change in total body mass. The effects of increasing total body mass and lactational transfer appear to more than offset possible changes in renal transporters under hormonal regulation or the cessation of menstruation that might otherwise reduce PFNA clearance during pregnancy and lactation.

Urinary Clearance versus Glomerular Filtration

Some mechanistic insight can be gained by comparing the clearance values (shown in Table 3-3 for rats and mice and estimated just above for humans) with species-specific glomerular filtration rate (GFR), with and without adjustment for serum protein binding. A detailed analysis is provided in Appendix E.3. In summary, GFR ranged from 2 to 4 orders of magnitude greater than the empirical clearance. After correction for serum binding (i.e., only assuming the fraction unbound in serum, f_{ub} can be cleared) GFR × f_{ub}, still overpredicts urinary CL by a factor of 4–60, except in female rats where observed urinary CL was approximately threefold higher than GFR × f_{ub}. Hence, it appears that serum protein binding is less limiting of both urinary clearance and tissue distribution than predicted by assuming these processes are strictly limited to the free fraction at equilibrium, but that renal resorption must extensively limit urinary and hence total clearance in most species and sexes.

Renal resorption was previously put forward as a general explanation for the slow clearance of per- and polyfluoroalkyl substances (PFAS) through the urine (Andersen et al., 2008).

In vitro experiments have since identified PFNA as a potential substrate for transporters in the OATP family, such as human OATP1B1, OATP1B3, and OATP2B1 (Zhao et al., 2017b). Another in vitro study identified rat organic anion transporters (OAT)3 and oatp1a1, as well (Weaver et al., 2010). Thus, active transport is a plausible and likely explanation for part of the difference between GFR, or GFR × f_{free} and urinary clearance of PFNA.

3.1.5. ADME Summary

Reported or calculated clearances, volumes of distribution, and half-lives for male and female rats, mice, and humans are listed in Table 3-3. Clearance and Vd values for rats and mice were derived from Bayesian analysis of the PK studies described previously (details in Appendix E). Human clearance and Vd values were based on the population GMs reported by Chiu et al. (2022).

Table 3-3. Key pharmacokinetic parameters in rats, mice, and humans

Sex and species	F_{abs}	CL (mL/kg-d)	Vd (mL/kg)	$T_{1/2}$[a]	References
Male rats	0.86	3.68	234.8	46.5 d	EPA analysis of Tatum-Gibbs et al. (2011); Ohmori et al. (2003); Iwabuchi et al. (2017); Kim et al. (2019)
Female rats	0.94	71.1	283.2	2.77 d	
Male mice	1	4.51	579.1	101.9 d	F_{abs} from Fujii et al. (2015). CL and Vd from EPA analysis of Tatum-Gibbs et al. (2011)
Female mice	1	4.89	319.6	46.5 d	
Women <12.4 or >40 yr & men	1[b]	0.090[c]	190	4.01 yr	Chiu et al. (2022)
Women 12.4–40 yr	1[b]	0.124[d]	190	2.91 yr	

[a]$T_{1/2}$ = (volume of distribution [mL/kg]) × ln (2) / (clearance [mL/kg-d]); for humans, divided by 365 d/yr.
[b]100% absorption is assumed for humans as a health-protective option due to lack of data.
[c]Lower 95% CI estimate of the population geometric mean from Chiu et al. (2022).
[d]Based on observed serum concentration in women vs. men 20–40 years of age from NHANES, EPA analysis, applied to the estimated CL for men and older women from Chiu et al. (2022).

According to EPA's $BW^{3/4}$ guidelines (U.S. EPA, 2011b) use of chemical-specific data for dosimetric extrapolation such as described above is preferable to the default method of $BW^{3/4}$ 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 values of 3.71 and 71.0 mL/kg-day in male and female rats, one would then predict clearances of 0.88 mL/kg-day in men and 16.9 mL/kg-day in women, which are respectively approximately 9.8 and 136 times higher than the respective estimated average human clearance values for men and nonreproductive age women (0.0.09 mL/kg-day) and for women between 12.4 and 40 years of age (0.124 mL/kg-day) in Table 3-3. Thus, based on the PFNA-specific PK data, use of $BW^{3/4}$ could lead to an overprediction of human elimination of 1 to 2 orders for magnitude, underpredicting effects to the same extent. Therefore, use of $BW^{3/4}$ as an alternative means of extrapolation was not considered further for PFNA.

3.1.6. Evaluation of Approaches for Pharmacokinetic Extrapolation, Including Physiologically Based Pharmacokinetic (PBPK) and Pharmacokinetic (PK) Modeling

The PFAS protocol (see 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. Given the empirical data available in rats, mice and humans, the application of a classical PK model with parameters set using species- and sex-specific PK model is considered as a particular instance of a data-informed extrapolation, which has a better potential for accounting for the accumulation of PFNA over the course of

toxicological studies or human lifetimes. Another option is to more directly use blood or tissue concentrations collected during a toxicological study in animals, i.e., without a formal PK model for the animal, to estimate corresponding human exposures. If chemical-specific information is not available, the protocol then recommends that doses be scaled allometrically using $BW^{3/4}$ methods. Selection from among 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 guidelines on using allometric scaling for the derivation of RfDs (U.S. EPA, 2011b). It preferentially prioritizes adjustments that result in reduced uncertainty in the dosimetric adjustments (i.e., preferring chemical-specific values to underpin adjustments versus use of default approaches).

Evaluation of Physiologically Based Pharmacokinetic (PBPK) Models

A PBPK model is available for PFNA in rats and humans in 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 PFNA, known physiology, and the accepted practices of PBPK modeling. Several flaws were found in the model (see Bernstein et al. (2021)). One flaw, an error in the balance of blood flow through the liver, had only a moderate impact on model predictions (i.e., less than 20%). 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 much lower than is otherwise supported by the empirical PK data. Details of the model's discrepancies are provided in Appendix E.2. When the model was used to simulate IV exposure, for which bioavailability is 100%, or the oral bioavailability was set to 90%, the model significantly overpredicted the observed blood concentration data. The conclusion of this analysis is that a key assumption on which the PBPK model of Kim et al. (2019) and most other existing PBPK models for PFAS are based, that distribution to tissues and clearance are strictly limited to the fraction unbound in blood (f_{ub}, as measured in vitro) is incorrect. While it is possible to relax this condition and fit specific parameters to obtain a rate of distribution to match the empirical data, EPA considers such an approach to effectively undermine a fundamental mechanistic assumption of these models in the absence of independent data to demonstrate the cause of the discrepancy. For example, if an independent experiment showed that the original estimate of f_{ub} was too low and is in fact close to that obtained by empirical fitting, the assumed model structure would be corroborated. But otherwise, the result of empirical fitting distribution parameters is considered by EPA to be the equivalent of using a classical PK model for which the volume of distribution (and rate of distribution to a "tissue" compartment) is empirically fitted, which is the option evaluated further below and described in detail in Appendix E.1. Given these findings, it was determined that the published model structure and underlying assumptions did not allow a sufficiently sound calibration of the PBPK model to the currently available PK data for use in this toxicological review and that empirical fitting of tissue distribution was required.

Fàbrega et al. (2015) also described a PBPK model calibrated for multiple PFAS in humans. However, this model makes use of the same key assumption regarding PFAS distribution as Kim et al. (2019), which EPA considers to be critically flawed. Further, Fàbrega et al. (2015) estimated the equilibrium blood:tissue partition coefficients by comparing tissue concentrations measured in cadavers (autopsy subjects) with blood concentrations from living donors reported 6 years later. EPA considers this comparison of blood and tissue levels in nonmatched subjects (albeit from the same geographic region), reported 6 years apart, to be a highly uncertain method for the estimation of tissue distribution. Hence, the PBPK model of Fàbrega et al. (2015) was not considered further for use in this review.

The high-throughput toxicokinetic (httk) PBPK model package (Breen et al., 2021; Pearce et al., 2017) contains a parameter set for PFNA but its prediction of renal clearance only accounts for f_{ub} in plasma. As described in Appendix E.3, the clearance rates predicted as GFR × f_u in human males and females are 3.8 and 3.1 mL/kg-day, which are 25- and 14-fold higher than the clearance values estimated by Chiu et al. (2022) (assumed applicable to men and nonreproductive age women) and by EPA for this review (see "Total clearance in human" section, above and Table 3-3). Hence, use of the httk PBPK package would significantly underpredict human internal dose for a given exposure and significantly overpredict HEDs related to a given POD obtained from toxicological studies in laboratory animals or human epidemiological studies using PFNA blood concentrations as the dose metric. Therefore, the httk PBPK model package is not considered further for use in this review.

Evaluation of Classical Pharmacokinetic (PK) Modeling and Study-Specific Measured PFNA Concentrations

Two other options for estimating the relationship between exposure (applied doses) and internal doses for dosimetric extrapolation are classical PK modeling and use of direct measurement of PFNA concentrations in experimental or epidemiological studies. When serum concentrations are measured as part of a toxicological study, such as occurred at the end of the NTP 28-day study in rats, those data can directly inform the internal dose associated with toxicity rather than relying on assumptions involved in a PK model, such as the assumption that the kinetics observed after a single dose in a PK study can be reliably extrapolated to multiple days of dosing. However, the response observed at the end of a toxicity study is typically assumed to result from the cumulative effect of a chemical on the tissue and organism being evaluated, not just the concentration occurring on a single day. A measure of the cumulative internal exposure that is often used is the average blood (serum) or tissue concentration, or AUC, calculated over the length of the study or a suitable length of time prior to the day of observation.

The concentration measured only on the final day of a study does not by itself provide a measurement of concentration over time that is needed to obtain the average concentration. While any PK model involves its own assumptions, these models predict the concentration time-course needed to determine average concentration or AUC. Given general knowledge of a chemical's PK,

such as its half-life, one might deduce or reasonably assume, for example, that day-to-day variation in a chemical's serum concentration will be minimal after the first few days of exposure, allowing interpretation of the measured end-of-study concentration as representative of the average. However, if serum concentrations are expected to vary from day to day over an entire study, the most straightforward way to predict this variation is through use of a PK model. Use of a PK model will also provide a more consistent means of comparing internal doses between studies among which the extent and timing of in-study serum or tissue concentrations measurements vary.

For example, Das et al. (2015) measured PFNA serum and tissue concentrations at multiple timepoints, while Wolf et al. (2010) only measured serum concentrations at the end of their mouse developmental study, 3 weeks after dosing ended. One might wish to use the serum concentrations in PND 1 mouse pups measured by Wolf et al. (2010) to extrapolate those observed endpoints, but comparable data are not available for Wolf et al. (2010) making any comparison between the studies on such a basis very complicated. Attempting to interpret effects seen in PND 1 mouse pups due to dosing during gestation based on serum concentrations measured on PND 21 (i.e., in a parallel cohort of mice) also seems fraught with issues. A PK model can account for the dosing schedule and predict the concentration time-course over the relevant period. Such results can then be used to interpret the effects observed at different postnatal timepoints in a way that is consistent between these studies (or between studies evaluating time-dependent toxicity in general). Hence, a PK model is the generally preferred tool for estimating internal doses for animal-to-human extrapolation.

Classical pharmacokinetic (PK) modeling in experimental animals

Bil et al. (2022) evaluated the PK of multiple PFAS, including PFNA, in male rats, with the objective of evaluating effects from combined exposure. While Bil et al. (2022) used a classical two-compartment PK model structure, for PFNA the PK data used (Tatum-Gibbs et al., 2011) were not sufficient to identify parameters for the second compartment. Hence, their effective model structure for PFNA was also identical to that evaluated by EPA for a single compartment (below).

EPA developed a classical PK model package that can be parameterized for one- or two-compartment models using the empirical PK parameters estimated above (see Table 3-3) or as detailed in Appendix E.1 for dosimetry in individual rats, mice, and humans and for the pregnant mother and fetus(es) during gestation. The model package includes paired one- or two-compartment PK models to describe dosimetry in nursing mothers and their offspring. While the PK models poorly predicted some mouse and rat PK data used to evaluate model performance, in particular gestational and lactational PK data for PFNA in mice from Das et al. (2015) and (Wolf et al., 2010), the results were still much closer to the observed mouse data than if one were to assume PFNA reached steady-state concentrations. (Given the half-life of PFNA in mice, an extended period of prenatal dosing would have been needed to achieve steady state and the PK model predictions reflect that dosing only occurred during gestation.) Further, because dosing in the mouse developmental studies only occurred during pregnancy, with endpoints evaluated in the pups up to

many weeks after birth, an assumption of steady state clearly would not match the internal dose time-course.

EPA's classical PK model predictions of the PFNA concentrations observed in male rats at the end of the NTP 28-day bioassay were considered adequate at low doses, i.e., at doses where corresponding PODs were identified and hence from which HEDs needed to be estimated, but systematically underpredicted the measured concentrations. Specifically, the mean of the model predictions was less than the mean observed concentration for every dose level, but the 90% model prediction interval included the mean observed concentration at the lowest and third dose and was within a few percent of the observed mean for the second dose (see Appendix E, Figure E-7, Table E-7). While the PK model predictions of PFNA concentrations in the male rats at the end of the NTP study were closer to the observed concentrations than assuming steady state, a third option in which the measured concentrations were simply interpolated was considered more accurate than either use of the PK model or assumption of steady state. Further, even though the estimated half-life in male rats is uncertain, its value is known well enough to state with high confidence that PFNA concentrations accumulated in the male rats throughout the NTP study, although in a slightly nonlinear manner due to concurrent changes in BW. Given the bias of assuming steady state or using the PK model, together with the expectation of near-linear PFNA accumulation in male rats exposed for 28 days, EPA concluded that the best estimate of the average concentration in male rats given the 28-day exposure would simply be one half of the final concentration calculated by linear interpolation between the measured concentrations.

However, use of classical PK model described in Appendix E.4 (with parameters estimated as described in Appendix E.1) is considered the best option for estimating internal doses during developmental studies in mice where the model also predicts internal doses in mouse pups. For the mouse developmental studies, the dosing only during gestation followed by endpoint observation at various postnatal times makes direct interpolation of the observed concentrations impractical and PK model predictions are clearly superior to the assumption of steady state (details in Appendix E.4.1).

PK model predictions were quantitatively further from the observed data than assuming steady state for female rats, while qualitatively the model predicted that female rats would have serum concentrations near to steady state and close to the observed end-of-study concentration over the course of the bioassay. Details are provided in Appendix E.4.1. Therefore, for extrapolation of endpoints from the NTP 28-day bioassay in female rats, EPA judged the best approach was to use the observed end-of-study serum concentrations. In particular, EPA assumed the end-of-study plasma concentrations provided a better estimate of the average internal dose in those females at the administered doses than obtained with either the PK model or calculated assuming steady state from the estimated clearance in female rats. Since the relationship between the applied dose and end-of-study plasma concentrations in female rats is close to linear (see Appendix E, Figure E-7),

the average internal dose in female rats at other dose levels (i.e., PODs identified by dose-response analysis) was simply estimated by linear interpolation between the measured concentrations.

Classical pharmacokinetic (PK) modeling in humans

Chiu et al. (2022) used a one-compartment PK model in their analysis. While the rigorous statistical analysis used with data curated from multiple human observational studies is notable and EPA has selected the resulting PK parameter values for use in calculating HEDs, the model structure is effectively the same as evaluated by EPA for its custom PK model when parameterized for a single compartment (further details below). Hence, while EPA is using its own model code for PK analyses, the application is considered technically identical to use of the Chiu et al. (2022) for one-compartment PK analysis.

Yao et al. (2023) applied their estimates of urinary clearance in infants using a one-compartment PK model to predict changes in serum levels during the first year of life. As with the previous two PK analyses, the model structure is essentially identical to the one evaluated by EPA for a single compartment. However, Yao et al. (2023) estimated infant intake of PFNA (and other PFAS) based on the milk ingestion per kg BW (mL/kg-day) in the first month of life, when this normalized ingestion rate is known to decline as the infant grows ((U.S. EPA, 2011a), https://www.epa.gov/sites/default/files/2015-09/documents/efh-chapter15.pdf, see Table 15-1) and the model did not account for the growth of the infant, which would dilute the initial body burden of PFNA. Hence, the model predictions are not considered accurate.

While limited, perinatal human data were identified and used to estimate developmental PK parameters for humans as described in human distribution during gestation (in Section 3.1.2), pregnancy in humans (in Section 3.1.4) and lactation in humans (in Section 3.1.4), data that can be used to validate the model assumptions for the perinatal period and otherwise in young children are limited. Specifically, independent longitudinal measurements of blood or serum PFNA levels in human subjects for whom ongoing exposure was known (e.g., essentially zero after a period of high exposure) that could be used to validate PK predictions for those individuals were not identified. (It is noted that the analysis of Chiu et al. (2022) involved some observations over time, in conjunction with corresponding changes in exposure, but the observations were primarily in adults.) Results from NHANES (e.g., as reported by Jain and Ducatman (2022)) can be used to evaluate predicted differences in PFNA dosimetry in men versus women, while those of Oh et al. (2022) can be compared with predicted changes during pregnancy and lactation. Koponen et al. (2018) observed longitudinal changes in serum concentrations of PFNA and other PFAS in children from 1 to 10 years old and evaluated the correlation of PFNA concentrations at age 1 year with duration of breastfeeding.

General parameters for changes in the BW of humans as a function of age, including during pregnancy, and milk ingestion rates have been described by Kapraun et al. (2022), and the PK model package described there was adapted for the analysis of PFAS PK. Although uncertainty exists in how PFNA PK may differ between (young) children and adults, use of a PK model allows

for the prediction of accumulation as a function of age and lifestage and in particular of a potential bolus of exposure due to breastfeeding that could not be accounted for otherwise.

As discussed previously, results of Yao et al. (2023) indicate that renal clearance in infants is about twofold lower than adults (Yao et al., 2023), although aspects of this study make its accuracy quite uncertain. On the other hand, EPA's PK model assumes that clearance in children is the same as in adults and when its results were compared with the data of Koponen et al. (2018) for children at 1 year of age, the model overpredicted the increase in PFNA concentration due to lactational transfer (see Appendix E.4.2). This overprediction by the EPA model indicates that clearance may be higher in young children than in adults, not lower. Neither the results of Yao et al. (2023) nor the results of EPA's PK modeling suggest that clearance in children is more than twofold different than in adults, hence it is within the range of uncertainty that the intrahuman uncertainty factor, UF_H, is intended to cover. Therefore, use of the clearance value obtained for adults should be reasonable for children as well. Specifically, for the purpose of simulation, total clearance in children from infancy on was assumed to equal that in adult men and older women. However, the Vd in the newborn was assumed to be twice that of adults, i.e., equal to that estimated for the first- and third-trimester fetus, and to then decline to adult values by age 10 years (Human distribution during gestation in 3.1.2).

Because of the limited PK data that might be used for model calibration and validation for PFNA during pregnancy and childhood, application of the PK model adapted from Kapraun et al. (2022) during these lifestages is considered quantitatively uncertain. For example, the transfer of PFNA from maternal serum to breast milk over the entire period of breastfeeding has not been evaluated. While EPA's PK model did predict the increase in serum PFNA of 1-year-olds as a result of breastfeeding reported by Koponen et al. (2018) reasonably well, this prediction involved adjusting the exposure level for children who were not breastfed to match model prediction for that group with the measured concentration (see Appendix E.4.2). Only the relative increase in serum PFNA that was due to each month of breastfeeding was then predicted, but not the absolute level. However, model simulations should provide a useful, qualitative prediction of the PFNA time-course in exposed humans, which can be compared with steady-state serum concentrations estimated using only human clearance (CL) values.

Children who are breastfed for 12 months are predicted by the PK model to experience a large bolus, relative to maternal levels, and to be above the corresponding (adult male and older female) steady state for several years, but then to be within 20% of that level by age 4 or 5 years, even when ingesting milk at a rate two standard deviations above the mean. The simulations shown in Appendix E, Figure E-11 used the mean milk-rate ingestion per kg body weight of the child. The bolus due to breastfeeding will be higher and the subsequent period of elevated concentration longer for children ingesting the upper percentile volume of milk, but from around age 4 to 12.4 years, they are still predicted to be less than 120% of the steady state for males and nonreproductive age women. Hence, applying the clearance for men and older women

(0.09 mL/kg-day) to estimate HEDs from serum concentrations of PFNA for children between ages 4 and 12 should not underestimate the corresponding exposure by more than 20%. Children who ingest an average volume of breastmilk are predicted to have their serum concentrations fall to about 70% of the nonreproductive steady state but this only indicates a modest degree of health protection for those individuals when using the steady-state estimate.

For a woman of reproductive age, the model indicates that her serum concentrations will be within 20% of the corresponding steady state (i.e., for CL = 0.124 mL/kg-day), through childbirth, although breastfeeding for a year would result in a significant decline such that she remains below that level for up to 5 years (given an upper percentile milk ingestion rate, see Appendix E, Figure E-11). Hence, use of the two human clearance values to estimate HEDs for corresponding lifestages should give predictions in a range that has minimal error compared with application of a PK model that involves specific assumptions that cannot be verified with existing data. Therefore, EPA considers use of lifestage-specific CL values to calculate HEDs to be a reasonable approach.

Summary of Physiologically Based Pharmacokinetic (PBPK) and Pharmacokinetic (PK) Model Evaluation

Although PBPK modeling was judged too unreliable for use, a custom PK model with parameters identified through empirical fitting was determined to adequately describe the PK in male rats and to provide the best alternative for estimation of internal doses in female mice and their pups during developmental toxicity studies. Direct interpolation of PFNA concentrations measured in female rats at the end of the NTP 28-day bioassay was determined to be the best approach for estimating the corresponding average PFNA serum concentrations. Further, while the custom PK model was determined adequate for male rats, because it systematically underpredicted the NTP data, interpolation of the NTP data (informed by model predictions) was likewise considered the best option for estimating PFNA dosimetry in adult male rats under that study design. But because of the study design (dosing schedule) and availability of PK data used for developmental bioassays in mice, direct interpolation of measured concentrations was considered impractical and likely unreliable for those animal subjects and while the custom PK model did not match the available mouse PK data as well as preferred, it was shown to be superior to an assumption of steady-state serum concentrations, which is implicit in the application of derived extrapolation factor (DDEFs) based on species-specific clearance.

Human CL can be applied to estimate HEDs from internal dose PODs identified from animal toxicological studies using the PK approaches described above, to estimate HEDs from epidemiological studies where human serum concentrations were the exposure metric, or as part of a DDEF applied to external dose PODs from animal studies. Results of simulations shown in Appendix E.4.2, indicate that a young woman will be within 20% of steady state (given the reproductive age clearance for women) by age 12.4 years and remain in that range except during the latter part of pregnancy, during breastfeeding and for the following 4 years, when her serum concentrations may fall more than 20% below the steady state. If a woman breastfeeds for less than

12 months (for breastmilk ingestion rates below the upper percentiles), her PFNA serum levels would remain higher.

DDEFs were also considered for animal-human extrapolation. DDEFs can be calculated from the fraction absorbed (F_{abs}) and the CL for male and female rats and mice, and men and women (see Table 3-3), but use of these implicitly assumes that animals are near steady state given bioassay exposure regimens. Given the estimated half-lives of PFNA, this assumption is probably not correct for male rats and male and female mice in the toxicity studies evaluated. While female rats are predicted to reach steady state much more quickly, the steady-state serum concentrations estimated for female rats were considerably lower than the concentrations measured in the NTP bioassay (see Appendix E, Figure E-7). Hence, use of a DDEF appears to be less accurate than the alternative PK approaches in all cases.

In general, the uncertainty in use of a one- or two-compartment classical PK model for estimation of internal doses in mice may be judged no greater than use of DDEFs for extrapolation of PODs for that species, since both approaches rely on the estimated CL and F_{abs}, while the DDEF relies on the assumption of steady state. The PK model also relies on the estimated Vd but accounts for the study-specific dose schedule and was shown to better predicted the observed concentrations from mouse (and male rat) bioassays than assuming steady-state concentrations.

On the other hand, large uncertainties remain for the possible application of the PK model for human dosimetry in young children, while the remaining predictions are mostly within 20% of the steady-state value predicted using only the estimated human clearance values. Hence animal-to-human extrapolation of internal dose PODs to HEDs will be conducted using a hybrid PK approach for all endpoints, where:

3) internal doses (average serum concentrations) for endpoints obtained in mice are estimated using the PK model,

4) internal doses in rats are interpolated from the end-of-study concentrations measured in the NTP 28-day bioassay, and

5) HEDs are then calculated from the corresponding internal dose PODs using lifestage appropriate values of human CL.

When epidemiological analyses are used to directly identify human serum concentration internal doses PODs, these will be likewise converted to HEDs using lifestage appropriate values of human CL.

For the specific parameters, F_{abs} is generally high (over 80%) for PFAS and the quantitative uncertainty in those values is likely to be small. However, the uncertainty in the CL values is larger and so is discussed here.

3.1.7. Uncertainty in Clearance in Experimental Animals

The 90% confidence interval (CI) for clearance (n-PFNA) in male rats (see Table 3-2) was 2.3–5.0 mL/kg-day, or just over twofold and indicates that the true value is unlikely to be more than a factor of 1.5 greater than the mean value of 3.68. The discrepancy between Tatum-Gibbs et al. (2011) and other studies does not appear to be a matter of strain. Tatum-Gibbs et al. (2011) used Sprague-Dawley rats, but the results reported by Kim et al. (2019), who also used Sprague-Dawley rats, are much more consistent with those of Iwabuchi et al. (2017) and Ohmori et al. (2003), who used Wistar rats. Thus, the discrepancy can only be noted as an uncertainty at this time, the source of which is not known (Kim et al., 2019; Iwabuchi et al., 2017; Tatum-Gibbs et al., 2011; Ohmori et al., 2003). The overall population mean CL estimated (3.68 mL/kg-day) is considered adequate for cross-species extrapolation, given that it is less than twofold below the highest study- and dose-specific mean value estimated by EPA's analysis.

For female rats, EPA's clearance CI has a much smaller range, 1.25-fold, compared with 2.2-fold in male rats, but PK model predictions and estimated steady-state levels from this CL in females underpredicted the concentrations measured at the end of the NTP 28-day bioassay. To match the observed concentrations, using F_{abs} = 0.94 and parameters and simulations otherwise as described in Appendix E.4.1, the female rat CL had to be reduced by a factor of 1.6 (results not shown).

The range of estimated CL is 2.2-fold iTable n male mice and 1.5-fold in female mice, but the results in male and female mice are based only on data from Tatum-Gibbs et al. (2011). Fujii et al. (2015) did not provide data in a way that EPA could extract and use. Moreover, Fujii et al. (2015) only observed the PK for 24 hours, so even if available, the data are not expected to be highly informative. The discrepancy between the CL values calculated from the PK model and parameters of Fujii et al. (2015) and the range estimated here likely reflect that this short observation window is not adequate for a compound with such slow elimination.

If one considers reported half-lives, there appears to be larger variability. For example, Tatum-Gibbs et al. (2011) reported a beta-phase half-life of 32 days in female rats for IV doses of 1–10 mg/kg evaluated over 50 days while Kim et al. (2019) reported a half-life of only 4.44 days for an i.v. dose of 3 mg/kg in female rats evaluated over 60 days. The discrepancy of over sevenfold in the apparent half-life must represent interlaboratory differences in experimental or analytic methods, given that the experiments were otherwise so similar. However, from EPA's analysis it appears that much of this discrepancy is due to the method used for calculating PK parameters. When EPA calculated average CL as dose/AUC, the dose-specific values of CL from Tatum-Gibbs et al. (2011) only ranged from 2.07 to 2.76 mL/kg-day, a 33% difference, and the calculated CL from Kim et al. (2019) was 2.76 mL/kg-day. When an average half-life is then calculated as $t_{1/2}$ = ln (2) *Vd/CL, the values from Tatum-Gibbs et al. (2011) were all 20.1 day (varying in the 2nd decimal place) and from Kim et al. (2019) was 26.8 day, only a 33% difference. The similarity among these results likely reflects the fact that AUC calculation is much less sensitive to noise in particular

measurements, the timing of blood samples, and the method used to calculate the half-life, indicating that the underlying PK data are in fact quite consistent, without major study-to-study variation.

EPA does note that (in male rats) the half-life for iso-PFNA was estimated to be 33%–50% lower than n-PFNA (see Table 3-3; (Benskin et al., 2009; De Silva et al., 2009a)), so some variation among studies may have occurred due to variation in the extent to which the branched and linear forms were present in the test sample, and variation in human data may occur in part due to variation in the form present. However, this variation between congeners is less than the variation in CL among studies and dose levels for each species and sex, and hence the uncertainty in the estimates of average CL for male and female rats and mice is judged to be less than a factor of 2 (i.e., the true average CL for each animal species and sex is within a factor of 2 of the mean values reported in Table 3-2).

3.1.8. Uncertainty in Clearance in Humans

It is difficult to evaluate the total uncertainty in the human elimination rate, given the limited data. Fujii et al. (2015) used paired serum and 24-hour urine samples from five male and five female healthy volunteers to estimate an overall rate of urinary clearance of 0.038 ± 0.01 (mean ± SD) mL/kg-day. While 10 subjects is a relatively small number, the 24-hour urine collection provides a robust measurement of that excretion rate, not requiring extrapolation from a spot sample. However, there was much greater variability in the estimated fecal clearance (0.024 ± 0.024 mL/kg-day), which was based on biliary clearance measured (24-hour collection) in three male and two female subjects, of which one female was being treated for pancreatic cancer and the other four subjects were being treated for gallstones present in the bile duct (choledocholithiasis). Fujii et al. (2015) then used an estimate of 98% resorption for PFAS excreted in the bile based on data for PFOA and PFOS. When Kim et al. (2019) measured urinary and fecal excretion in rats, they found fecal excretion to be 9% of urinary excretion, while the estimates for humans from Fujii et al. (2015) predict that fecal elimination is 63% of urinary clearance. Hence, the fecal excretion estimated by Fujii et al. (2015) may significantly overpredict human fecal elimination, perhaps because of the disease status or the medical intervention used (nasobiliary drainage, percutaneous transhepatic biliary drainage or percutaneous transhepatic gallbladder drainage; (Fujii et al., 2015)).

Zhang et al. (2013c) reported blood or serum and morning urine concentrations of PFNA for two demographic groups: (1) women ≤50 years of age (n = 16); and (2) men (all ages) combined with women >50 years of age (n = 50). This grouping arises from a presumption that urinary clearance in men is not age dependent, while it is in women, and that urinary clearance in older women is similar to men. While the number of subjects evaluated was much larger than in Fujii et al. (2015), use of spot urine samples introduces some uncertainty since these need to be extrapolated to 24-hour urinary elimination rates. Also, whole blood concentrations were converted to serum concentrations by dividing the blood concentration by the population-average

hematocrit (for men and women, respectively), effectively assuming zero extraction from blood cells (Zhang et al., 2013c). The estimated median urinary clearance was 0.20 mL/kg-day in the younger women and 0.094 mL/kg-day in men and older women, but in the population as a whole the median (95% CI) was 0.097 (0.028–0.54) mL/kg-day [digitized from Figure 2 of Zhang et al. (2013c)]. The mean for the entire population (calculated as the weighted mean of the reported values for younger women and men and older women) is 0.174 mL/kg-day. While, as noted above, the almost 20-fold range (95% CI) of CL estimated by Zhang et al. (2013c) includes interindividual variability, which does not directly indicate uncertainty in the population mean, that the population mean urinary CL is 4.5 times greater than the value estimated by Fujii et al. (2015) does indicate a comparable degree of uncertainty in the urinary clearance.

Chiu et al. (2022) estimated a total CL of 0.153 (median estimate of GM) (0.090–0.255, 95% CI of GM) mL/kg-day, which is intermediate between the mean urinary CL estimated for Zhang et al. (2013c) (0.174 mL/kg-day) and by Fujii et al. (2015) (0.038 mL/kg-day). Since Chiu et al. (2022) evaluated total CL for PFNA based only on estimates of total exposure and observed blood concentrations in healthy adults (not relying on read-across data from other PFAS nor Vd estimated in other species) EPA considers those results to be the best estimate of total clearance in human adults, while recognizing that the population evaluated was primarily men and older women. The 95% CI for the GM estimated by Chiu et al. (2022) is also considered a sound measure of uncertainty in the average CL of this population, indicating an uncertainty range in the population GM of +70%/−40%, although the 98% CI for CL values of individuals ranged roughly 3.5 times above and below the GM.

To account for uncertainty in the human clearance of PFNA, EPA chose to use the lower 95% CI for the population GM estimated by Chiu et al. (2022), 0.090 mL/kg-d, for men and older women. This value is twofold higher than the 1st percentile CL in their entire sampled population (the 1st percentile random individual CL is 0.045 mL/kg-d). While use of the lower CI on the population GM CL addresses uncertainty in that GM, application of the portion of the uncertainty factor for human interindividual differences typically attributed to PK, $UF_{H,PK}$ = 3, should then be sufficiently protective of the entire population, accounting for variability in individual CL around the GM. Specifically, application of $UF_{H,PK}$ = 3 corresponds to use of CL = 0.03 mL/kg-day, which is 1.5 times lower than that estimated for the 1st percentile random individual by Chiu et al. (2022).

In comparison with results from other studies, 0.090 mL/kg-day, is slightly below the median urinary CL estimated by Zhang et al. (2013c) for men and older women, 0.094 mL/kg-day (mean = 0.15 mL/kg-day), although it is higher than the mean urinary CL reported by Fujii et al. (2015), 0.038 mL/kg-day. It is expected that total clearance, including fecal elimination, is higher than urinary clearance, but some of the difference between the results of Fujii et al. (2015) and those of Chiu et al. (2022) and Zhang et al. (2013c) may be due to sampling variability, since Fujii et al. (2015) only tested 10 subjects and the mean is near the 1st percentile individual estimate for total CL from Chiu et al. (2022), 0.045 mL/kg-day. A lower bound of urinary CL among human

adults could be estimated from Fujii et al. (2015) as mean −2 × SD = 0.036 mL/kg-day, which is still within a factor of 3 of the 0.09 mL/kg-day.

The 95% upper CI for urinary CL from Zhang et al. (2013c) for men and older women was 0.20 mL/kg-day and that for younger women was 0.37 mL/kg-day, while the 99th percentile random individual total CL reported by Chiu et al. (2022) was 0.55 mL/kg-day. Hence, the upper CI individual value from Chiu et al. (2022) can be considered a reasonable upper bound for all studies, while it is 6 times higher than 0.09 mL/kg-day. Use of a human CL of 0.09 mL/kg-day is therefore presumed by EPA to provide a reasonable average for prediction of the population of males and older women.

For children, results in Figure 3-1 indicate that girls at age 12 have CL similar to boys the same age. As discussed in the "Urinary clearance in humans" section, above, Yao et al. (2023) evaluated urinary clearance in infants and while the approach seemed rather uncertain, they obtained a median (25th, 75th percentiles) of 0.047 (0.028, 0.100) mL/kg-day. While the 25th percentile falls slightly below the range of values reported for adults by other authors, including Fujii et al. (2015), and since this rate does not include fecal or other routes of excretion, it still seems likely that total CL in infants is within a factor of 3 of 0.09 mL/kg-day. Hence, the overall uncertainty in total CL among human males of all ages and nonreproductive age females is judged to be within a factor of 3 below 0.0.9 mL/kg-day. While individual CL values could be as much as 6 times higher than 0.09 mL/kg-day, those individuals would be at lower risk than estimated using this value.

For reproductive age women (12.4–40 years) CL was estimated to be 0.124 mL/kg-day (see "Total clearance in humans" section, above), which is well within the range of uncertainty described above and in fact below the median estimated GM CL from Chiu et al. (2022) (0.153 mL/kg-day) and slightly below the lower 95% CI reported for urinary CL in young women by Zhang et al. (2013c), 0.13 mL/kg-day. Assuming the variance in CL in young women is the same as reported by Chiu et al. (2022), the uncertainty in this CL for young women is likewise judged to be within a factor of 3 below and no more than 6 above 0.124 mL/kg-day.

Summary of Uncertainty in Human Clearance

The overall uncertainty in the total CL values selected or estimated to represent population averages for humans (0.09 mL/kg-day in all males and females below age 12.4 and above age 40, and 0.124 mL/kg-day in females 12.4–40 years of age) is judged to be less than a factor of 3 from a risk analysis perspective, since total CL in the entire population appears unlikely to be less than 0.03 mL/kg-day and total CL in women 12.4–40 years of age appears unlikely to be less than 0.04 mL/kg-day. While distributional estimates from Chiu et al. (2022) indicate that CL values of some individuals may be 0.545 mL/kg-day, 6 times higher than 0.09 mL/kg-day, and by extrapolation CL values for young women may be as high as 0.744 mL/kg-day, the risk to high CL individuals will be less than estimated using 0.09 mL/kg-day.

3.2. NONCANCER EVIDENCE SYNTHESIS AND INTEGRATION

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. Each synthesis describes the available endpoints evaluated across studies. For this section, evidence to inform organ/system-specific effects of PFNA in animals following developmental exposure are discussed in the individual organ/system-specific sections (e.g., liver effects in animals after gestational exposure are discussed in the Section 3.2.3 but cross-referenced in Section 3.2.2). General toxicity, including effects on body weights and survival, are summarized first in Section 3.2.1 to aid in interpretation of other potential health effects (in Sections 3.2.2 to 3.2.11), given associations between PFNA exposure and overt toxicity in some animal studies. Evidence synthesis and integration judgments are not drawn for general toxicity.

3.2.1. General Toxicity

General Toxicity in Animal Studies

Three *high*, six *medium*, and one *low* confidence animal studies reported effects relevant to general toxicity, mostly for survival and body weight. Several of these studies reported dose-dependent reductions in body weight after PFNA exposure. Body weight changes, depending on the magnitude and precision, can make interpreting study results for hazard outcomes challenging, as they may be indicative of nonspecific overt toxicity. Thus, the possibility that declines in body weight could affect the interpretation of organ/system-specific endpoints is discussed in each hazard section below where appropriate, with a more detailed description of the body weight data provided.

The study by Kinney et al. (1989) was judged to be *low* confidence due to deficiencies in sensitivity, duration of exposure, and results reporting (i.e., group variability). Kennedy (1987) was considered *uninformative*, largely due to the lack of quantitative results for body weight changes (and was also *uninformative* for all other assessed endpoints) and was not considered further. The study evaluation judgments are shown in Figure 3-2 and details are available in HAWC. Additionally, a *high* confidence 28-day study by NTP (2018), NTP (2019) reported high mortality at the two highest doses tested in adult male and female rats that hindered results interpretation (for all other outcomes, including body weight changes). Results for these higher dose groups in the NTP study are generally not presented in the assessment with the exception of the histopathology,

where necropsy was performed after chemical-induced death (termed natural death as distinguished from moribund sacrifice).[9]

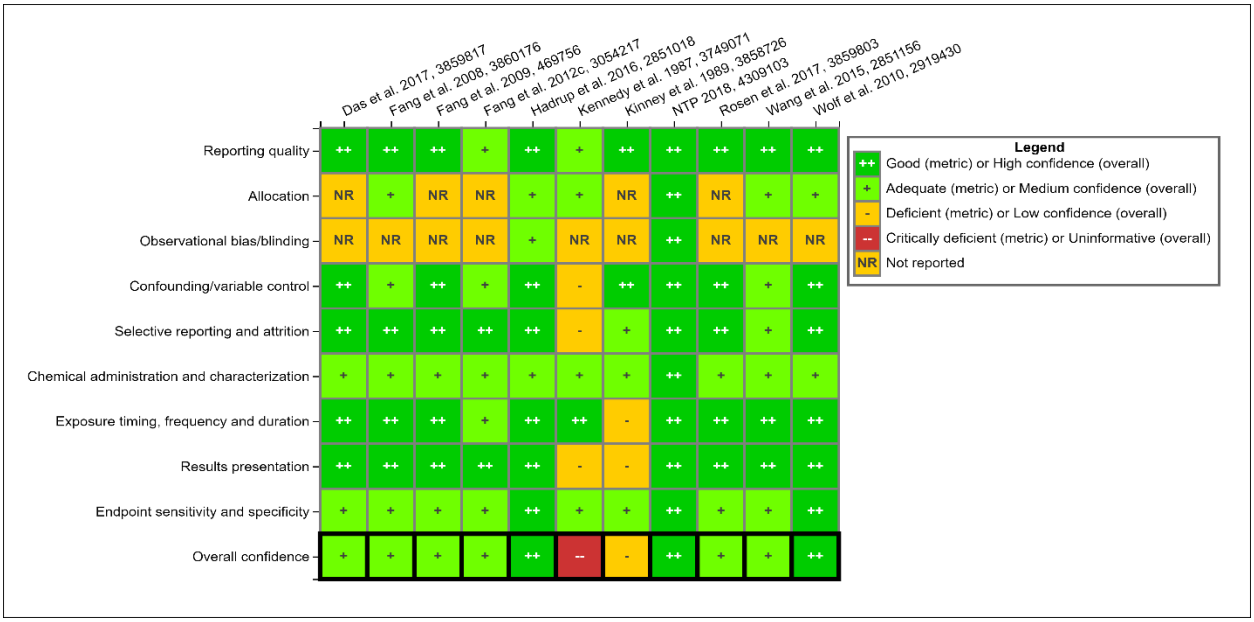


Figure 3-2. Summary of animal study evaluations that examined PFNA effects on general toxicity endpoints (mortality and body weight).[a] See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/General-toxicity/.

[a]Body weights for gestational exposures are reported in Section 3.2.2, but (Wolf et al., 2010) reported body weight for nonpregnant females, which is included here.

Adult rodent mortality

Animal mortality in the nondevelopmental studies was only observed in the NTP study (NTP, 2019, 2018), which was the longest in duration at 28 days and included 10 animals per dose group. At study termination (28 days), NTP reported complete mortality in both male and female SD rats in the highest dose groups, 10 mg/kg-day and 25 mg/kg-day respectively (NTP, 2018). In the second highest dose groups, at terminal sacrifice, male survival was limited to two animals at 5 mg/kg-day and female survival to one animal at 12.5 mg/kg-day. The average life spans of males in the 5 and 10 mg/kg-day groups were 25.5 and 16.5 days, respectively. For females, the average life spans in the 12.5 and 25 mg/kg-day dose groups were 19.9 and 12.4 days, respectively. Survival

[9]Concerns that histopathological results might have been influenced by potential tissue degradation after natural death were alleviated by the lack of evidence that these observations were influenced by post mortem autolysis. Specifically, (1) there was a general lack of correlation between nonneoplastic lesion incidence and the number of early death animals; and (2) animals were checked twice per day with dead animals placed in cold storage (−60C) (NTP, 2018, 2011). It should also be noted that a revised version of the NTP 2019 report was issued in July 2022, but had no substantial effects on the NTP reporting of the 28-day study on PFNA (NTP, 2019).

was 100% in all other dose groups for both sexes at terminal sacrifice (males: 0, 0.625, 1.25, 2.5 mg/kg-day; females: 0, 1.56, 3.12, 6.25 mg/kg-day). Except for histopathological results, where observations were recorded at necropsy, results for the two highest dose groups are not included in the evidence synthesis due to either complete mortality or an insufficient number of animals to provide meaningful results.

Adult rodent body weight

Body weight changes, specifically weight reductions, were common across PFNA short-term toxicology studies in rats and mice at higher doses (see Figure 3-3), and a 10% or greater decrease in body weight was generally considered a minimally biologically sensitive response (U.S. EPA, 2012a, 2005) in the absence of data regarding how body weight may affect specific parameters (e.g., endpoints, organs, or systems). However, scientifically based values can be used to inform judgments on whether effects on body weight are likely to affect results interpretation. For example, body weight reductions less than 30% are generally not expected to affect reproductive parameters although responses may vary somewhat depending on sex, species, and test model (Creasy and Chapin, 2018; U.S. EPA, 1996).

In the study with the longest duration of PFNA exposure in adult rats (28-days), NTP reported significant reductions in rat body weights, with more severe effects in males (NTP, 2018). Specifically, at terminal sacrifice male body weights were statistically significantly reduced by 17% at 1.25 mg/kg-day and by 44% at 2.5 mg/kg-day. In females, body weight was unaffected at 1.56 mg/kg-day and was statistically significantly reduced by 6% at 3.12 mg/kg-day and by 10% at 6.25 mg/kg-day (generally not interpreted as biologically significant).

In shorter, 14-day studies in male rodents generally consistent body weight reductions occurred at doses ≥3 mg/kg-day. Fourteen-day oral dosing studies by Fang and coauthors reported significant reductions in body weight in male BALB/c mice (Fang et al., 2008) and SD rats (Fang et al., 2009) at 3 and 5 mg/kg-day PFNA (15% and 18% respectively for mice, 17% and 36% respectively for rats). A third study in rats by the same group showed significant reductions only at the highest dose of 5 mg/kg-day (21% lower than control) (Fang et al., 2012c). Fang and coauthor studies by other groups also found significant decreases in the average body weight of male Wistar rats (31%) and BALB/c mice (26%) after 14 days of treatment with 5 mg/kg-day but no change at lower doses (Hadrup et al., 2016; Wang et al., 2015a). Additionally, a study including nonpregnant female mice indicated no statistically significant changes in adult body weight at doses up to 2 mg/kg-day (Wolf et al., 2010). In two 7-day studies, no effects on body weight in male rodents were observed after exposures up to 10 mg/kg-day PFNA (Das et al., 2017; Rosen et al., 2017), suggesting duration of exposure is an important factor in PFNA-induced body weight decreases.

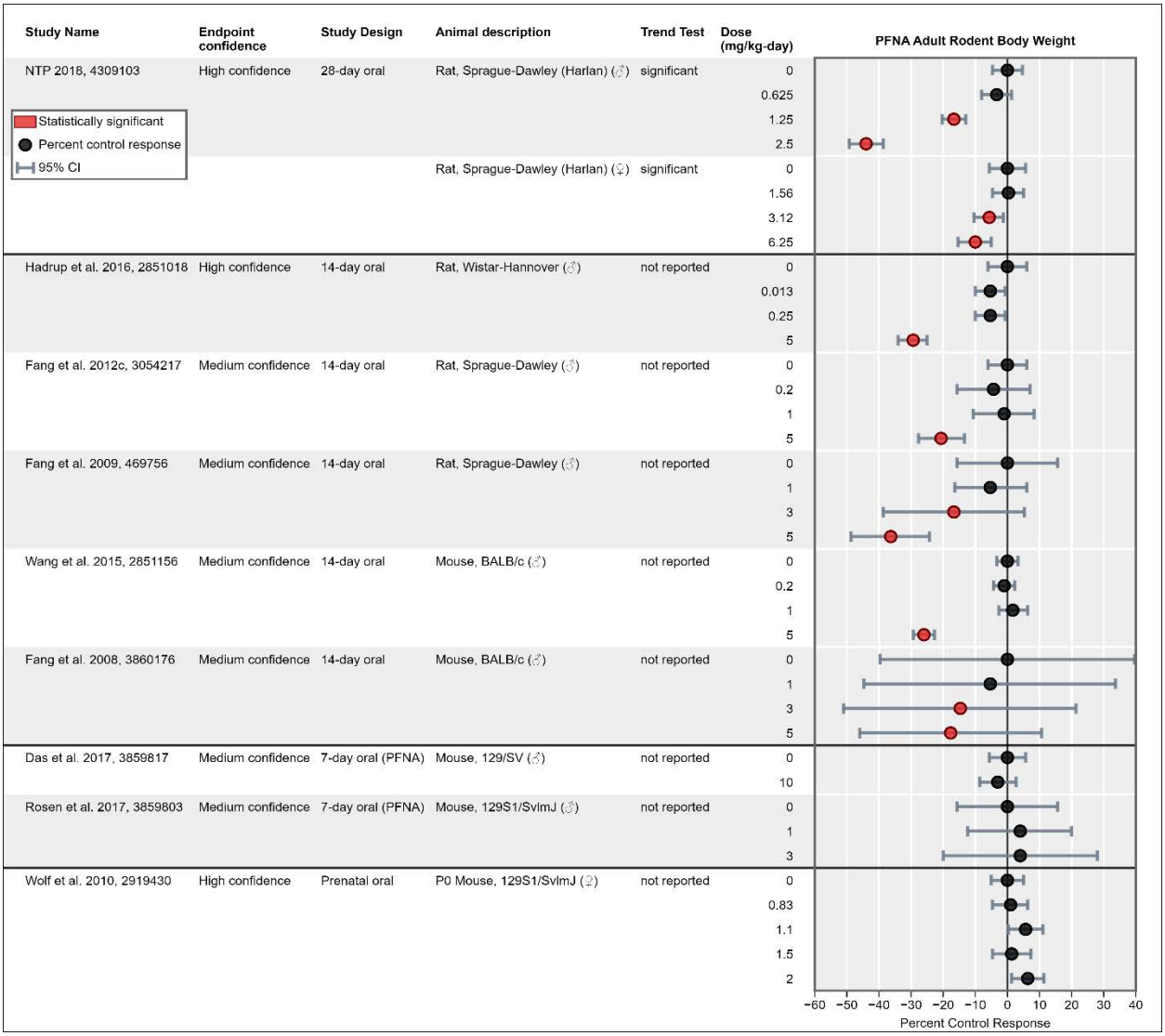


Figure 3-3. PFNA effects on adult rodent body weight displayed as percent control values. See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-adult-rodent-body-weight/.

*Results for nonpregnant mice are displayed for Wolf et al. (2010).

3.2.2. Developmental Effects

This section describes the evidence informative to assessing the potential for developmental toxicity attributable to PFNA exposure during preconception, prenatally or postnatally up to the time of sexual maturation, and effects that may manifest throughout life. As such, this section has some overlap with evidence summaries for other health systems where studies evaluated effects of developmental exposure (see Sections 3.2.3, 3.2.4, 3.2.5, 3.2.6, 3.2.8, and 3.2.10 on hepatotoxicity, male reproductive toxicity, female reproductive toxicity, immunotoxicity, neurotoxicity, and urinary system toxicity, respectively).

Human Studies

Epidemiological studies of PFNA evaluated the following developmental outcomes: fetal and childhood growth restriction, gestational duration (i.e., preterm birth and gestational age), birth defects, fetal loss, and anogenital distance. The fetal growth restriction endpoints include studies evaluating mean and standardized measures of birth weight, birth length, and head circumference, as well as binary measures of low birth weight and small for gestational age. Primary outcomes across studies overlap somewhat, for example, some of the longitudinal studies considered both neonatal and childhood growth. Given the voluminous available data, a meta-analysis of mean birth weight and PFNA exposure was also conducted by EPA and published by (Wright et al., 2023) (see Appendix C.1.5 for meta-analyses results and methodology). The results of this meta-analysis were used to inform the evidence synthesis and integration decisions in this section and were considered for use in dose-response analysis (see Section 5.2, "Modeling results in humans (decreased birth weight) for further discussion of toxicity value derivations).

Study evaluation considerations

As detailed in the PFAS Systematic Review Protocol (see Appendix A), multiple outcome-specific considerations for study evaluation were influential on the domain ratings and the overall study confidence. For the confounding domain, fetal growth studies were downgraded when key confounders such as parity were not considered. Some hemodynamic factors related to physiological changes during pregnancy were also considered as potential confounders (e.g., GFR and blood volume changes over the course of pregnancy), because these factors may be related to both PFNA exposure levels and the developmental endpoints examined here. In general, less uncertainty exists for studies that sampled biomarkers earlier in pregnancy. Thus, more confidence was placed in the epidemiologic studies that adjusted for biomarker sample timing, GFR, or related measures in their regression models, or if they limited this potential source of confounding by sampling PFNA levels earlier in pregnancy. An additional source of uncertainty was the potential for confounding by other PFAS (and other co-occurring contaminants). Although scientific consensus on how best to address PFAS co-exposures remains elusive, this was considered in both the study evaluations and as part of the overall weight-of-evidence determination (see Appendix D for additional discussion of these issues).

For the exposure domain, most of the available studies analyzed PFNA in maternal or cord serum plasma or whole blood using standard methods. One study examined placental PFNA and infant heel stick measures. Given the long half-life of PFNA (see Section 3.1, Table 3-3) and lack of clarity on an MOA (see "Mechanistic and Supplemental Information," below), samples collected during any of the three trimesters (and at or shortly after birth) were considered representative of the critical in utero exposure window(s) for fetal growth and gestational duration measures. Many of the cross-sectional studies relied on umbilical cord measures collected shortly after birth. Exposure measures collected close to when the outcomes are quantified were considered

etiologically relevant and acceptable for these developmental endpoints; thus, exposure measurement ratings were not downgraded for timing of measurement. Measures of postnatal growth were included on the basis of an assumed fetal programming mechanism (i.e., Barker hypothesis) where in utero exposures or other perturbations, such as poor nutrition, can lead to developmental effects such as fetal growth restriction and, eventually, adult-onset disorders (Perng et al., 2016a; De Boo and Harding, 2006). For example, for non-PFAS chemicals some 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., 2016a) and may also be related to metabolic disorders. Therefore, a primary critical exposure window for measuring changes in postnatal (and early childhood) weight and height was assumed to be in utero. Postnatal studies were considered good in the exposure domain on the basis of in utero measures, while others were downgraded to adequate if samples were only collected during childhood or concurrently with outcome assessment (i.e., cross-sectional analyses). While some uncertainty and potentially reduced sensitivity is associated with these postnatal exposure measures, the long PFNA half-life suggests that these measures would capture some exposure experiences during earlier windows. For example, biomarker samples collected at a common age of examination are expected to adequately preserve relative ranking across broad exposure categories during pregnancy.

Studies were downgraded for study sensitivity if they had limited exposure contrasts and/or small sample sizes, since this can impact the ability of studies to detect associations that may be present. Although studies were not penalized for examination of differences across groups, smaller sample sizes across stratum-specific results can preclude detection of statistically significant effect estimates across groups (e.g., sex-specific results) if they lack adequate statistical power.

In the outcome domain, considerations address validation and accuracy of specific endpoints and for binary outcomes. For example, birth weight measures have been shown to be accurate and precise, while other fetal and early childhood anthropometric measures may result in more uncertainty due to some anticipated measurement error. 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, most notably if ascertained after the first trimester. Often, this type of error can result in bias toward the null if ascertainment of fetal loss is not associated with PFNA exposure (i.e., nondifferential). In some situations, differential loss is possible, which could result in a bias away from the null and even manifest as an apparent protective effect. Other binary endpoints such as birth defects were examined. Given anticipated etiologic heterogeneity across birth defects, broad groupings of all defects were downgraded due to the loss of specificity.

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). In both humans and animals, it is associated with other male reproductive tract abnormalities, including hypospadias and cryptorchidism (Liu et al., 2014; Sathyanarayana et al., 2010; Salazar-Martinez et al., 2004). The primary outcome-specific criteria in epidemiological studies 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.

Fetal and childhood growth restriction were examined using 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, mean weight, or related standardized measures evaluated either at birth or later during childhood. One childhood growth study examined rapid weight gain defined as an increase in weight z-score >0.67 standard deviations anytime between 4 weeks and 2 years of age (Shoaff et al., 2018; Perng et al., 2016a; De Boo and Harding, 2006). Given that sufficient *high* and *medium* confidence evidence is available for a set of related endpoints, the developmental effects synthesis is largely focused on these studies (i.e., classified as good in the outcome domain). Overall, birth weight and birth weight-derived measures are considered the most accurate and reliable of the endpoints considered here. These measures were also obtained predominately from medical records; therefore, more confidence was placed on these developmental endpoints. Some potentially adverse endpoints of interest examined include fetal growth restriction endpoints based on birth weight, such as mean birth weight or its variations (e.g., standardized birth weight z-scores), as well as categorical measures such as SGA (e.g., lowest decile of birth weight stratified by gestational age and other covariates) and LBW (i.e., typically <2,500 grams; 5 pounds, 8 ounces) in neonates. Sufficient details on SGA percentile definitions and stratification factors, as well as relevant sources of standardization for z-scores, were necessary for these endpoints to achieve a good rating. LBW is a less preferred measure of fetal growth restriction than SGA, especially if analyses include both term and preterm neonates. This preference is because birth weight is dependent on both the rate of fetal growth and gestational duration, and perturbation in each endpoint may arise from different etiologies.

Gestational duration measures were examined in epidemiological studies as either continuous (i.e., per gestational week) or binary 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 preterm birth. 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 exists for studies that compare and adjust for differences between last menstrual period and ultrasound measurements. In the absence of any direct evidence that suggests otherwise, any sources of error noted in the classification of these endpoints are anticipated to be

nondifferential with respect to PFNA exposure. This, therefore, would not be considered a major concern for risk of bias, but could impact precision and study sensitivity. As noted above, 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 IRIS PFAS Systematic Review Protocol (see Appendix A).

Study inclusion

Sixty-one epidemiological publications (across 59 different studies) examining PFNA exposure in relation to developmental endpoints were identified in the literature search. Several studies examined multiple endpoints that are captured in separate subsections below: 12 studies on postnatal growth, 18 studies on gestational duration, 5 studies on fetal loss, 3 studies on anogenital distance, 2 studies on birth defects, and 41 studies (across 42 publications) that examined fetal growth restriction. Among the fetal growth restriction studies, 8 studies examined either binary SGA and LBW endpoints, 20 studies examined birth length, and 17 studies examined head circumference.

Data processing

The study results shown below in various forest plots sorted by confidence and sample timing are detailed according to whether they examined sex-specific results and/or the overall population. Related endpoints are also examined together when common units of observation are available. The forest plots that summarize the data here can be used to evaluate patterns and trends in results across studies and to compare the magnitude of the individual associations between studies and across different common subgroups. These analyses considered differences in results across various study population and design features such as exposure contrasts and measures of centrality, use of different exposure measures and reference groups, type and timing of biomarker sampling, type of study, and study confidence.

For graphical depiction of results alone reported in some studies, EPA digitized the data to obtain a quantitative estimate of the effect estimate and confidence levels. In addition, study results were rescaled and re-expressed, when possible, to common endpoint measures (e.g., grams versus kilograms) and exposure measures (e.g., converting effect estimates based on $\log_2$ and $\log_{10}$ to a per ln-unit PFNA increase) to allow for similar scaling and a more direct comparison across studies in forest plots. For example, results beta coefficients (βs) and confidence intervals (CIs from several studies (Kashino et al., 2020; Xu et al., 2019a; Meng et al., 2018b; Shi et al., 2017; Valvi et al., 2017) were rescaled from either gram mean birth weight (BWT) or centimeter (mean birth length) changes per $\log_{10}$ and $\log_2$ transformed unit (ng/mL) to ln-unit changes. Since nearly all the published studies are based on the log scale of PFNA exposure, EPA re-expressed study results

reported in nature scale to per ln-unit changes of PFNA exposures (as detailed in Appendix C.1.7). This enabled pooling of data for meta-analytical evaluations and allowed a direct comparison of results on the same scale to evaluate between-study consistency and heterogeneity of results. For evaluation of patterns, study results were examined by overall confidence levels as well as study sensitivity and other study design and domain considerations (e.g., exposure sample timing). In addition, cross-sectional analyses are grouped with cross-sectional studies that used maternal serum/plasma, umbilical cord, or placental postpartum PFNA measures in relation to gestational duration even if the data were derived from prospective cohort or nested case-control studies (e.g., (Hall et al., 2022; Yang et al., 2022a; Gyllenhammar et al., 2018b)).

Fetal growth restriction – neonatal anthropometric measures[10]

Birth weight – Background of studies

Forty-one studies were identified that examined PFNA exposures in relation to different birth weight measures. Three publications where similar results were already reported from the same cohort are not considered independently in evidence synthesis (i.e., referred to as overlapping publications here given data are from the same study cohort). For example, the Woods et al. (2017) study used the same study population (Health Outcomes and Measures of the Environment cohort) as Shoaff et al. (2018). Similarly, the Rokoff et al. (2018) study overlaps with the Project Viva study by Sagiv et al. (2018). For consistency, birth outcome measures reported in Manzano-Salgado et al. (2017a) were preferred to in utero growth estimates in the Costa et al. (2019) study from the same Environment and Childhood – Infancia y Medio Ambiente (INMA) birth cohort. The smaller population subset from the Bjerregaard-Olesen et al. (2019) study is from the same Aarhus Birth Cohort as Bach et al. (2016). However, the Bjerregaard-Olesen et al. (2019) study provides additional sex-specific data not examined in the Bach et al. (2016) study for some endpoints. Any differences in results for these endpoints are highlighted in the syntheses below where applicable, but only one study is plotted for each endpoint to aid the evaluation of consistency across studies. When results for both continuous and categorical PFNA are available in one study, these results are plotted together for continuity. Findings on birth weight, length, and head circumference were consistent in the overall population and across sexes when reported in multiple publications, so focus was limited to the primary publication. The only exception was for birth weight results among girls in the Bjerregaard-Olesen et al. (2019) subset where both sets of results are provided in the text.

[10]New epidemiological studies on this outcome continue to be published. The most recent literature update, completed in April 2023, found 12 new studies examining the association between PFNA and developmental effects since April 2022, including 6 studies with new data on the outcome of primary interest, fetal growth restriction. As described in Appendix B.2, none of these newer studies were interpreted to have a material impact on the synthesis judgments or dose-response decisions. These studies are documented in Appendix B.2 but not yet incorporated into the text and figures of the evidence synthesis.

Among the 38 studies available for study evaluation, 3 (Maekawa et al., 2017; Lee et al., 2016; Monroy et al., 2008) were considered *uninformative* largely due to deficiencies in participant selection, confounding, and study sensitivity (see Figure 3-4). Twenty-seven of the remaining 35 informative birth weight studies were prospective birth cohorts, and 8 were cross-sectional studies (Hall et al., 2022; Xu et al., 2019a; Gyllenhammar et al., 2018b; Li et al., 2017; Shi et al., 2017; Callan et al., 2016; Kwon et al., 2016; Chen et al., 2012) (see Figures 3-5 to 3-10). Six of the 35 PFNA 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; Chen et al., 2012). The study by Hall et al. (2022) based its exposure characterization on PFNA placental measures sampled at birth, while Gross et al. (2020) was based on infant heal stick samples. Twenty-seven studies had maternal blood measures that were sampled preconception (Robledo et al., 2015) or during the first trimester (Buck Louis et al., 2018; Lind et al., 2017a; Bach et al., 2016) or third trimester (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 (Chang et al., 2022; Chen et al., 2021; Eick et al., 2020; Hjermitslev et al., 2020; Wikström et al., 2020; Marks et al., 2019; Workman et al., 2019; Meng et al., 2018b; Sagiv et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Lenters et al., 2016), or after delivery (Gyllenhammar et al., 2018b). The study by Meng et al. (2018a) used exposure data from two study populations in the Danish National Birth Cohort, one using PFNA measures from the baby's umbilical cord and one using measures from maternal blood samples collected either in the first or second trimester (see Figure 3-6). For comparability with other studies of mean birth weight, EPA examined data only from one measure, such as umbilical cord or maternal serum concentrations, and when necessary, relied on other related publications (e.g., Gyllenhammar I (2017)) or additional information or data provided by study authors. As noted above, EPA converted effect estimates that were based on continuous PFNA measures to a 1 ln-unit increase to enhance comparability across studies. For example, the results employing a common unit of measurement were also used for the birth weight meta-analysis conducted by EPA (see Wright et al. (2023) and Appendix C for details on the methods employed).

Of the 35 informative studies examining different birth weight measures, 15 were considered *high* confidence overall (Gardener et al., 2021; Luo et al., 2021; Yao et al., 2021; Eick et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Buck Louis et al., 2018; Sagiv et al., 2018; Shoaff et al., 2018; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Bach et al., 2016; Wang et al., 2016), 11 were *medium* confidence (Chang et al., 2022; Hall et al., 2022; Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018b; Meng et al., 2018b; Kwon et al., 2016; Lenters et al., 2016; Robledo et al., 2015; Chen et al., 2012), and 9 were *low* confidence largely due to deficiencies related to participant selection and confounding (Gross et al., 2020; Gao et al., 2019; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Li et al., 2017; Shi et al., 2017; Callan et al., 2016). Eight of the nine *low* confidence studies were deficient in the study sensitivity domain. The *medium* confidence study by

Robledo et al. (2015) and the *high* confidence study by Wang et al. (2016) had good sensitivity, while the remaining 24 studies were considered adequate. As noted above, the evidence syntheses for mean BWT differences detailed below primarily emphasize the results from the 26 *high* and *medium* confidence studies.

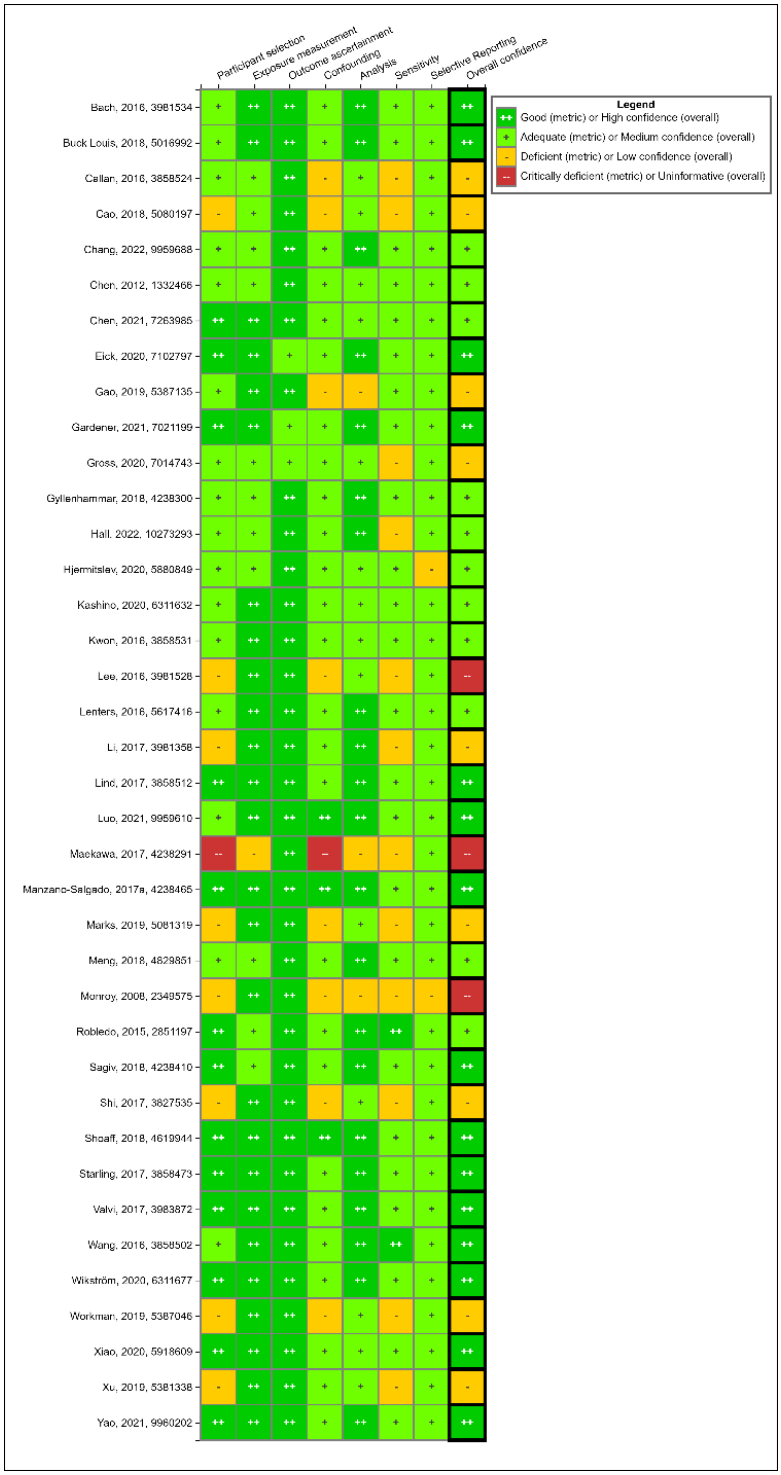


Figure 3-4. Study evaluation heat map of 38 epidemiologic studies of birth weight and PFNA exposure. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-Birth-Weight/.

Birth weight – Mean and standardized studies – Background

Twelve of the 35 informative studies (Gardener et al., 2021; Eick et al., 2020; Gross et al., 2020; Wikström et al., 2020; Workman et al., 2019; Xiao et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018b; Sagiv et al., 2018; Shoaff et al., 2018; Bach et al., 2016; Wang et al., 2016) reported standardized measures along with mean birth weight (in grams) differences in relation to PFNA. Three (Gardener et al., 2021; Gross et al., 2020; Xiao et al., 2019) other studies reported only standardized birth weight measures, with Gardener et al. (2021) not plotted with the others in Figure 3-9 given an atypical, dichotomized effect estimate with different scaling. Of the 32 epidemiological studies with mean birth weight data, in relation to PFNA exposures, four (Hall et al., 2022; Lind et al., 2017a; Wang et al., 2016; Robledo et al., 2015) only reported sex-specific findings in both sexes (i.e., no results in the overall population). Fifteen different studies reported sex-specific results in both sexes, while a 16th study reported data among boys only (Marks et al., 2019).

Mean birth weight – Overall population results

Twenty-seven studies (11 *high,* 9 *medium,* and 7 *low* confidence) examined mean BWT in the overall population (i.e., both girls and boys analyzed together) (see Figures 3-5 and 3-6) with 19 studies reporting some inverse associations in relation to PFNA. Most of the inverse associations were detected in the *medium* and *high* confidence studies including 8 of 9 *medium* and 8 of 11 *high* confidence studies. The *low* confidence studies were not as consistent with three of the seven studies showing some inverse associations (two studies each were null and showed larger mean BWT with increasing PFNA exposures). Half the associations among the *medium* confidence studies and five of eight among the *high* confidence subset reported statistically significant inverse associations based on either continuous or categorical (i.e., the primary focus here is on the upper quantiles) (see Figure 3-6).

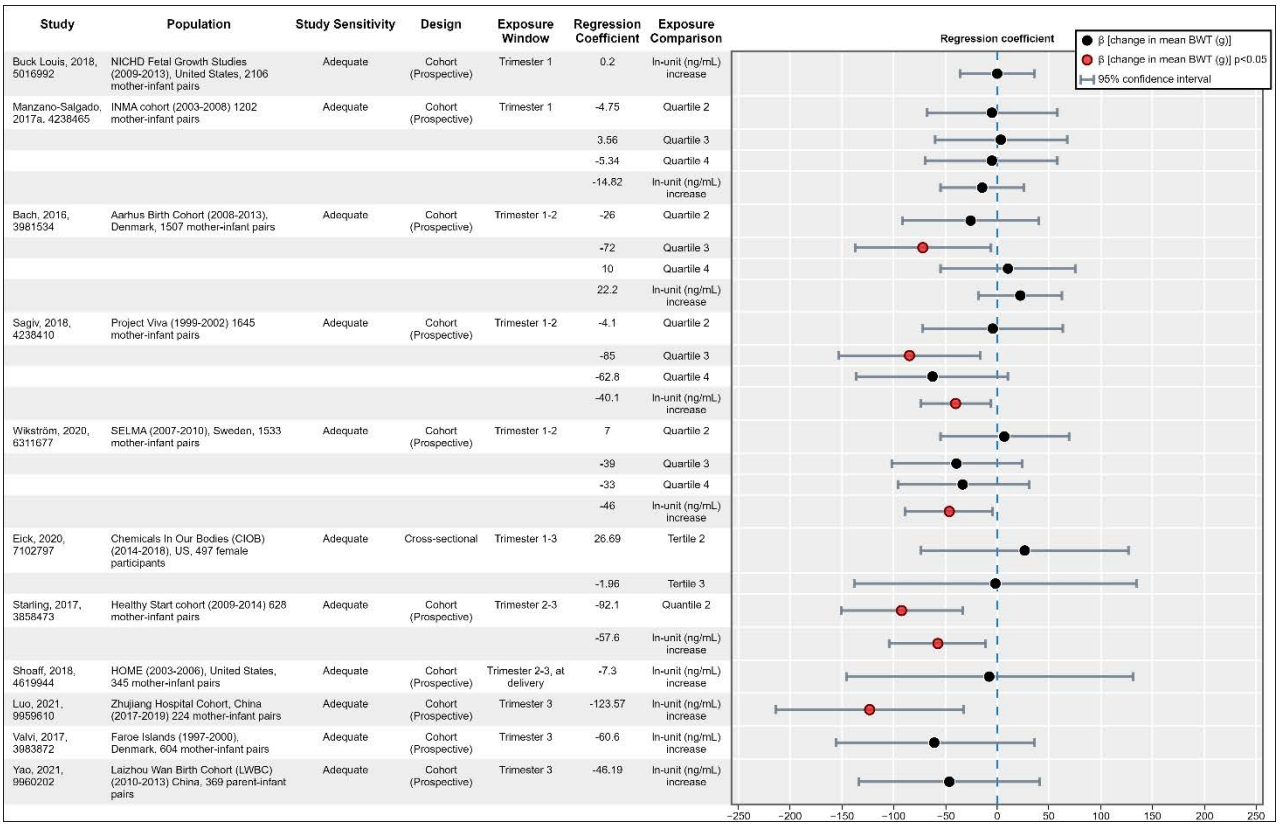
As noted above, 8 of 11 *high* confidence studies showed inverse associations, and 3 were null (Eick et al., 2020; Buck Louis et al., 2018; Shoaff et al., 2018). The Buck Louis et al. (2018) study reported null results for mean birth weight in the overall population, but their race-stratified results showed imprecise birth weight deficits for white (β = −38 g; 95% CI: −183, 107) and Black neonates (β = −84 g; 95% CI: −217, 43 per standard deviation increase in PFNA). Another study (Bach et al., 2016) with mixed results showed some suggestion of nonsignificant increase in mean BWT with increased PFNA based on a continuous exposure measure (β = 22.2 g; 95% CI: −18.2, 62.6 per ln-unit increase) but did see a statistically significant reduction in their quartile 3 (β = −72 g; 95% CI: −137, −6) comparison with quartile 1. Considerable variability in effect size was noted in two of the *high* confidence studies with the largest association (β = −123.6 g; 95% CI: −214.4, −32.7 per ln-unit PFNA increase) detected in the *high* confidence study by Luo et al. (2021). The smallest association (β = −14.8 g; 95% CI: −55.0, 25.4 per ln-unit increase) was seen in Manzano-Salgado et al. (2017a); they showed null results for the categorical data. The five

remaining *high* confidence studies showing inverse associations (Yao et al., 2021; Wikström et al., 2020; Sagiv et al., 2018; Starling et al., 2017; Valvi et al., 2017) showed results consistent in magnitude based on a per ln-unit increase (β range: −40 to −61 g). There was no evidence of any impact of sample timing among the eight *high* confidence studies, as four of them were based on early biomarker sampling.

Eight of nine *medium* confidence studies showed inverse associations and one was null (Chen et al., 2012). There was consistency in the magnitude across these eight studies with the exception of Gyllenhammar et al. (2018b) and Kwon et al. (2016) studies that reported larger statistically significant mean BWT decreases (βs= −108 g; 95% CI: −188, −28; β = −77 g; 95% CI: −135, −19 per ln-unit changes, respectively). Similar to deficits among the *high* confidence studies, the BWT deficits ranged from −30 to −59 g per ln-unit PFNA increase in the other six *medium* confidence studies. There was no evidence of any impact of biomarker sample timing among the eight *medium* confidence studies, as four of them were based on early sampling.

Overall, only two of 10 studies with categorical data showed exposure-response relationships that would support the findings based on continuous PFNA exposures. For example, both of the *medium* studies that reported inverse associations based on reported categorical data showed exposure-response relationships. The Meng et al. (2018b) study detected an exposure-response relationship with a large deficit (β = −81 g; 95% CI: −147, −15) in quartile 4 (>0.6 ng/mL) compared with the quartile 1 referent (<0.4 ng/mL). Similar results were seen for the Chang et al. (2022) study (range: −41 to −106 g). No evidence of dose-dependence was shown in the *low* or *high* confidence studies, although the *high* confidence Starling et al. (2017) study reported a statistically significant result for their dichotomized categorical analysis of PFNA levels exceeding the median exposure of 0.5 ng/mL (β = −92.1 g; 95% CI: −150.6, −33.6) compared with ≤0.4 ng/mL. Another *high* confidence study (Sagiv et al., 2018) reported large deficits in quartiles 3 (β = −85 g; 95% CI: −154, −17) and 4 (β = −63 g; 95% CI: −136, 10) compared with quartile 1 (referent: ≤0.4 ng/mL).

Overall, few patterns were evident across different comparisons of the mean BWT studies examining the overall population. For example, no evidence of any impact of sample timing was shown among the 16 *medium* and *high* confidence studies, as 8 of these were based on early biomarker sampling. The six null studies did not appear related to exposure contrasts or levels or to overall study sensitivity, as five of them had adequate sensitivity (Eick et al., 2020; Buck Louis et al., 2018; Meng et al., 2018b; Shoaff et al., 2018).



Study	Population	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Buck Louis, 2018, 5016992	NICHD Fetal Growth Studies (2009-2013), United States, 2106 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1	0.2	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1	-4.75	Quartile 2
					3.56	Quartile 3
					-5.34	Quartile 4
					-14.82	ln-unit (ng/mL) increase
Bach, 2016, 3981534	Aarhus Birth Cohort (2008-2013), Denmark, 1507 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-2	-26	Quartile 2
					-72	Quartile 3
					10	Quartile 4
					22.2	ln-unit (ng/mL) increase
Sagiv, 2018, 4238410	Project Viva (1999-2002) 1645 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-2	-4.1	Quartile 2
					-85	Quartile 3
					-62.8	Quartile 4
					-40.1	ln-unit (ng/mL) increase
Wikström, 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-2	7	Quartile 2
					-39	Quartile 3
					-33	Quartile 4
					-46	ln-unit (ng/mL) increase
Eick, 2020, 7102797	Chemicals In Our Bodies (CIOB) (2014-2018), US, 497 female participants	Adequate	Cross-sectional	Trimester 1-3	26.69	Tertile 2
					-1.96	Tertile 3
Starling, 2017, 3858473	Healthy Start cohort (2009-2014) 628 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 2-3	-92.1	Quantile 2
					-57.6	ln-unit (ng/mL) increase
Shoaff, 2018, 4619944	HOME (2003-2006), United States, 345 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 2-3, at delivery	-7.3	ln-unit (ng/mL) increase
Luo, 2021, 9959610	Zhujiang Hospital Cohort, China (2017-2019) 224 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 3	-123.57	ln-unit (ng/mL) increase
Valvi, 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 3	-60.6	ln-unit (ng/mL) increase
Yao, 2021, 9960202	Laizhou Wan Birth Cohort (LWBC) (2010-2013) China, 369 parent-infant pairs	Adequate	Cohort (Prospective)	Trimester 3	-46.19	ln-unit (ng/mL) increase

Figure 3-5. PFNA and birth weight (overall population) *high* confidence only.[a-d]
See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Birth-Weight-Overall-Population-High/.

BWT = birth weight; IQR = interquartile range.
[a]Studies are sorted first by overall study confidence level, then by Exposure Window(s) examined.
[b]Regression coefficients for continuous exposure expressed as change in birth weight(g) per increase in ln-transformed exposure concentration (ng/mL) in exposure concentration.
[c]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).

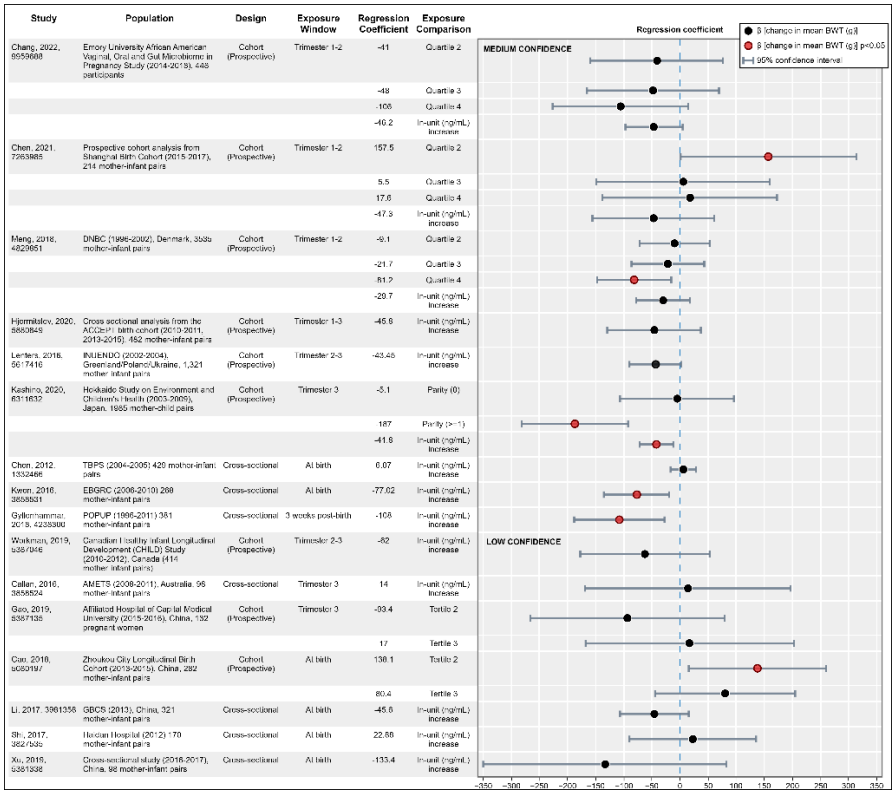


Figure 3-6. PFNA and birth weight (overall population) *medium* and *low* confidence only[a-d]. See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Birth-Weight-Overall-Population-Medi-4ef6/.

Abbreviations: BWT = birth weight.

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

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

[d]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).

Summary of meta-analysis of PFNA effects on birth weight

The EPA meta-analysis of the 27 studies estimated a statistically significant decrease in mean birth weight of 33 grams per ln-unit increase in PFNA (β = −32.9 g; 95% CI: −47.0, −18.7) (Wright et al., 2023); see Appendix C.1. This overall result was similar in magnitude when studies were restricted to just the *high* confidence studies (β = −28.0 g; 95% CI: −49.0, −6.9) or the *medium* and *high* confidence studies combined (β = −32.9 g; 95% CI: −48.0, −17.8). However, similar to previously published meta-analyses for PFOA and PFOS, differences in deficits were detected on the basis of sample timing. The stratum-specific results based on PFNA studies with earlier pregnancy sampling periods (e.g., first trimester only, or first and second trimester) showed smaller deficits (β = −22.0 g; 95% CI: −40.1, −4.0) compared with mid- and late-pregnancy periods (β = −48.4 g;

95% CI: −67.7, −29.0) as well as postpartum samples (β = −42.9 g; 95% CI: −88.0, 2.2). In contrast to the other meta-analytical findings for PFOS and PFOA reported in the literature, EPA's analyses indicated that, while some differences by timing exist, each sampling period showed birth weight deficits in excess of 22 grams per ln-unit increase in PFNA. Overall, these results were statistically robust across study confidence judgments, sample timing, and different scaling efforts based on natural-scale versus natural log-scale modeling.

Similar to the overall evidence detailed in the synthesis based on the categorical and continuous birth weight results presented above, the meta-analysis results examined here provide additional evidence of an overall inverse association on birth weight from maternal exposure to PFNA. The findings here were also robust to different stratification approaches suggesting that sample timing was not the sole determinant of the associations. In conclusion, based on this meta-analysis, the epidemiological studies are supportive of adverse effects on birth weight despite some variation by sample timing noted above.

Mean birth weight – Sex-specific results

Fourteen of the 16 mean birth weight studies with sex-specific analyses reported reductions in at least one sex, with only two not showing any inverse associations in either sex. These studies include 11 of the 16 in boys, including 3 (Wikström et al., 2020; Lind et al., 2017a; Valvi et al., 2017) of 6 *high*, 5 (Hall et al., 2022; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018b; Meng et al., 2018b) of 6 *medium*, and 3 (Marks et al., 2019; Li et al., 2017; Shi et al., 2017) of 4 *low* confidence studies. Ten of the 15 studies in girls also reported inverse associations including 4 (Wikström et al., 2020; Valvi et al., 2017; Bach et al., 2016; Wang et al., 2016) of 6 *high*, 5 (Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018b; Meng et al., 2018b; Robledo et al., 2015) of 6 *medium,* and 1 (Li et al., 2017) of 3 *low* confidence studies. Five of these studies in total reported statistically significant associations (three *high* confidence studies in girls and two *medium* confidence studies in boys), despite more limited statistical power for stratified analyses (see Figures 3-6 and 3-7).

Five of the largest birth weight deficits in girls were found in three *high* and two *medium* confidence studies. These results based on continuous PFNA exposure expressions were clustered in magnitude with four studies (three *medium* and one *low* confidence) ranging from −27 to −43 g and four others (two *high* and two *medium* confidence) ranging from −52 to −66 g per ln-unit increase. The Wang et al. (2016) study reported the largest deficit per ln-unit increase (β = −80 g; 95% CI: −160, −0.01), as well as an exposure-response relationship with very large (>250 g) birth weight decrements across all three upper quartiles (β range: −150 to −260 g) compared with quartile one (≤0.84 ng/mL) in girls only. Among girls only, the *high* confidence study by Bach et al. (2016) reported larger BWT decrements for quartile 2 (β = −97 g; 95% CI: −193, −2) and 3 (β = −123 g; 95% CI: −218, −29) than seen for quartile 4 (β = −35 g; 95% CI: −133, 63). Per ln-unit increase, their results were null (β = −8.1 g; 95% CI: −76.7, 62.6) in girls, although a deficit was

observed (β = −56 g; 95% CI: −118, 5) in the smaller subset examined by Bjerregaard-Olesen et al. (2019) [data not plotted].

The *low* confidence study of boys only by Marks et al. (2019) was the only other sex-specific study to show evidence of an exposure-response relationship. Compared with PFNA quartile 1 (< 0.2 ng/mL), they detected statistically significant mean birth weight deficits of −96 (95% CI: −238, 47) and −133 (95% CI: −265, −1) grams reported for tertiles 2 (0.3–0.4 ng/mL) and 3 (0.4–1.6 ng/mL), respectively. The birth weight difference based on a rescaling of their continuous exposure result was −68.4 g (95% CI: −180.9, 44.1) per ln-unit increase. The only other study with just categorical sex-specific data (Hall et al., 2022) did not show an exposure-response but did report a large but somewhat imprecise birth weight deficit for tertile 3 among boys only (β = −187.1 g; 95% CI: −400.1, 25.8).

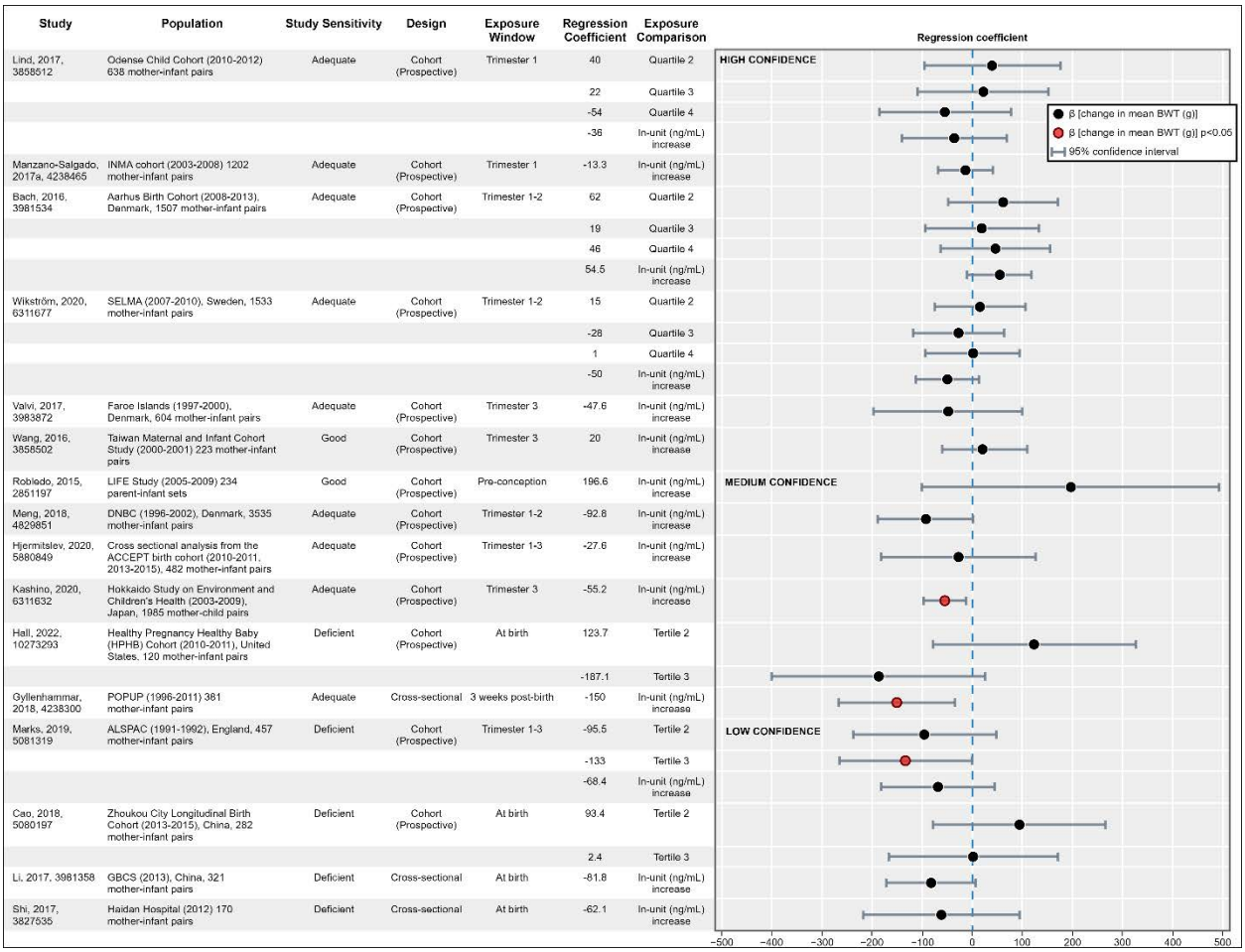
Nine of the 11 studies in boys that showed inverse associations were based on continuous PFNA exposure expressions; the magnitude of associations was quite variable. They ranged from a deficit of −28 g (95% CI: −182, 127) per each ng/mL increase in the *medium* confidence (Hjermitslev et al., 2020) study to −150 g (95% CI: −266, −34) per each ln-unit increase in the *medium* confidence (Gyllenhammar et al., 2018b) study. Few discernible patterns were observed across these studies, although the largest five deficits were seen in *medium* and *low* confidence studies.

Five studies found birth weight deficits in both sexes, including two *high* confidence studies by Valvi et al. (2017) and Wikström et al. (2020) that reported similar deficits among both girls and boys (all reported values in both studies were between −48 and −56 g per ln-unit increase in PFNA exposure). Two *high* confidence studies (Bach et al., 2016; Wang et al., 2016) and one *medium* confidence study (Robledo et al., 2015) reported associations among girls only. The latter study was notable for imprecision of their effect estimate. The *medium* confidence (Hall et al., 2022) study reported larger associations among girls compared with boys, while three *medium* confidence studies (Kashino et al., 2020; Gyllenhammar et al., 2018b; Meng et al., 2018b) and one *low* confidence study (Li et al., 2017) reported larger associations among boys. One *high* (Lind et al., 2017a), one *medium* (Hall et al., 2022), and two *low* confidence studies saw deficits only in boys (Marks et al., 2019; Shi et al., 2017).

Few patterns were evident across sexes in the mean birth weight studies. Although an equal number of studies in girls with inverse associations had early- and late-pregnancy sampling, the majority of studies among boys (6 of 11) were based on late biomarker sampling. This pattern was not evident among *medium* and *high* confidence studies only, as 4 of 8 studies in boys and 5 of 9 studies in girls had early sampling. Although statistical power may be somewhat limited in sex-specific analyses, there was no clear evidence that study sensitivity impacted the few null findings.

Overall, nearly all 16 sex-specific studies showed some evidence of inverse associations between PFNA and mean birth weight in either or both sexes, which included 8 of 12 *medium* or *high* confidence studies in boys and 9 of 12 in girls. Although deficits were more consistent among

girls, quite variable results were reported, including some very large birth weight deficits across different quantiles (up to −187 g in boys and −260 g in girls) and per ln-unit change (up to −150 g in boys and −80 g in girls). There was some evidence of sex-specific birth weight results in boys being more likely to occur in later biomarker sampled studies. This may indicate an impact of pregnancy hemodynamics; however, the number of early- and late-pregnancy studies among the *medium* and *high* confidence studies was comparable across both sexes. Although statistically significant results were detected in five of these studies, the lack of consistent patterns and often insufficient power preclude drawing any definitive sex-specific conclusion for this subset of data.



Study	Population	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Lind, 2017, 3858512	Odense Child Cohort (2010-2012) 638 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1	40	Quartile 2
					22	Quartile 3
					-54	Quartile 4
					-36	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1	-13.3	ln-unit (ng/mL) increase
Bach, 2016, 3981534	Aarhus Birth Cohort (2008-2013), Denmark, 1507 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-2	62	Quartile 2
					19	Quartile 3
					46	Quartile 4
					54.5	ln-unit (ng/mL) increase
Wikström, 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-2	15	Quartile 2
					-28	Quartile 3
					1	Quartile 4
					-50	ln-unit (ng/mL) increase
Valvi, 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 3	-47.6	ln-unit (ng/mL) increase
Wang, 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001) 223 mother-infant pairs	Good	Cohort (Prospective)	Trimester 3	20	ln-unit (ng/mL) increase
Robledo, 2015, 2851197	LIFE Study (2005-2009) 234 parent-infant sets	Good	Cohort (Prospective)	Pre-conception	196.6	ln-unit (ng/mL) increase
Meng, 2018, 4829851	DNBC (1996-2002), Denmark, 3535 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-2	-92.8	ln-unit (ng/mL) increase
Hjermitslev, 2020, 5880849	Cross sectional analysis from the ACCEPT birth cohort (2010-2011, 2013-2015), 482 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-3	-27.6	ln-unit (ng/mL) increase
Kashino, 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-child pairs	Adequate	Cohort (Prospective)	Trimester 3	-55.2	ln-unit (ng/mL) increase
Hall, 2022, 10273293	Healthy Pregnancy Healthy Baby (HPHB) Cohort (2010-2011), United States. 120 mother-infant pairs	Deficient	Cohort (Prospective)	At birth	123.7	Tertile 2
					-187.1	Tertile 3
Gyllenhammar, 2018, 4238300	POPUP (1996-2011) 381 mother-infant pairs	Adequate	Cross-sectional	3 weeks post-birth	-150	ln-unit (ng/mL) increase
Marks, 2019, 5081319	ALSPAC (1991-1992), England, 457 mother-infant pairs	Deficient	Cohort (Prospective)	Trimester 1-3	-95.5	Tertile 2
					-133	Tertile 3
					-68.4	ln-unit (ng/mL) increase
Cao, 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	Deficient	Cohort (Prospective)	At birth	93.4	Tertile 2
					2.4	Tertile 3
Li, 2017, 3981358	GBCS (2013), China, 321 mother-infant pairs	Deficient	Cross-sectional	At birth	-81.8	ln-unit (ng/mL) increase
Shi, 2017, 3827535	Haidan Hospital (2012) 170 mother-infant pairs	Deficient	Cross-sectional	At birth	-62.1	ln-unit (ng/mL) increase

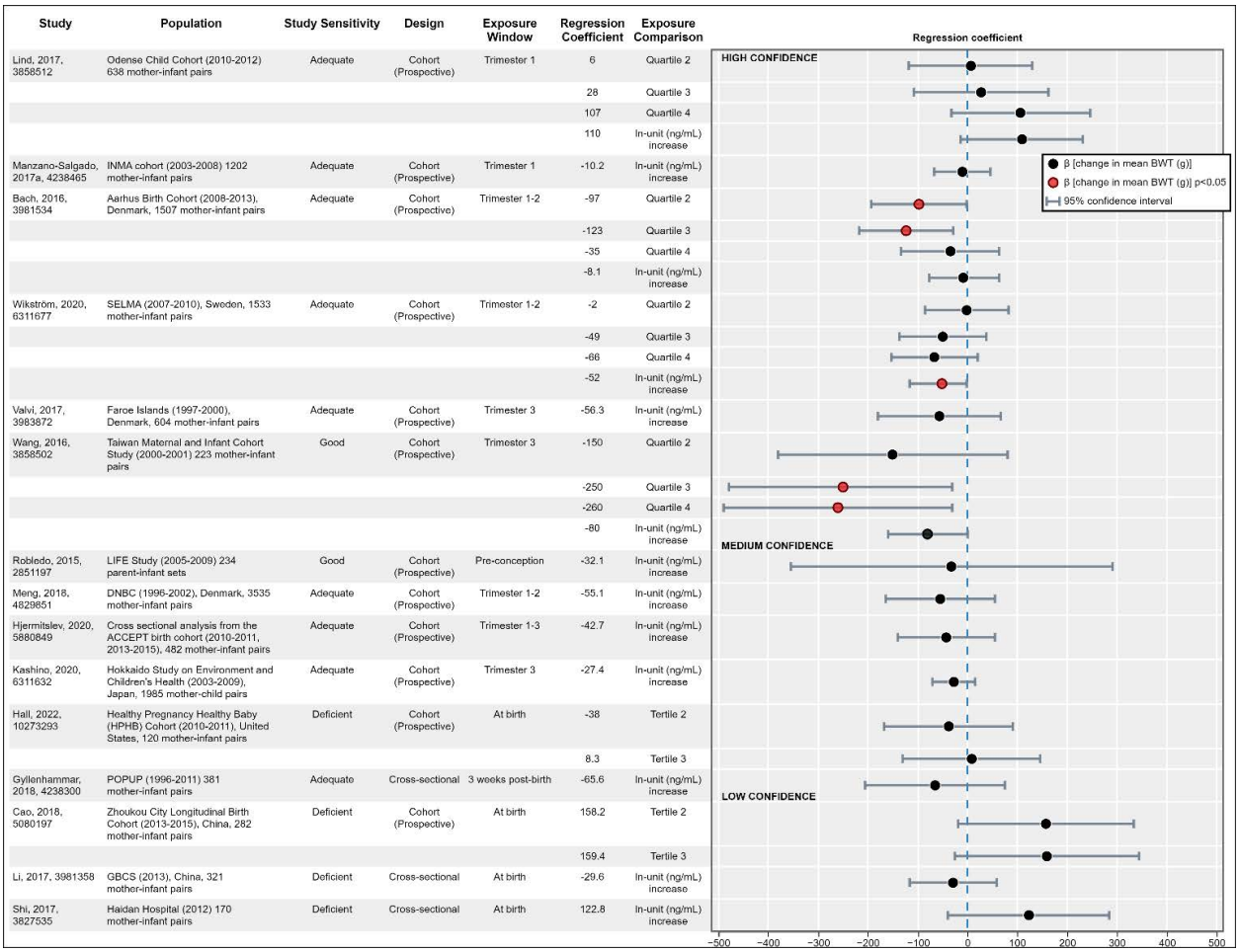
Figure 3-7. PFNA and birth weight (sex-stratified: boys only).[a–e] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Birth-Weight-Sex-Stratified-Boys-Only/. Sex-Stratified.

Abbreviations: BWT = birth weight.

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

[b]Meng et al. (2018b) 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]Gyllenhammar et al. (2018b) results are displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort (Swedish Environmental Protection Agency, 2017) compared with a smaller sample size of 381 in their 2018 publication.
[d]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).
[e]Hall et al. (2022) birth weight differences estimated from digitization of their Figure S4.



Study	Population	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Lind, 2017, 3858512	Odense Child Cohort (2010-2012) 638 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1	6	Quartile 2
					28	Quartile 3
					107	Quartile 4
					110	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1	-10.2	ln-unit (ng/mL) increase
Bach, 2016, 3981534	Aarhus Birth Cohort (2008-2013), Denmark, 1507 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-2	-97	Quartile 2
					-123	Quartile 3
					-35	Quartile 4
					-8.1	ln-unit (ng/mL) increase
Wikström, 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-2	-2	Quartile 2
					-49	Quartile 3
					-66	Quartile 4
					-52	ln-unit (ng/mL) increase
Valvi, 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 3	-56.3	ln-unit (ng/mL) increase
Wang, 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001) 223 mother-infant pairs	Good	Cohort (Prospective)	Trimester 3	-150	Quartile 2
					-250	Quartile 3
					-260	Quartile 4
					-80	ln-unit (ng/mL) increase
Robledo, 2015, 2851197	LIFE Study (2005-2009) 234 parent-infant sets	Good	Cohort (Prospective)	Pre-conception	-32.1	ln-unit (ng/mL) increase
Meng, 2018, 4829851	DNBC (1996-2002), Denmark, 3535 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-2	-55.1	ln-unit (ng/mL) increase
Hjermitslev, 2020, 5880849	Cross sectional analysis from the ACCEPT birth cohort (2010-2011, 2013-2015), 482 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 1-3	-42.7	ln-unit (ng/mL) increase
Kashino, 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-child pairs	Adequate	Cohort (Prospective)	Trimester 3	-27.4	ln-unit (ng/mL) increase
Hall, 2022, 10273293	Healthy Pregnancy Healthy Baby (HPHB) Cohort (2010-2011), United States, 120 mother-infant pairs	Deficient	Cohort (Prospective)	At birth	-38	Tertile 2
					8.3	Tertile 3
Gyllenhammar, 2018, 4238300	POPUP (1996-2011) 381 mother-infant pairs	Adequate	Cross-sectional	3 weeks post-birth	-65.6	ln-unit (ng/mL) increase
Cao, 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	Deficient	Cohort (Prospective)	At birth	158.2	Tertile 2
					159.4	Tertile 3
Li, 2017, 3981358	GBCS (2013), China, 321 mother-infant pairs	Deficient	Cross-sectional	At birth	-29.6	ln-unit (ng/mL) increase
Shi, 2017, 3827535	Haidan Hospital (2012) 170 mother-infant pairs	Deficient	Cross-sectional	At birth	122.8	ln-unit (ng/mL) increase

Figure 3-8. PFNA and birth weight (sex-stratified: girls only).[a–e] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Birth-Weight-Sex-Stratified-Girls-Only/.

Abbreviations: BWT = birth weight.
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Meng et al. (2018b) 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]Gyllenhammar et al. (2018b) results are displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort (Swedish Environmental Protection Agency, 2017) compared with a smaller sample size of 381 in their 2018 publication.
[d]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in the Study Design column).
[e]Hall et al. (2022) birth weight differences estimated from digitization of their Figure S4.

Mean birth weight – Summary

Among the 32 unique studies of mean birth weight for the overall population, or either/both sexes, most reported some inverse associations with roughly half showing statistically significant findings. The associations were at times not always consistent across different exposure metrics examined within the same study, and some variation in magnitude of results was noted across sexes. Nonetheless, 14 of 16 studies with sex-specific results showed some evidence of mean birth weight differences in either or both sexes.

Among the 27 studies that examined mean weight differences in relation to either continuous or categorical PFNA data in their overall population, 19 studies reported some inverse associations. Inverse associations were seen in 16 of 20 *high* and *medium* confidence studies that examined mean birth weight in the overall population. The evidence in the overall population was less consistent in the *low* confidence studies, and several studies were marked by imprecision despite some showing large birth weight deficits (e.g., Xu et al. (2019a)). Inverse associations were also noted for both sexes (10 of 15 in girls and 11 of 16 in boys) similar to the proportion seen in the overall population. There was no evidence of any impact of sample timing among the 16 *medium* and *high* confidence studies examining the overall population, as 8 of them were based on early biomarker sampling. There was also no evidence of sample timing impacts among the findings in girls (5 of 10 had early sampling), although most studies among boys (6 of 11) were based on late biomarker sampling. This finding was not evident among *medium* and *high* confidence studies only, as 4 of 8 studies in boys and 5 of 9 studies in girls were based on early sampling.

There was a wide range of mean birth weight differences in the overall population (β range: −15 to −133 g) showing inverse associations per ln-unit PFNA increase. Yet, notably, 12 of the 18 studies with inverse associations based on continuous PFNA data ranged from −40 to −62 g, and 9 of them ranged from −40 to −47 g. The differences among 9 studies (including 8 *medium* and *high*) studies in girls ranged from −27 to −80 g per ln-unit increase with larger results generally among the *high* confidence studies. Results were more variable among 10 studies (including 7 *medium* and *high*) in boys (β range: −28 to −150 g) especially in the *medium* confidence studies. Results based on 11 studies with categorical PFNA data were largely in agreement with those continuous exposure results expressed per ln-unit increase. However, only 4 different mean birth weight studies that reported inverse associations showed exposure-response relationships with increasing birth weight across exposure quantiles. This included 1 of 5 studies in girls, 1 of 6 in boys and 2 of 10 studies in the overall population.

Nearly half the studies showing inverse associations reported statistically significant findings suggesting that collectively random error is not a large concern here. As anticipated given the smaller sample sizes per stratum, statistical significance was seen in fewer studies reporting race- or sex-specific analyses, which may be due to limited sensitivity as only two of these studies were considered to have good overall study sensitivity (Wang et al., 2016; Robledo et al., 2015). Five of the six null studies examining the overall population had adequate study sensitivity and

seemed to have sufficient PFNA exposure contrasts. Few other patterns were evident across different subgroups for all mean birth weight studies with sex-specific analyses including among the few null studies. Although an equal number of studies in girls had early- and late-pregnancy sampling, the majority of studies among boys (6 of 11) were based on late biomarker sampling. This was not evident among *medium* and *high* confidence studies only, as 4 of 8 studies in boys and 5 of 9 studies in girls had early sampling.

Birth weight – Standardized – Background

Ten of 11 studies in the overall population reported continuous standardized birth weight scores in relation to different PFNA measures (see Figures 3-9 and 3-10), while the Gardener et al. (2021) study (not included on the forest plot) examined a binary measure of odds of being in the lowest standardized birth weight category (versus the top three birth weight z-score quartiles). Five of the 11 studies also reported sex-specific results (Eick et al., 2020; Gross et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Wang et al., 2016), while Gardener et al. (2021) only examined interactions across sex for associations between PFNA and standardized birth weight measures.

Among the 11 studies that examined PFNA exposure in relation to standardized birth weight scores in the overall population, seven were *high* (Gardener et al., 2021; Eick et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Sagiv et al., 2018; Shoaff et al., 2018; Bach et al., 2016), two each were *medium* (Gyllenhammar et al., 2018b; Meng et al., 2018b) and *low* (Gross et al., 2020; Workman et al., 2019) confidence. Five studies had good (Wikström et al., 2020; Gyllenhammar et al., 2018b; Meng et al., 2018b; Sagiv et al., 2018; Shoaff et al., 2018) study sensitivity ratings, while four were adequate (Gardener et al., 2021; Eick et al., 2020; Xiao et al., 2019; Bach et al., 2016) and two were deficient (Gross et al., 2020; Workman et al., 2019).

Standardized birth weight measures – Overall population results

Six of the 11 studies examining standardized birth weight scores showed some inverse associations in the overall population including 5 of 10 studies plotted in Figure 3-9. The *high* confidence Gardener et al. (2021) study reported null results for their lowest standardized birth weight category (versus the top 3 BWT z-score quartiles) for PFNA quartile 4 exposures, but slight nonsignificant increased odds among participants in quartiles 2 and 3 (OR range: 1.39 to 1.68) compared with quartile 1. They also found no statistically significant interactions for their birth weight z-score measures by sex. Null associations between PFNA exposure and standardized birth weight scores were reported in five studies (Eick et al., 2020; Workman et al., 2019; Meng et al., 2018b; Shoaff et al., 2018; Bach et al., 2016) (see Figure 3-9).

The *high* confidence Sagiv et al. (2018) study reported statistically significant deficits in birth weight-for-gestational age z-scores per ln-unit increase (β = −0.09; 95% CI: −0.16, −0.01), as well as larger non-monotonic decreases for the third (β = −0.19; 95% CI: −0.32, −0.06) and fourth (β = −0.15; 95% CI: −0.30, −0.01) quartiles. The *medium* confidence study by Gyllenhammar et al.

(2018b) also showed a decrease in birth weight standard deviation score for each ln-unit PFNA increase (β = −0.16; 95% CI: −0.34, 0.0) in the overall population. Statistically significant decreased birth weight standard deviation scores were reported in overall population (β = − 0.11; 95% CI: −0.21, −0.02 per ln-unit increase) in the *high* confidence study by Wikström et al. (2020). Results similar in magnitude were reported across both sexes per ln-unit PFNA increase, but categorical results were evident among quartiles 3 and 4 (β range; −0.11 to −0.15) in girls only compared with quartile 1. Larger associations were seen in the *high* confidence study by Xiao et al. (2019) (β = −0.49; 95% CI: −1.07, 0.09 per ln-unit) and the *low* confidence study by Gross et al. (2020) (β = −0.42; 95% CI: −0.77, −0.07 among those with PFNA levels greater than the mean level of dried-blood spot samples).

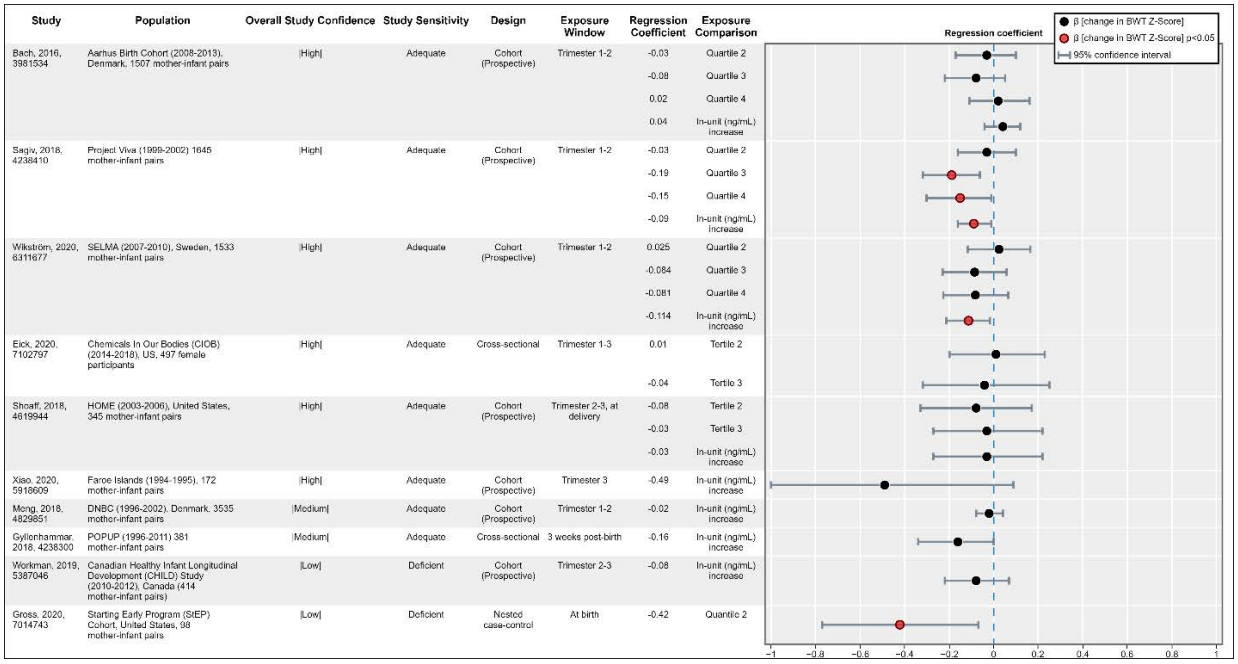


Figure 3-9. PFNA and birth weight z-score (overall population). See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Birth-Weight-Z-Score-Overall-Population/.

Abbreviations: BWT = birth weight.
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Xiao et al. (2019) results are truncated; the complete 95% CI ranges from −1.07 to 0.09 grams.
[c]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).
[d]Gyllenhammar et al. (2018b) standardized birth weight differences estimated from digitization of their Figure 1.

Standardized birth weight measures – Sex-specific results

Among five sex-specific studies, two reported inverse birth weight z-scores among boys, while three did so in girls. Large statistically significant lower birth weight z-scores results were reported in the *low* confidence study by Gross et al. (2020) among girls (β = −0.77; 95% CI: −1.24,

−0.30) and boys (β = −0.60; 95% CI: −1.13, −0.07) in those with PFNA levels greater than the mean level of dried-blood spot samples. For each PFNA ln-unit increase, the *high* confidence study by Xiao et al. (2019) reported larger associations in girls (β = −0.76; 95% CI: −1.49, −0.03) than in boys (β = −0.22; 95% CI: −0.95, 0.52). Statistically significant results similar in magnitude were seen for girls (β = −0.11; 95% CI: −0.26, −0.04) and boys (β = −0.11; 95% CI: −0.24, −0.01) per ln-unit increase in the *high* confidence study by Wikström et al. (2020). Their categorical results were similar in magnitude for quartiles 3 (β = −0.11; 95% CI: −0.31, 0.08) and 4 (β = −0.15; 95% CI: −0.34, 0.05) among girls only, as results were largely null among boys.

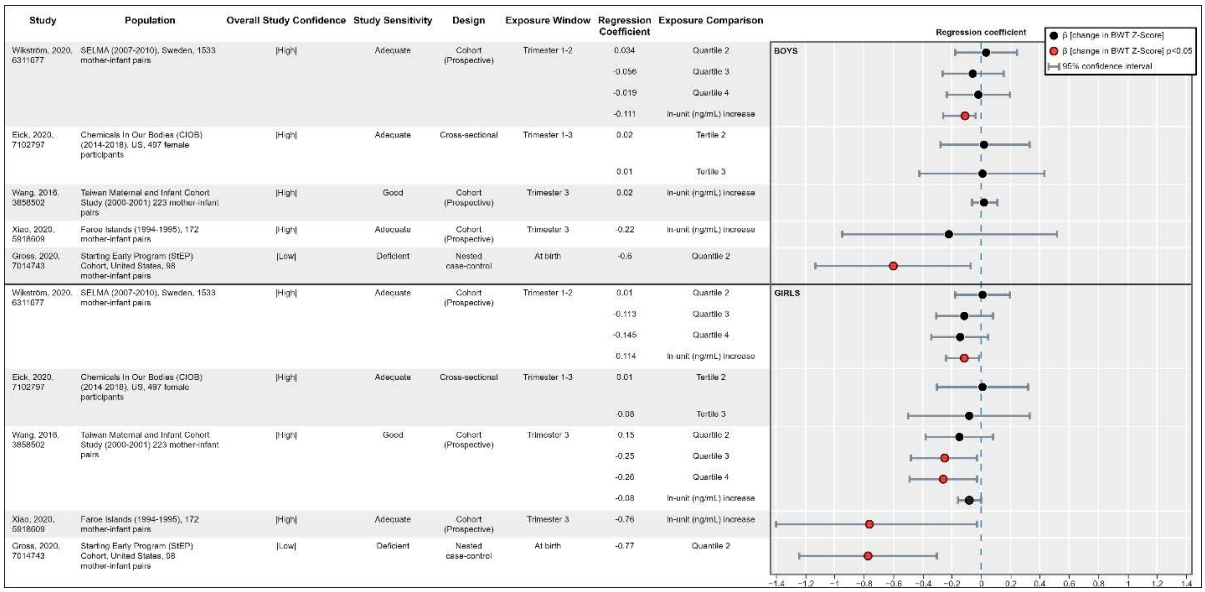


Figure 3-10. PFNA and birth weight z-score (sex-stratified: boys above reference line, girls below).[a–c] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Birth-Weight-Z-Score-Sex-Stratified--7b39/.

Abbreviations: BWT = birth weight.

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

[b]Xiao et al. (2019) results are truncated; the complete 95% CI ranges from −1.49 to −0.03.

[c]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).

Standardized birth weight results – Summary

Overall, 7 of 12 studies showed some evidence of inverse associations between PFNA and standardized birth weight measures in the overall population or in either sex. This included 6 of 11 in the overall population with one based on a binary endpoint. Although most of these studies were on the lower end of the distribution, the range on to birth weight score range per ln-unit PFNA exposure was from −0.08 to −0.76. None of the five studies with categorical data in the overall population showed evidence of inverse exposure-response relationships with standardized birth weight scores. One of three studies in girls also showed an exposure-response relationship. Overall,

three of five studies in boys and four of five in girls showed inverse associations between PFNA and standardized birth weight scores. Sex-specific results in both girls and boys from two studies were larger in magnitude than that observed for the overall population.

Small for gestational age and low birth weight – Background

Eight epidemiological studies included here examined associations between PFNA exposure and different binary fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints) (Chang et al., 2022; Wikström et al., 2020; Xu et al., 2019a; Manzano-Salgado et al., 2017a; Wang et al., 2016; Chen et al., 2012) or LBW (Hjermitslev et al., 2020; Meng et al., 2018b; Manzano-Salgado et al., 2017a; Chen et al., 2012) (Figure 3-11). Three studies were *high* confidence (Wikström et al., 2020; Manzano-Salgado et al., 2017a; Wang et al., 2016), three were *medium* confidence (Hjermitslev et al., 2020; Meng et al., 2018b; Chen et al., 2012), and two were *low* confidence (Chang et al., 2022; Xu et al., 2019a). One study had good study sensitivity (Wang et al., 2016), six had adequate study sensitivity (Chang et al., 2022; Hjermitslev et al., 2020; Wikström et al., 2020; Meng et al., 2018b; Manzano-Salgado et al., 2017a; Chen et al., 2012) while one was deficient (Xu et al., 2019a). Seven of the eight studies reported results in the overall population, two (Wikström et al., 2020; Manzano-Salgado et al., 2017a) provided results in both the overall population and across sexes, and Wang et al. (2016) just reported sex-specific findings.

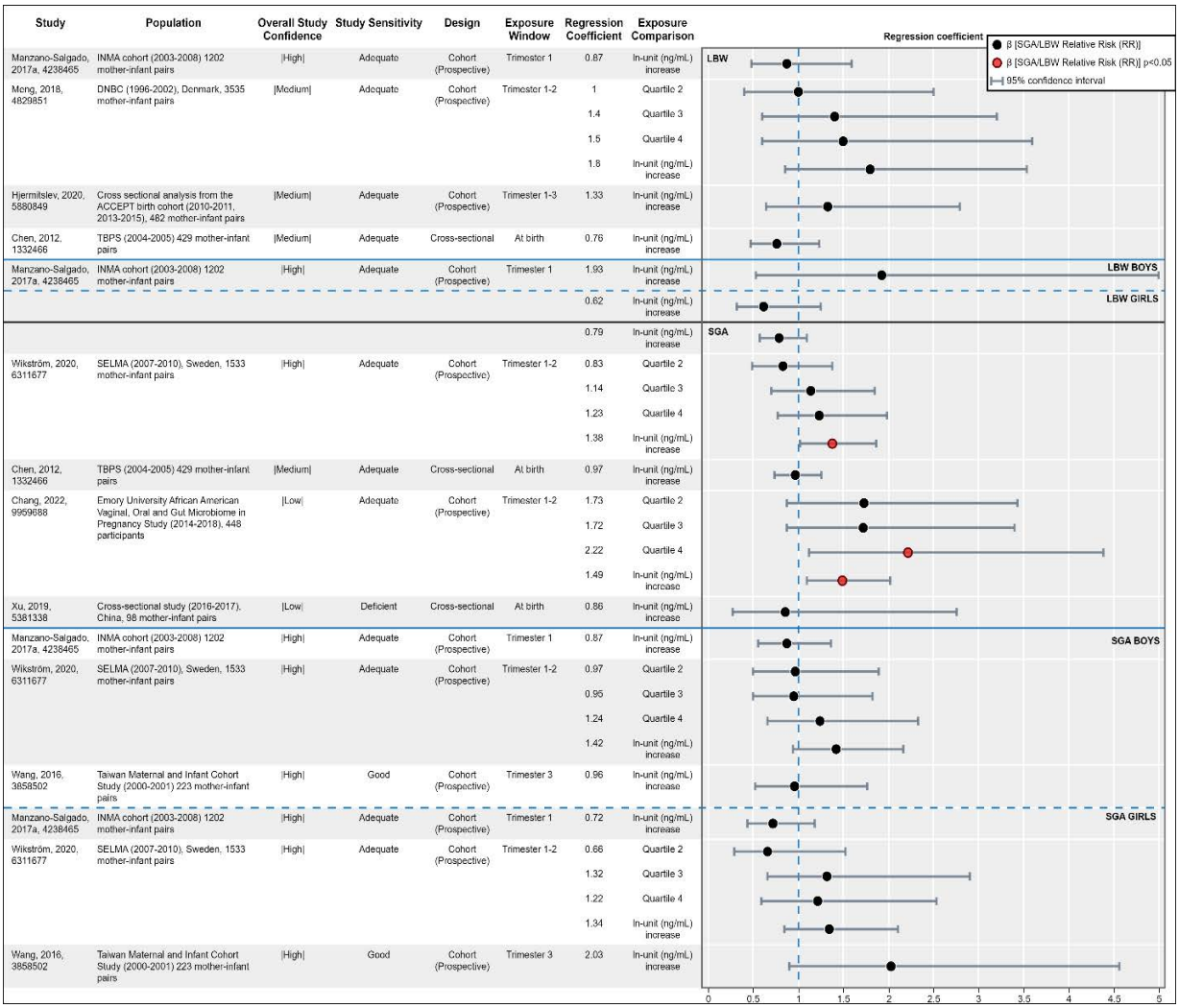
Small for gestational age and low birth weight – Results

Three (two *high* and one *low* confidence) different studies (Chang et al., 2022; Wikström et al., 2020; Wang et al., 2016) (out of six) showed some increased risks of SGA in relation to PFNA exposures. A statistically significant increased risk for SGA was reported per ln-unit (OR = 1.49; 95% CI: 1.10, 2.02) as well as for the upper quartile (OR = 2.22; 95% CI: 1.12, 4.38) in the *low* confidence study by Chang et al. (2022). Nonsignificant increased risks were also seen in the lower two quartiles (OR range: 1.72–1.73). The *medium* confidence study by Wikström et al. (2020) showed that PFNA was associated with SGA based on a continuous measure (OR =1.38; 95% CI: 1.02, 1.87 per ln-unit increase), as well as categorical exposures (Q4 OR = 1.23; 95% CI: 0.77, 1.99 compared with Q1 referent). Results were similar in magnitude for boys (OR = 1.42) and girls (OR = 1.34) per ln-unit increase and for quartile 4 results (OR range: 1.22–1.24). Although results were null for boys (OR = 0.96; 95% CI: 0.52, 1.76), the *high* confidence study by Wang et al. (2016) reported an increased OR (2.03; 95% CI: 0.90, 4.56) for SGA per ln-unit PFNA increase among girls. The *high* confidence study by Manzano-Salgado et al. (2017a) reported null results among boys (OR = 0.87; 95% CI: 0.48, 1.59), but slight nonsignificant decreased risk of SGA among girls (OR = 0.72; 95% CI: 0.44, 1.18) and the overall population (OR = 0.79; 95% CI: 0.57, 1.10). Results were also null in the Chen et al. (2012) and Xu et al. (2019a) studies.

Three of the four studies showed increased risk of LBW in relation to PFNA exposures in either the overall population and/or either sex. The *medium* confidence Meng et al. (2018b) study reported an OR of 1.80 (95% CI: 0.86, 3.54) for LBW and a very LBW (i.e., <2,260 grams) measure

(OR = 1.63; 95% CI: 0.72, 4.2) in the overall population per ln-unit increase as well as increased ORs of 1.4 (95% CI: 0.6, 3.2) and 1.5 (95% CI: 0.6, 3.6) in quartiles 3 and 4, respectively, compared with the quartile 1. A nonstatistically significant risk (OR = 1.33; 95% CI: 0.64, 2.79 per ln-unit increase) in the overall population was reported between LBW and PFNA in the *medium* confidence study by Hjermitslev et al. (2020). Although it was not statistically significant, there was an inverse association (OR = 0.76; 95% CI: 0.47, 1.23) seen in the *medium* confidence Chen et al. (2012) study. A similar finding in girls (OR = 0.62; 95% CI: 0.32, 1.25) was observed in the *high* confidence Manzano-Salgado et al. (2017a) study, while increased risks were not observed in girls or the overall population (OR = 0.87; 95% CI: 0.48, 1.59). They did report a nearly twofold increased risk among boys (OR = 1.93; 95% CI: 0.53, 7.21) per ln-unit increase.

Overall, six of eight different studies showed increased risk of either SGA, LBW, or very LBW in relation to PFNA exposures. The magnitude of changes per each ln-unit increase across studies was consistent (OR range: 1.3 to 2.0) irrespective of whether results were examined for the overall population or for the sexes. Although none of the three studies with categorical data showed exposure-response relationships, the highest reported ORs in each were found in the highest quartile. No distinctive patterns appeared to explain the study results including similar results across sexes. In addition, all three studies that showed increased risks for LBW were based on early biomarker sampling as were two of three SGA studies. Study sensitivity did not seem to explain the limited null study findings for SGA or LBW.



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0.87	ln-unit (ng/mL) increase
Meng, 2018, 4829851	DNBC (1996-2002), Denmark, 3535 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-2	1	Quartile 2
						1.4	Quartile 3
						1.5	Quartile 4
						1.8	ln-unit (ng/mL) increase
Hjermitslev, 2020, 5880849	Cross sectional analysis from the ACCEPT birth cohort (2010-2011, 2013-2015), 482 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-3	1.33	ln-unit (ng/mL) increase
Chen, 2012, 1332466	TBPS (2004-2005) 429 mother-infant pairs	|Medium|	Adequate	Cross-sectional	At birth	0.76	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	1.93	ln-unit (ng/mL) increase
						0.62	ln-unit (ng/mL) increase
						0.79	ln-unit (ng/mL) increase
Wikström, 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1-2	0.83	Quartile 2
						1.14	Quartile 3
						1.23	Quartile 4
						1.38	ln-unit (ng/mL) increase
Chen, 2012, 1332466	TBPS (2004-2005) 429 mother-infant pairs	|Medium|	Adequate	Cross-sectional	At birth	0.97	ln-unit (ng/mL) increase
Chang, 2022, 9959688	Emory University African American Vaginal, Oral and Gut Microbiome in Pregnancy Study (2014-2018), 448 participants	|Low|	Adequate	Cohort (Prospective)	Trimester 1-2	1.73	Quartile 2
						1.72	Quartile 3
						2.22	Quartile 4
						1.49	ln-unit (ng/mL) increase
Xu, 2019, 5381338	Cross-sectional study (2016-2017), China, 98 mother-infant pairs	|Low|	Deficient	Cross-sectional	At birth	0.86	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0.87	ln-unit (ng/mL) increase
Wikström, 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1-2	0.97	Quartile 2
						0.95	Quartile 3
						1.24	Quartile 4
						1.42	ln-unit (ng/mL) increase
Wang, 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001) 223 mother-infant pairs	|High|	Good	Cohort (Prospective)	Trimester 3	0.96	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0.72	ln-unit (ng/mL) increase
Wikström, 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1-2	0.66	Quartile 2
						1.32	Quartile 3
						1.22	Quartile 4
						1.34	ln-unit (ng/mL) increase
Wang, 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001) 223 mother-infant pairs	|High|	Good	Cohort (Prospective)	Trimester 3	2.03	ln-unit (ng/mL) increase

Figure 3-11. PFNA and dichotomous fetal growth restriction (small for gestational age and low birth weight).[a–d] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Dichotomous-Fetal-Growth-Restriction-443b/.

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 data above black reference line, small for gestational age data below.

[c]Sex-stratified boy data above blue dotted line; sex-stratified girl data below.

[d]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).

Small for gestational age and low birth weight – Summary

Overall, six of eight different studies showed some increased risks for either SGA, LBW, or very LBW including two that demonstrated statistical significance. The relative risks reported in the four different SGA and LBW studies based on the continuous exposures (per unit increase) were moderate but consistent in magnitude for the overall population (OR range: 1.3 to 1.8) or either sex (OR range: 1.3 to 2.0). None of the three studies with categorical data based on the overall population showed exposure-response relationships, but the highest reported ORs in each were found in the highest quartile. The reported risks of SGA in relation to PFNA were more consistent and larger among girls, while the lone sex-specific findings for LBW was in boys. Five of the six studies with increased risks were based on early biomarker sampling, so no impact of pregnancy hemodynamics is expected. No other patterns were evident across study characteristics including that study sensitivity did not seem to explain null study findings.

Birth length – Background of studies

Twenty studies examined the relationship between PFNA exposures and birth length in the overall population or across sexes (see Figure 3-12). Eleven studies reported sex-specific findings among boys and 10 did so for girls. Three of the 11 studies reporting sex-specific findings did not report overall population results (Marks et al., 2019; Wang et al., 2016; Robledo et al., 2015). Two studies (Xiao et al., 2019; Gyllenhammar et al., 2018b) reported standardized birth length measures, while the remaining studies examined mean birth length differences in relation to PFNA. As noted above, results from two publications (Bjerregaard-Olesen et al., 2019; Bach et al., 2016) from the Aarhus Birth Cohort were comparable for both birth length and head circumference in the overall population. Thus, for more continuity with the sex-specific findings, the Bjerregaard-Olesen et al. (2019) results are only included in the forest plot below.

Six of the 20 PFNA studies examining birth length studies were classified as *medium* confidence (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018b; Robledo et al., 2015; Chen et al., 2012), while 7 each were *high* (Luo et al., 2021; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Wang et al., 2016) and *low* confidence (Gao et al., 2019; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016) largely due to concerns with participant, selection, confounding, and study sensitivity. Two studies were rated good for study sensitivity (Wang et al., 2016; Robledo et al., 2015), 12 were adequate (Chen et al., 2021; Luo et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Gao et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Gyllenhammar et al., 2018b; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Chen et al., 2012) and 6 were rated as deficient (Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016).

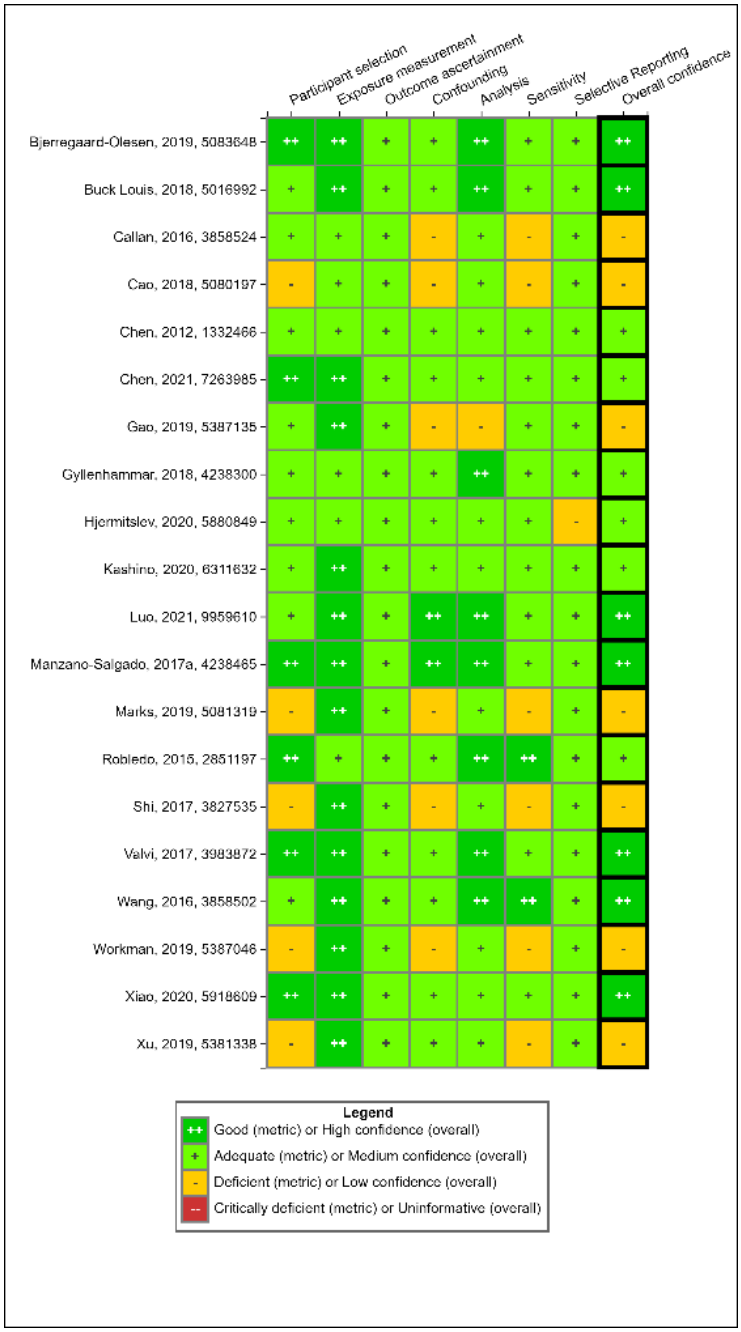


Figure 3-12. Study evaluation heat map of 20 epidemiologic studies of birth length and PFNA exposure. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-Birth-Length/.

Birth length – Overall population results

Eight of the 17 studies in the overall population were null (Hjermitslev et al., 2020; Bjerregaard-Olesen et al., 2019; Gao et al., 2019; Xu et al., 2019a; Manzano-Salgado et al., 2017a; Shi et al., 2017; Valvi et al., 2017; Callan et al., 2016) (see Figure 3-13). Two other studies (Cao et al., 2018; Chen et al., 2012) showed birth length increases in relation to PFNA exposures, while 7 of the 17 studies showed some evidence of inverse associations between PFNA and mean or standardized birth length differences. Three of the seven studies showed statistically significant inverse associations including both standardized birth length studies. For each ln-unit PFNA increase, the *high* confidence study by Xiao et al. (2019) reported decreased birth length z-scores in the overall population (β = −0.42; 95% CI: −0.95, 0.13), girls (β = −0.46; 95% CI: −1.14, −0.23), and boys (β = −0.36; 95% CI: −1.05, 0.33). Gyllenhammar et al. (2018b) reported a smaller association (β = −0.14; 95% CI: −0.34, −0.05 birth length standard deviation score) per ln-unit increase.

Six of the seven studies in the overall population that showed some evidence of inverse birth length associations with PFNA exposures were *medium* (Chen et al., 2021; Kashino et al., 2020; Gyllenhammar et al., 2018b) or *high* confidence (Luo et al., 2021; Xiao et al., 2019; Buck Louis et al., 2018). Similar statistically significant mean birth length deficits were observed in Buck Louis et al. (2018) (β = −0.24 cm; 95% CI: −0.45, −0.05 per ln-unit increase) and Kashino et al. (2020) (β = −0.21 cm; 95% CI: −0.37, −0.05 per ln-unit increase). The *high* confidence study by Luo et al. (2021) showed a larger mean birth length change (β = −0.37 cm; 95% CI: −0.83, 0.09 per ln-unit increase). The *medium* confidence study by Chen et al. (2021) reported mean birth length deficits similar in magnitude in quartile 4 (β = −0.28 cm; 95% CI: −0.73, 0.17) and per ln-unit increase (β = −0.34 cm; 95% CI: −0.65, −0.03). A larger association with PFNA exposure was noted in the *low* confidence Workman et al. (2019) study (β = −0.65 cm; 95% CI: −1.45, 0.15 per ln-unit increase).

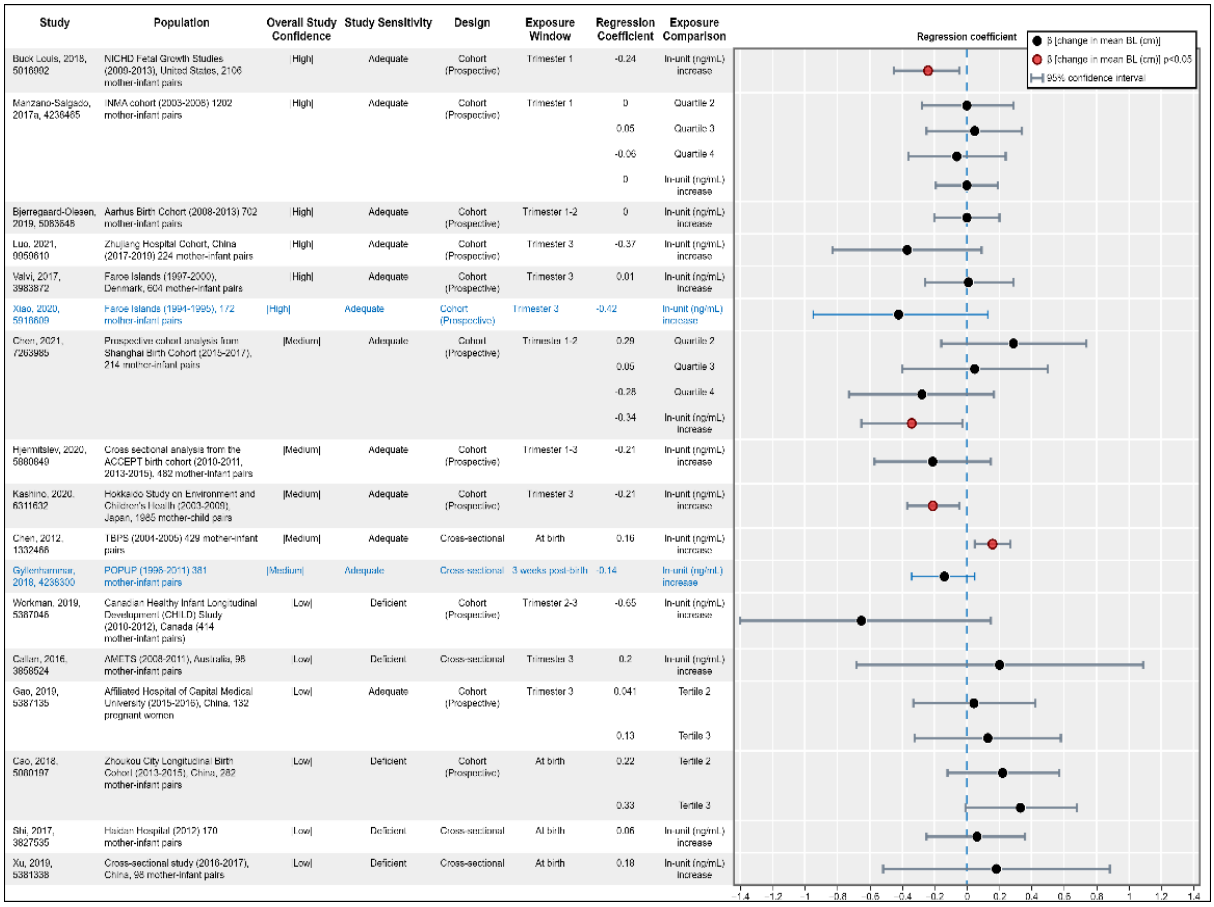


Figure 3-13. PFNA and birth length.[a-d] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Birth-Length-Overall-Population/.

Abbreviations: BWT = birth weight.

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

[b]Xiao et al. (2019) results are truncated; the complete 95% CI ranges from −1.07 to 0.09 grams.

[c]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).

[d]Gyllenhammar et al. (2018b) standardized birth weight differences estimated from digitization of their Figure 1.

Birth length – Sex-specific results

Six (Kashino et al., 2020; Marks et al., 2019; Xiao et al., 2019; Shi et al., 2017; Wang et al., 2016; Robledo et al., 2015) of 11 studies reported inverse associations in boys (2 each were *high, medium,* and *low* confidence), while 5 (Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Wang et al., 2016; Robledo et al., 2015) of 10 studies did so in girls (3 *high*; 2 *medium* confidence) (see Figure 3-14). The range in the six mean birth length studies for boys was −0.16 to −0.53 cm, including one standardized birth length and five mean birth length studies (based on continuous and categorical data). Three of the four studies with inverse associations in both sexes showed stronger results among girls. Associations among girls were also greater on average in four mean birth length studies (β range: −0.13 to −0.70 cm) with three mean birth length studies and one standardized birth length study ranging from −0.38 to −0.48 per ln-unit PFNA increase. Both studies with sex-specific categorical data showed some evidence of inverse exposure-response relationships (one of two studies in each boys and girls) that offered additional support of the continuous exposure results.

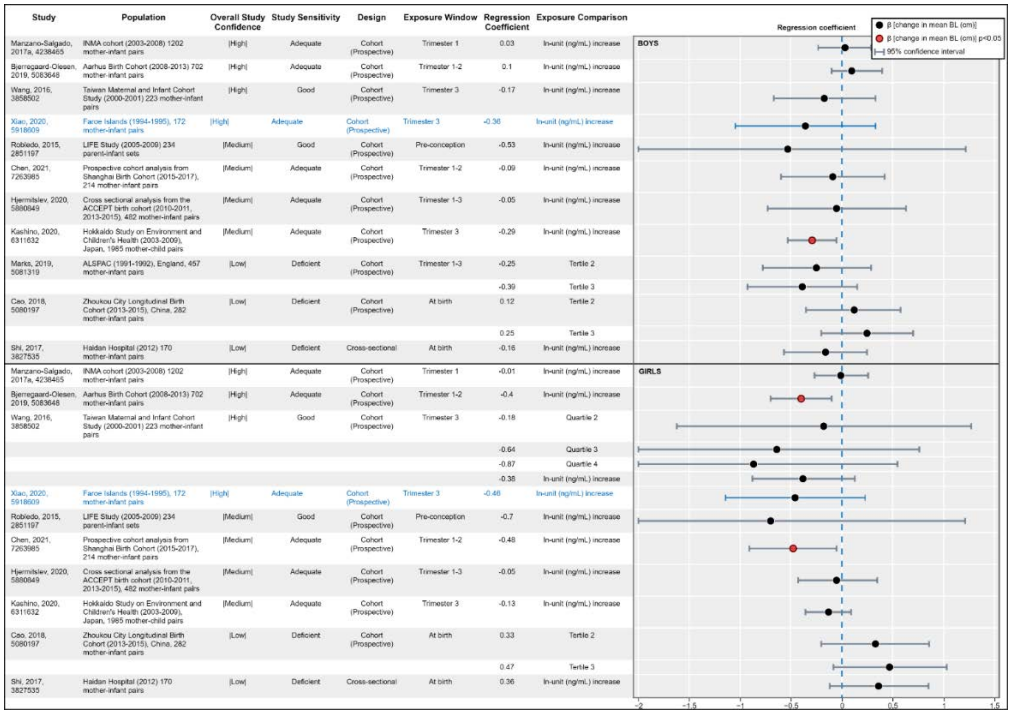


Figure 3-14. PFNA and birth length (sex-stratified: boys above reference line; girls below). [a–c] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Birth-Length-Sex-Stratified-Boys-Abo-1b01/.

Abbreviations: BL = birth length.

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

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

[c]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).

Birth length results – Summary

Overall, 12 of 20 different studies showed some evidence of inverse associations between PFNA and standardized or mean birth length measures in the overall population or in either sex. This included 7 of 17 in the overall population including 6 of 11 *medium* and *high* confidence studies. Nine of 11 studies also showed an inverse association in at least one sex including six of eight *medium* and *high* confidence studies in boys and five of eight *medium* and *high* confidence studies in girls. Similar proportions of studies reported inverse associations in boys (6 of 11 studies; 2 each were *high, medium,* and *low* confidence) and girls (5 of 10 studies; 3 *high,* 2 *medium* confidence). However, three of the four studies with inverse associations in both sexes showed stronger results among girls. Results were more variable in magnitude (β = −0.16 to −0.53 cm) among the five studies in boys reporting associations for either continuous or categorical PFNA exposures. Three of the five studies of mean birth length difference in girls reported consistent findings (β range= −0.38 to −0.48 cm), as did one unitless standardized measure study. One of the two studies with categorical data each among boys and girls showed some evidence of inverse exposure-response relationships that supported the continuous exposure results. Three studies each among the overall population and for the sex-specific findings were statistically significant, despite presumably limited sample sizes in the latter.

As noted above, nearly all the associations detected in the overall population were among *medium* and *high* confidence studies (6 of 11 in total). Although the two studies with standardized birth length measures both showed inverse associations in the overall population, the results were more variable (β range: −0.14 to −0.42 per ln-unit PFNA increase) than the mean birth length studies. For example, while the range across 5 mean birth length studies was −0.21 to −0.65 cm per ln-unit PFNA increase, the four *medium* and *high* confidence studies ranged from −0.21 to −0.37 cm.

None of the five studies with categorical data in the overall population showed evidence of inverse exposure-response relationships. Among the 12 different studies demonstrating birth length deficits, 7 relied on late biomarker sampling suggesting some potential impact of pregnancy hemodynamics. This seemed more evident among the overall population results (5 of 7 studies had late sampling) than the sex-specific findings (5 of 9 studies had late sampling). Few other patterns were evident across study characteristics. For example, five of the eight studies null in the overall population had adequate study sensitivity.

Head circumference – Background of studies

Seventeen studies examined PFNA exposures in relation to head circumference (see Figure 3-15) including four studies that reported sex-specific findings only. Two studies (Xiao et al., 2019; Gyllenhammar et al., 2018b) reported standardized head circumference measures, while the remaining studies examined mean head circumference differences in relation to PFNA. Six studies were *high* confidence (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018;

Manzano-Salgado et al., 2017a; Valvi et al., 2017; Wang et al., 2016) and seven were *medium* confidence (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018b; Lind et al., 2017a; Robledo et al., 2015; Chen et al., 2012). All four of the *low* confidence studies were considered deficient for study sensitivity (Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Callan et al., 2016). Two were rated as good (Wang et al., 2016; Robledo et al., 2015), and 11 had adequate study sensitivity (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Gyllenhammar et al., 2018b; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Chen et al., 2012).

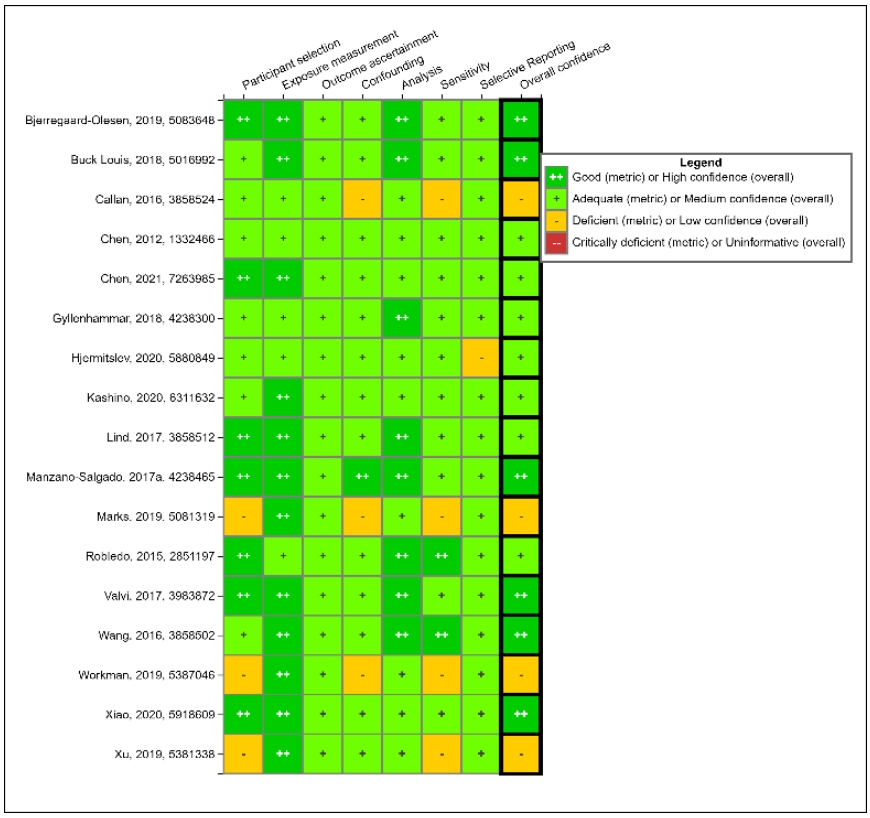


Figure 3-15. Study evaluation heat map of 17 epidemiological studies of head circumference and PFNA exposure. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-Head-Circumference/.

Head circumference – Overall population results

Four (3 *high*, 1 *medium* confidence) of 13 studies showed some inverse head circumference associations with PFNA exposures in the overall population (see Figure 3-16). The *high* confidence Manzano-Salgado et al. (2017a) study was largely null (β = −0.06 cm; 95% CI: −0.19, 0.07) per ln-unit increase, but some monotonicity was suggested, especially in the upper two quartiles (range: −0.06 to −0.16 cm). The *high* confidence Hjermitslev et al. (2020) study reported a statistically significant inverse association in analyses restricted to term births only (β = −0.22 cm; 95% CI:

−0.44, −0.00 per ng/mL increase). Although not statistically significant, the *medium* confidence Kashino et al. (2020) study reported that head circumference reductions were related to each PFNA ln-unit increase (β = −0.13 cm; 95% CI: −0.27, 0.01). The *high* confidence study by Xiao et al. (2019) reported lower head circumference z-scores in the overall population although that result was also not statistically significant (β = −0.36; 95% CI: −0.98, 0.26). Null associations were detected in 8 (Chen et al., 2021; Bjerregaard-Olesen et al., 2019; Xu et al., 2019a; Buck Louis et al., 2018; Gyllenhammar et al., 2018b; Valvi et al., 2017; Callan et al., 2016; Chen et al., 2012) of the 13 studies that examined the overall population including 6 of the 10 *high* or *medium* confidence studies, while another study showed an imprecise increased head circumference in relation to PFNA (Workman et al., 2019).

Overall, limited evidence of associations was noted in 4 (3 *high*, 1 *medium*) of 13 studies including 4 of 10 *high* and *medium* confidence studies among the overall population. There was limited evidence of exposure-response relationships based on categorical analyses, as monotonic differences were seen only in the upper two quartiles of one of two studies in the overall population. Similarly, one of two sex-specific studies also showed dose-dependence in boys. There was no evidence of any impact of pregnancy hemodynamics on these findings, as two of the four studies with inverse associations between PFNA exposures and head circumference were based on early biomarking sampling. Seven of the eight null studies had adequate study sensitivity but few other patterns among the results were evident.

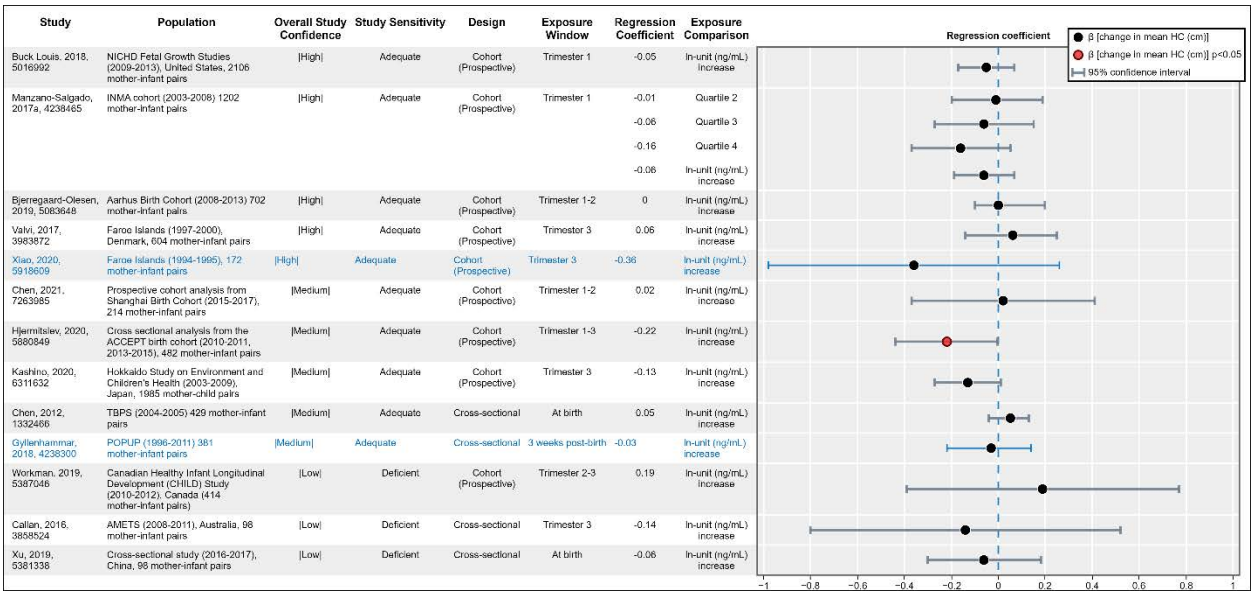


Figure 3-16. PFNA and head circumference (overall population).[a–d] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Head-Circumference-Overall-Population/.

Abbreviations: HC = head circumference.
[a]Studies are sorted first by overall study confidence level, then by exposure window(s) examined.

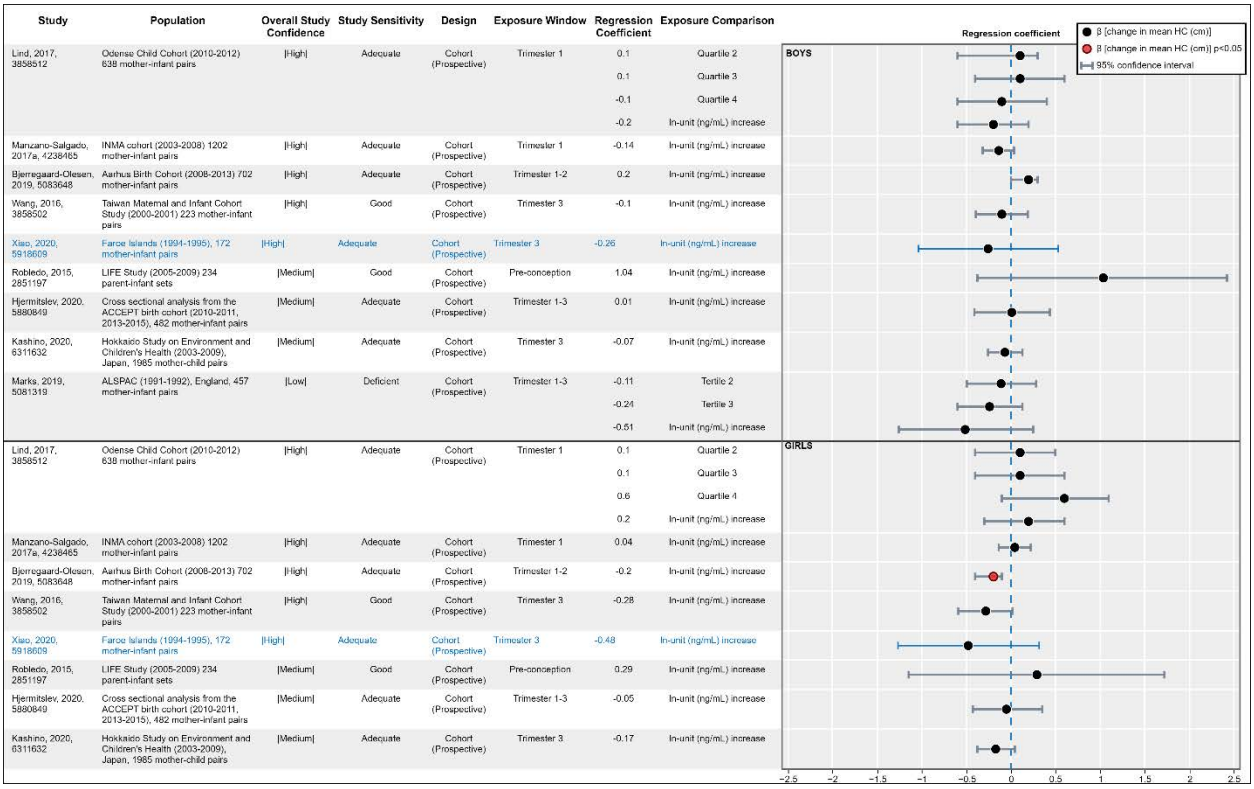
[b]Xiao et al. (2019) and Gyllenhammar et al. (2018b) shown in blue text reported head circumference z-score data.
[c]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).
[d]Gyllenhammar et al. (2018b) standardized head circumference differences estimated from digitization of their Figure 1.

Head circumference – Sex-specific results

The sex-specific results were fairly mixed and at times imprecise, but seven of nine different studies showed inverse associations between PFNA and different head circumference measures at birth including one study that showed some decreases in both sexes (see Figure 3-17). Although neither was statistically significant, the *high* confidence study by Xiao et al. (2019) reported larger head circumference z-scores in girls (β = −0.48; 95% CI: −1.27, 0.32 per ln-unit) than in boys (β = −0.26; 95% CI: −1.04, 0.53). The *high* confidence study by Lind et al. (2017a) reported comparable nonsignificant associations in the opposite direction for boys (β = −0.2 cm; 95% CI: −0.6, 0.2) and girls (β = 0.2 cm; 95% CI: −0.3, 0.6) per each ln--unit increase. The *high* confidence study by Manzano-Salgado et al. (2017a) found a nonsignificant head circumference difference among boys only (β = −0.14 cm; 95% CI: −0.32, 0.03). The *low* confidence study by Marks et al. (2019) reported a nonsignificant head circumference decrease per ln-unit increase (β = −0.51 cm; 95% CI: −1.26, 0.25). They reported an exposure-response relationship for tertile 2 (β = −0.11 cm; 95% CI: −0.50, 0.28) and tertile 3 (β = −0.24 cm; 95% CI: −0.60, 0.13) compared with the tertile 1 referent (≤0.2 ng/mL). In contrast, the *medium* confidence study by Robledo et al. (2015) reported larger increased mean head circumference in relation to PFNA for both boys (β = 1.04 cm; 95% CI: −0.38, 2.42) and girls (β = 0.29 cm; 95% CI: –1.15, 1.72) per ln-unit increase.

Among girls, the three studies showed inverse mean head circumference differences in relation to each ln-unit increase that were comparable in magnitude (β range: −0.17 to −0.28 cm) and precision. The *high* confidence study by Bjerregaard-Olesen et al. (2019) reported a statistically significant decreased mean head circumference for each ln-unit increase in PFNA (β = −0.2 cm; 95% CI: −0.4, −0.1). Although not statistically significant, the *medium* confidence Kashino et al. (2020) study reported a value similar in magnitude in girls only (β = −0.17 cm; 95% CI: −0.38, 0.04 each PFNA ln-unit increase). The *high* confidence study by Wang et al. (2016) also reported inverse associations only among girls (β = –0.28 cm; 95% CI: –0.59, 0.02 per ln-unit increase).

Overall, seven different sex-specific studies of nine showed some inverse associations between PFNA and head circumference in either sex. However, only one of these seven showed deficits in both sexes. Results were mostly comparable in magnitude across sexes for the mean head circumference differences as four of eight studies showed inverse associations in girls compared with four of nine studies in boys. There was some additional evidence of exposure-response relationships in categorical analyses, as monotonic differences were seen in one study of boys (of two in total). Four of the seven studies examined here were based on early biomarker sampling suggesting the impact of pregnancy hemodynamics was likely not a strong determinant of these results.



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
BOYS							
Lind, 2017, 3858512	Odense Child Cohort (2010-2012) 638 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0.1	Quartile 2
						0.1	Quartile 3
						-0.1	Quartile 4
						-0.2	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	-0.14	ln-unit (ng/mL) increase
Bjerregaard-Olesen, 2019, 5083648	Aarhus Birth Cohort (2008-2013) 702 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1-2	0.2	ln-unit (ng/mL) increase
Wang, 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001) 223 mother-infant pairs	|High|	Good	Cohort (Prospective)	Trimester 3	-0.1	ln-unit (ng/mL) increase
Xiao, 2020, 5918609	Faroe Islands (1994-1995), 172 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 3	-0.26	ln-unit (ng/mL) increase
Robledo, 2015, 2851197	LIFE Study (2005-2009) 234 parent-infant sets	|Medium|	Good	Cohort (Prospective)	Pre-conception	1.04	ln-unit (ng/mL) increase
Hjermitslev, 2020, 5880849	Cross sectional analysis from the ACCEPT birth cohort (2010-2011, 2013-2015), 482 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-3	0.01	ln-unit (ng/mL) increase
Kashino, 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-child pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 3	-0.07	ln-unit (ng/mL) increase
Marks, 2019, 5081319	ALSPAC (1991-1992), England, 457 mother-infant pairs	|Low|	Deficient	Cohort (Prospective)	Trimester 1-3	-0.11	Tertile 2
						-0.24	Tertile 3
						-0.51	ln-unit (ng/mL) increase
GIRLS							
Lind, 2017, 3858512	Odense Child Cohort (2010-2012) 638 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0.1	Quartile 2
						0.1	Quartile 3
						0.6	Quartile 4
						0.2	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0.04	ln-unit (ng/mL) increase
Bjerregaard-Olesen, 2019, 5083648	Aarhus Birth Cohort (2008-2013) 702 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1-2	-0.2	ln-unit (ng/mL) increase
Wang, 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001) 223 mother-infant pairs	|High|	Good	Cohort (Prospective)	Trimester 3	-0.28	ln-unit (ng/mL) increase
Xiao, 2020, 5918609	Faroe Islands (1994-1995), 172 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 3	-0.48	ln-unit (ng/mL) increase
Robledo, 2015, 2851197	LIFE Study (2005-2009) 234 parent-infant sets	|Medium|	Good	Cohort (Prospective)	Pre-conception	0.29	ln-unit (ng/mL) increase
Hjermitslev, 2020, 5880849	Cross sectional analysis from the ACCEPT birth cohort (2010-2011, 2013-2015), 482 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-3	-0.05	ln-unit (ng/mL) increase
Kashino, 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-child pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 3	-0.17	ln-unit (ng/mL) increase

Figure 3-17. PFNA and head circumference (sex-stratified: boys above reference line, girls below).[a–c] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Head-Circumference-Sex-Stratified-Bo-9385/.

Abbreviations: HC = head circumference.
[a]Studies are sorted first by overall study confidence level then by exposure window examined.
[b]As indicated by the blue text, Xiao et al. (2019) reported head circumference z-score data.
[c]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).

Head circumference summary

Although the overall results across studies were mixed, 8 different studies (of 17 total) among the overall population or either/both sexes showed some inverse associations between PFNA and head circumference. This included 7 of 13 *medium* and *high* confidence studies. More limited evidence of inverse associations in the overall population was found in 4 (3 *high*, 1 *medium* confidence) of 13 studies including 4 of 10 *medium* and *high* confidence studies. Effects estimates were often precise, and some differences were close or reached statistical significance (at the alpha level of 0.05). Seven different sex-specific studies of nine showed some inverse associations between PFNA and head circumference in either sex including one that reported associations in both sexes. The magnitude of results was comparable and fairly precise in most studies, and there was no strong evidence of sexual dimorphism.

Some additional evidence among two of the four studies with categorical analyses among the overall population or either sex supported dose-dependence seen in the studies based on continuous exposure measures. Overall, five of eight head circumference studies that showed some evidence of inverse associations in the overall population or either sex were based on early biomarker sampling suggesting limited impact of pregnancy hemodynamics. Other study characteristics also did not appear related to the patterns of findings across studies. For example, study sensitivity also did not seem to explain the studies reporting null associations.

Fetal growth restriction summary

Among 35 different studies included in the synthesis here that examined different birth weight-related measures (i.e., SGA, LBW, and standardized of mean birth weight measures), 27 showed some evidence of inverse associations with different birth weight measures and/or increased risk of SGA or LBW in either or both sexes (Table 3-4). Nearly half of these studies showed statistically significant differences, supporting that many studies were sufficiently powered to detect associations in the PFNA exposure ranges examined in the available studies. This was more applicable to the analyses in the overall population, as study sensitivity may have been more limited among the sex-specific analyses with smaller sample sizes.

Six of 11 studies (5 of 9 *medium* and *high* confidence) showed inverse associations between PFNA exposures and standardized birth weight measures in the overall population. Among five sex-specific studies examining standardized birth weight, three reported inverse birth weight z -scores in relation to PFNA among boys (2 *high*; 1 *low* confidence), while four did so in girls (3 *high*; 1 *low* confidence). This included associations seen in three of four *high* confidence studies in girls with each demonstrating some statistically significant results irrespective of confidence level. Although the magnitude of differences varied somewhat across and within studies, three of the standardized birth weight studies showed associations in the overall population and both sexes.

As noted above, 22 of the 32 unique studies of mean birth weight among the overall population, or either/both sexes reported some inverse associations. This included 19 of 27 studies (and 15 of the 20 *high* and *medium* confidence studies) that reported some mean birth weight differences in their overall population in relation to categorical or continuous PFNA data expressions. Twenty-four studies with continuous exposure data expressed results on a per ln-unit PFNA change, of which18 showed inverse mean BWT associations. Among these 18, there was a wide range in mean birth weight differences for the overall population across all confidence levels (range: −15 to −133 g) with 12 studies ranging from −40 to −62 g per ln-unit PFNA increase.

Fourteen of 16 studies with sex-specific results showed some evidence of mean birth weight differences in either or both sexes, including similar proportions as in the overall population for both girls (10 of 15) and boys (11 of 16). The mean birth weight differences among 10 studies (including 9 *medium* and *high* studies) in girls ranged from −27 to −80 g per ln-unit increase. Results were more variable among 11 studies (including 8 *medium* and *high* studies) in boys

(range: −24 to −150 g). Most of the variability occurred among boys in the *medium* confidence studies. Results based on 11 studies with categorical PFNA data were largely in agreement with those continuous exposure results expressed per ln-unit increase.

The studies with categorical data were largely supportive of those measures based on continuous exposure measures, but only four different mean birth weight studies (one in girls; one in boys; two in the overall population) that reported inverse associations showed exposure-response relationships with increasing birth weight across exposure quantiles. Although more studies overall (11 of 19) that showed inverse associations were based on later sampling, evidence was limited for any impact of sample timing among the 16 *medium* and *high* confidence studies or among the sex-specific findings irrespective of confidence level.

EPA's meta-analysis of birth weight (Wright et al., 2023) and in Appendix C.1.5 showed statistically robust results across analyses of different study confidence and sample timing stratifications. For example, except for two strata with small sample sizes, statistically significant mean BWT deficits across every study confidence and sampling timing stratum examined showed birth weight deficits in excess of 22 grams per ln-unit increase in PFNA. These results were fairly consistent in magnitude irrespective of stratum and sample size with a range of −22 to −49 grams per ln-unit increase and supportive of the mean birth weight and other fetal growth restriction findings across all studies examined. The findings across different sample timing strata showed associations even among the early biomarker group, which reduces concern about the potential impact of biomarker sample timing as an explanatory factor for the overall results.

As noted above, six different studies of eight showed some increased risks for either SGA, LBW, or very LBW including two that demonstrated statistical significance. Although none of the three studies with categorical data showed exposure-response relationships, the highest reported ORs in each were found in the highest quartile from the overall population. No distinctive patterns appear to explain the study results including similar results across sexes across both endpoints, although SGA findings were more consistent and larger among girls. For example, five of the six studies with increased risks were based on early biomarker sampling suggesting that pregnancy hemodynamics is not a likely explanatory factor here.

Compared with fetal birth weight, other anthropometric measures of fetal growth such as head circumference and birth length were more mixed. For example, 12 of 20 different studies showed some evidence of inverse associations between PFNA and standardized or mean birth length measures in the overall population or in either sex. These studies included 7 (1 *low* and 3 each *medium* and *high* confidence studies) of 17 in the overall population and 9 of 11 studies in at least one sex. There was limited overall evidence of inverse exposure-response relationships in either the overall population or either sex. Although similar proportions of studies reported inverse associations in boys (6 of 11 studies; 2 each were *high*, *medium*, and *low* confidence) and girls (5 of 10 studies; 3 *high*; 2 *medium* confidence), three of the four studies with inverse associations in both sexes showed stronger results among girls. Few patterns were evident to explain the inverse birth

length associations although more of them relied on late biomarker sampling suggesting some potential impact of pregnancy hemodynamics among both the overall population results (5 of 7 studies) and the sex-specific findings (5 of 9 studies).

A similar proportion of studies as found for birth length showed some evidence of inverse associations between PFNA and head circumference. Eight different studies (of 17 in total) among the overall population or either/both sexes showed some inverse associations including 7 of 13 *medium* and *high* confidence studies. More limited evidence of inverse associations in the overall population was shown based on only 5 (3 *high*, 1 *medium*, 1 *low* confidence) of 13 studies. More consistent results were seen across the sex-specific analyses, but sexual dimorphism was not evident. Additional evidence to support dose-dependence demonstrated from the continuous PFNA exposure measures was seen among two of the four studies with categorical analyses in the overall population or either sex. Among the eight studies in total (across the overall population or either sex) that showed some evidence of inverse associations between PFNA exposures and head circumference, five were based on early biomarking sampling. This suggests pregnancy hemodynamics alone (nor other factors examined) was not the sole determinant of the inverse study results.

Table 3-4. Summary of 35 epidemiologic studies of PFNA exposure and fetal growth restriction

Author	Study location/years	Sample size	Median (range) in ng/mL	SGA/LBW	Birth weight	Birth length	Head circumference
***High* confidence studies**[a]							
Wang et al. (2016)	Taiwan, 2000–2001	223	1.55–Boys[b] 1.58–Girls[b] (0.08, 10.30–All)	↑ SGA (Girls) ∅ (Boys)	– Girls*[c] ∅ Boys	– Girls[c]/ Boys	– Girls ∅ Boys
Shoaff et al. (2018)	OH, USA, 2003–2006	299	0.9 (0.01, 2.9)[b]		∅ All		
Bjerregaard-Olesen et al. (2019); Bach et al. (2016)	Denmark, 2008–2013	1,533	0.8 (LOD, 4.69)[b]		– All*[d] – Girls* + Boys	∅ All/Boys – Girls*	∅ All + Boys – Girls*
Sagiv et al. (2018)	E. MA, USA 1999–2003	1,645	0.7 (0.3, 1.49)[b,e]		– All*		
Lind et al. (2017a)	Denmark, 2010–2012	638	0.7 (0.2, 4.4)[b]		+ Girls – Boys		+ Girls – Boys
Manzano-Salgado et al. (2017a)	Spain, 2003–2008	1,202	0.66 (0.03, 5.51)[b]	↑ LBW (Boys)[b] ∅ LBW (All/Girls) ↓ SGA (All/Girls) ∅ SGA (Boys)	– All/ ∅ Boys/ Girls	– All/Boys ∅ Girls	– All/Boys ∅ Girls
Buck Louis et al. (2018)	USA, 2009–2013	2,106	0.76 (N/A)[b]		∅ All/Hispanic/ Asian – Black + White	– All*/Black*/ White/Asian /Hispanic	∅ All/Hispanic /White – Black + Asian
Valvi et al. (2017)	Denmark,	604	0.59 (N/A)[b]		– All/Girls/	∅ All	∅ All

Author	Study location/years	Sample size	Median (range) in ng/mL	SGA/LBW	Birth weight	Birth length	Head circumference
	1997–2000				Boys		
Starling et al. (2017)	CO, USA, 2009–2014	598	0.39 (LOD, 6.0)[b]		– All*		
Luo et al. (2021)	China, 2021	224	0.5 (N/A)[b]		– All	– All	
Wikström et al. (2020)	Sweden, 2007–2010	1,533	0.53 (LOD, 1.02)[b]	↑ SGA (All/Girls/Boys)	– All/Boys/ Girls		
Yao et al. (2021)	China, 2010–2013	369	0.81 (0.15, 7.76)[b]		– All		
Xiao et al. (2019)	Faroe Islands, 1994–1995	172	0.6 (0.1, 1.6)[b,g]		– All/Boys/ Girls	– All/Boys/ Girls	– All/Boys/ Girls
Eick et al. (2020)	USA, 2014–2018	506	0.30 (N/A)[b]		∅ All/Boys/ Girls		
Gardener et al. (2021)	USA, 2009–2013	354	0.7 (LOD, 3.68)[b]		↑ All[k] – binary z-scores		
***Medium* confidence studies**							
Chen et al. (2012)	Taiwan, 2004–2005	429	2.36 (N/A)[h]	∅ SGA (All) ∅ LBW (All)	∅ All	+ All*	∅ All
Robledo et al. (2015)	MI/TX, USA, 2005–2009	234	1.21–Boys[i] 1.57–Girls[i]		+ Boys – Girls	– Boys/Girls	+ Boys/Girls
Lenters et al. (2016)	Ukraine/Poland/Greenland, 2002–2004	1,321	0.56–0.69[b]		– All		
Meng et al. (2018b)	Denmark, 1996–2002	3,535	0.5 (N/A)[b,h]	↑ LBW (All)	– All*[c]/Girls/ Boys		
Gyllenhammar et al. (2018b); (Swedish	Sweden, 1996–2001	381	0.41 (0.06, 2.4)[b]		– All*	– All	∅ All

Author	Study location/years	Sample size	Median (range) in ng/mL	SGA/LBW	Birth weight	Birth length	Head circumference
Environmental Protection Agency, 2017)[j]							
Kwon et al. (2016)	S. Korea, 2006–2010	268	0.20 (0.04, 0.78)[h]		– All*		
Hjermitslev et al. (2020)	Greenland, 2010–2015	266	1.15 (0.21, 7.87)[b]	↑ LBW (All)	– All/Girls/ Boys	∅ All/ Boys/Girls	– All*/ ∅ Girls/Boys
Hall et al. (2022)	Canada, 2010	120	0.11 (0.0CF0.60)[k]		– Boys ∅ Girls		
Chen et al. (2021)	China, 2013–2015	214	2.33 (N/A)[b]		– All	– All/Girls* ∅ Boys	∅ All
Kashino et al. (2020)	Japan, 2003–2009	1,591	1.2 (N/A)[b]		– All*/Boys*/ Girls	– All*/Boys*/ Girls	– All/Girls ∅ Boys
Chang et al. (2022)	USA, 2014–2018	370	0.27 (LOD–2.27)[b]	↑ SGA[l]	– All[c]		
***Low* confidence studies**							
Callan et al. (2016)	W. Australia, 2003–2004	98	0.30 (0.05, 1.3)[b]		∅ All	∅ All	∅ All
Li et al. (2017)	China, 2013	321	0.2 (N/A)[h]		– All/Boys/ Girls		
Shi et al. (2017)	China, 2012	170	0.19 (0.04, 0.81)[h]		+ All/Girls – Boys	+ All/Girls – Boys	
Xu et al. (2019a)	China, 2016–2017	98	0.26 (N/A)[h]	∅ SGA (All)	– All	∅ All	∅ All
Cao et al. (2018)	China, 2013–2015	337	0.11 (0.04, 0.23)[h,m]		– Boys + Girls	+ All	

Author	Study location/years	Sample size	Median (range) in ng/mL	SGA/LBW	Birth weight	Birth length	Head circumference
Marks et al. (2019)	England, 1991–1992	447	0.4 (0.1, 1.6)[b]		– Boys[c]*	– Boys[c]	– Boys[c]
Gao et al. (2019)	China, 2015–2016	132	0.57 (LOD, 3.98)[b]		∅ All	∅ All	
Gross et al. (2020)	NY, USA, 2012–2014	98	0.15 (N/A)[n]		– All/Boys/ Girls		
Workman et al. (2019)	Canada, 2010–2011	414	0.37 (LOD, 4.0)[b]		– All	– All	+ All

Abbreviations: LOD = limit of detection; SGA = small for gestational age; LBW = low birth weight.
/Denotes multiple groups with the same direction of associations.
*Statistically significant results based on $p < 0.05$; ∅ = represents a null association; + = represents a positive association; – = represents a negative (inverse) association; ↑ = represents increased odds ratio; ↓ = represents decreased odds ratio.
Note: "adverse effects" are indicated by both increased ORs (↑) for dichotomous outcomes and negative associations (–) for the other outcomes.
[a]Overall confidence judgment varies by growth-related outcomes but is summarized here for the birth weight endpoints; mean BWT association is primarily shown here with exception of Xiao, Gross, and Gardener which reported on standardized results.
[b]Median levels in maternal blood.
[c]Exposure-response detected.
[d]Results were not consistent across PFAS measures, e.g., associations only seen in lowest two quartiles.
[e]5th to 95th percentiles.
[f]Increased odds ratios among term births only.
[g]Geometric mean provided.
[h]Median levels in cord blood.
[i]Geometric mean levels in maternal blood.
[j]Swedish Environmental Protection Agency (2017) and Gyllenhammar et al. (2018b) results are included here (both analyzed the POPUP cohort).
[k]Placental levels measured in ng/g.
[l]*Medium* confidence for BWT and *low* confidence for SGA
[m]10th to 90th percentiles.
[n]Dried Blood Spots from Infant Heel Stick Sample.

Postnatal growth restriction

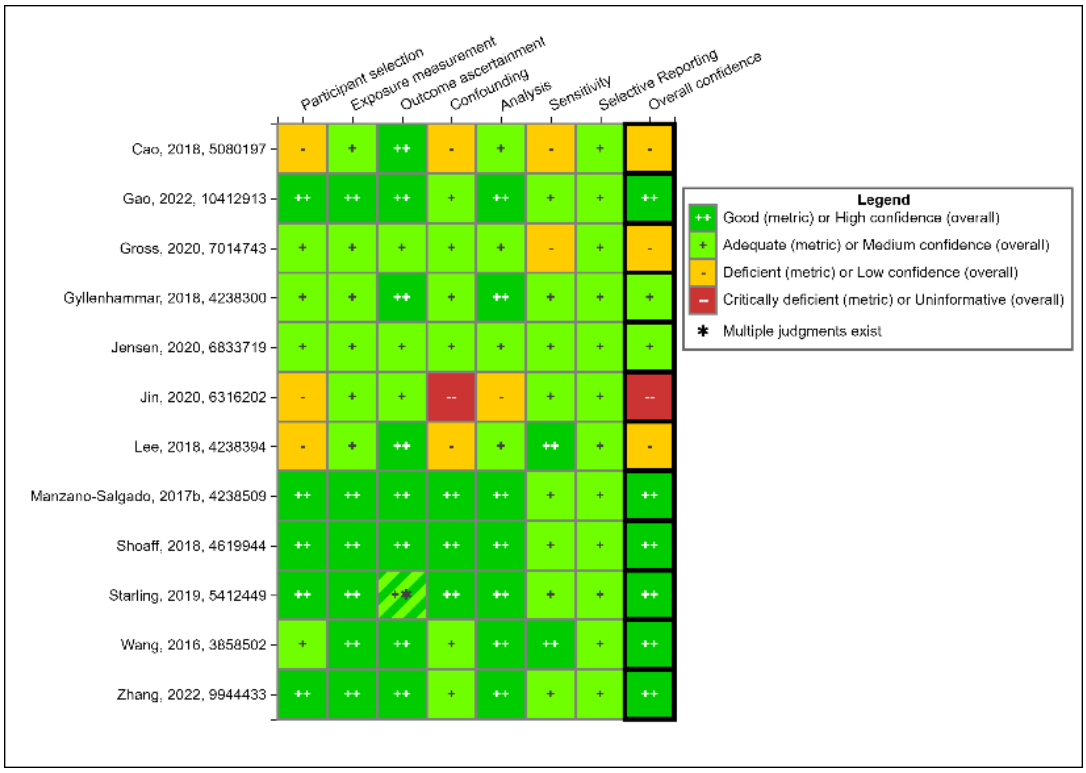


Figure 3-18. Study evaluation heat map of 12 epidemiological studies of childhood growth (e.g., weight, height, adiposity, and rapid weight gain) and PFNA exposure. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-PNG-Measures/.

Postnatal weight and height – Background of studies

Twelve studies examined different postnatal growth measures in relation to PFNA, although one study (Jin et al., 2020) not further considered was rated *uninformative* due to deficiencies and critical deficiencies or in confounding, analysis, and participant selection (see Figure 3-18). Endpoints included in this synthesis were early childhood weight, height, rapid weight gain, and different measures of adiposity in relation to PFNA exposures. Six of the 11 included studies were *high* confidence (Zhang et al., 2022; Gao et al., 2019; Starling et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b; Wang et al., 2016), two were *medium* (Jensen et al., 2020a; Gyllenhammar et al., 2018b) and three were *low* confidence (Gross et al., 2020; Cao et al., 2018; Lee et al., 2018) (see Table 3-5; Figure 3-18). Seven studies had adequate (Zhang et al., 2022; Jensen et al., 2020a; Gao et al., 2019; Starling et al., 2019; Gyllenhammar et al., 2018b; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) study sensitivity, while two each were considered good (Lee et al., 2018; Wang et al., 2016) or deficient (Gross et al., 2020; Cao et al., 2018).

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, Wang et al. (2016) and Lee et al. (2018) examined postnatal growth at 2 years, while analyses in Cao et al. (2018) were based on a mean of 19 months in participants. Gyllenhammar et al. (2018b) had serial measures of postnatal growth at 3, 6, 12, and 18 months. Adiposity was measured at 5 months of age by Starling et al. (2019) and at 3 and 18 months by Jensen et al. (2020a). Gross et al. (2020) measured overweight status at 18 months old (defined as weight-for-length z-scores ≥85th percentile). Manzano-Salgado et al. (2017b) assessed weight gain z-scores at 6 months, while age- and sex-specific z-scores for body mass index and waist circumference were measured in children aged 4 and 7 years. Shoaff et al. (2018) repeated early childhood measures based on three measures based on examination at approximately 4 weeks and 1 and 2 years of age. Postnatal examinations in Zhang et al. (2022) were based on four measures during the first 12 months (3 days, 42 days, 6 months, 12 months) from participants recruited from the Shanghai Birth Cohort between 2013 and 2016. Using data from the same participants, Gao et al. (2022) assessed early-life growth trajectories during the first 2 years of life.

Postnatal weight results

Eight studies examined childhood standardized (n = 6) or mean weight (n = 2) measures in relation to PFNA including five *high*, one *medium*, and two *low* confidence studies. Among the six studies with standardized weight measures, one (Wang et al., 2016) reported data only in the sexes. Two standardized weight studies reported data only in the overall population (Gyllenhammar et al., 2018b; Shoaff et al., 2018), while the remaining three examined the overall population and both sexes (Zhang et al., 2022; Starling et al., 2019; Manzano-Salgado et al., 2017b). Two of seven studies examining the overall population were based on mean weight changes (Cao et al., 2018; Lee et al., 2018), while one of four sex-specific studies were based on mean weight measures.

Both of the *low* confidence mean weight studies showed some evidence of inverse associations for the overall population or either sex (see Figure 3-19). Despite limited sensitivity, the study by Lee et al. (2018) detected statistically significant mean weight reductions for quartile 4 (≥1.81 ng/mL) compared with quartile 1 (<0.77 ng/mL) among measurements at age 2 (β = −580 g; 95% CI: −960, −210) and for changes from birth to age 2 years (β = −280 g; 95% CI: −600, 40); this included exposure-response relationships across the quartile categories for the age 2 endpoint. They also reported decreased mean weight (β = −320 g; 95% CI: −480, −150) at age 2, as well as a large change in weight (β = −260 g; 95% CI: −440, −100) from birth to age 2 with each ln-unit increase. The study by Cao et al. (2018) reported a null finding for postnatal weight in the overall population for tertile 3 (β = −31 g; 95% CI: −485, 423) and an imprecise decrease in tertile 2 (β = −193 g; 95% CI: −636.7, 251.1). They reported a large weight increase in girls for tertile 3 (β = 294 g; 95CI: −457, 1045) but a decrease of similar magnitude and imprecision in tertile 2

(β = −225; 95% CI: −957, 507). Exposure-response decreases across PFNA tertiles 2 (β = −143 g; 95% CI: −701, 416) and 3 (β = −309 g; 95% CI: −877, 258) were also detected among boys only.

One study of six showed standardized weight increases in the overall population (Gyllenhammar et al., 2018b), while two other studies were null in both the overall population and both sexes (Zhang et al., 2022; Manzano-Salgado et al., 2017b). Three (all *high* confidence) of the six standardized weight studies showed some weight deficits in relation to PFNA exposures (see Figure 3-20). The study by Starling et al. (2019) reported largely null results in the overall population and both sexes for weight-for-age z-scores measured at age 5 months. They did report small nonsignificant weight-for-age and weight-for-length z-score differences in opposite directions for boys (β range: 0.10 to 0.13) and girls (β range: −0.10 to −0.17) for each ln-unit increase in PFNA. The study by Wang et al. (2016) detected a reduction in average childhood weight *z-scores* (β = −0.20; 95% CI: −0.42, 0.01) from ages 2 to 11 years among girls only, but age-specific results were null at ages 2, 5, 8, and 11 years. The average change across childhood was null in boys (β = 0.04; 95% CI: −0.10, 0.19), but they reported a reduction at age 2 years (β = −0.19; 95% CI: −0.59, 0.20). Although their results based on each ln--unit increase were null, the study by Shoaff et al. (2018) detected monotonic nonstatistically significant weight-for-age differences across tertiles (β range: −0.14 to −0.19) for weight-for-age z-scores measured from 4 weeks to 2 years of age. Their weight-for-length results were smaller in general, but they saw differences around −0.1 for both tertile 3 and their continuous analysis per ln-unit increase.

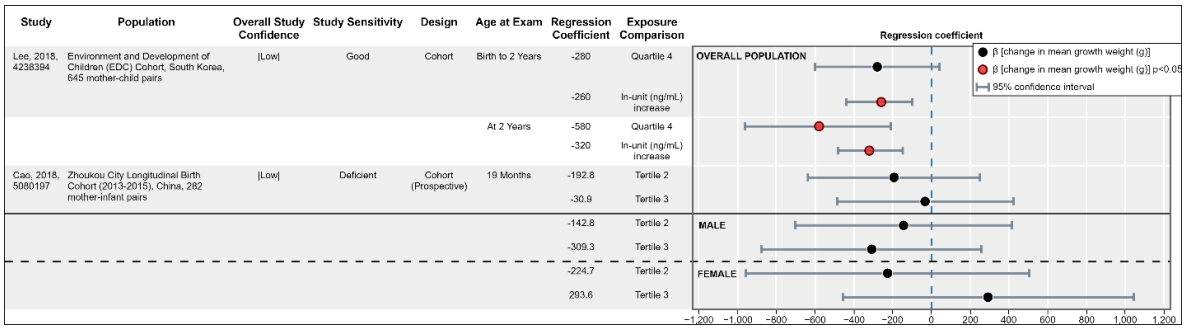


Figure 3-19. PFNA and postnatal growth mean weight.[a–d] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Postnatal-Growth-Mean-Weight/.

Abbreviations: CI = confidence interval.

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

[b]Data for overall population are found above the reference line; sex-stratified data are found below the black reference line.

[c]For Cao et al. (2018), sex-specific data are found below the black reference line. Above the dashed line is data for boys; below the dashed line is data for girls.

[d]While decreased weight was reported for multiple quartiles in relation to weight at 2 years in Lee et al. (2018), the 95% CIs were not estimable and only quartile 4 is plotted here.

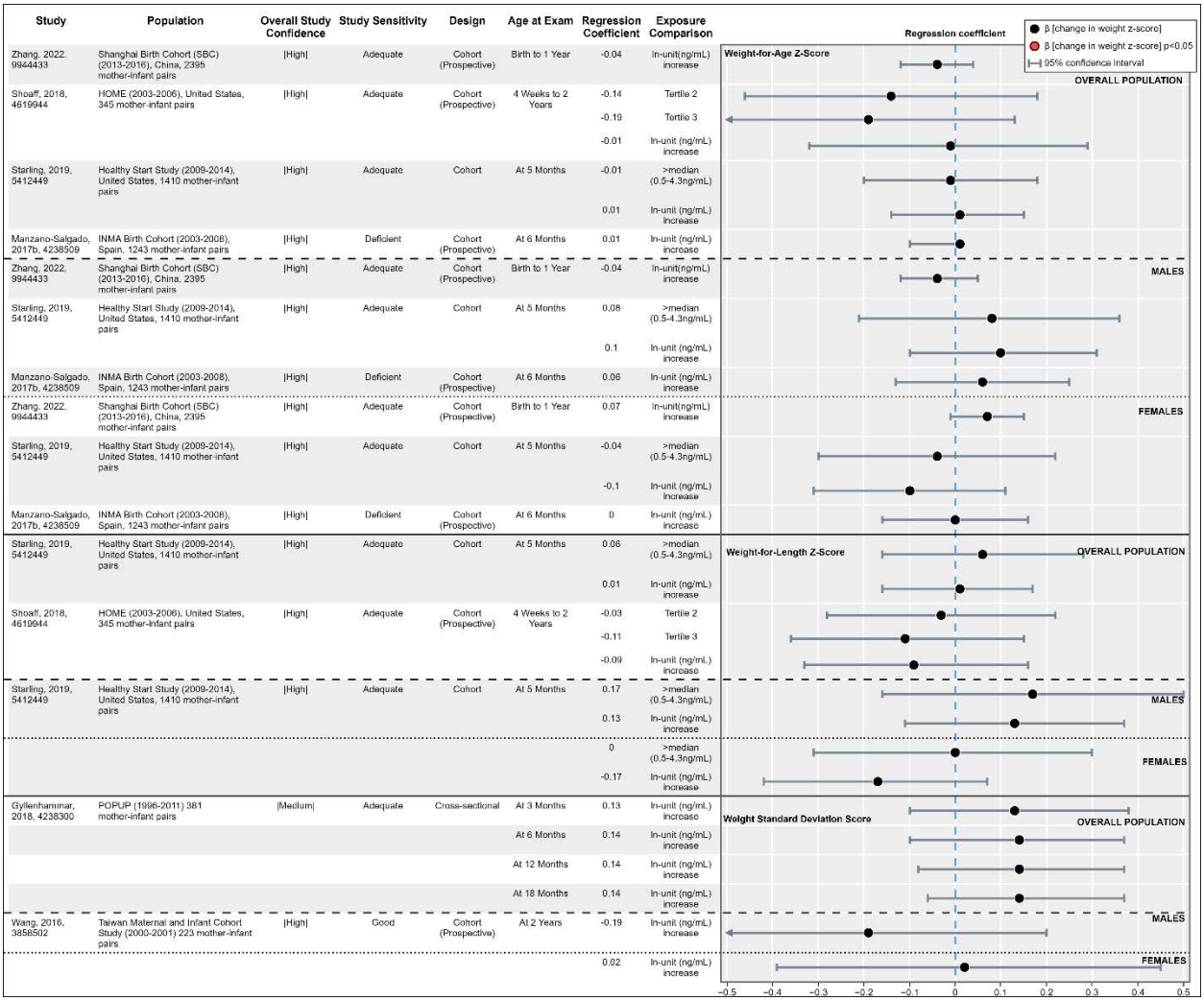


Figure 3-20. PFNA and postnatal growth weight standardized measures.[a–d] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Postnatal-Growth-Weight-Standardized-acff/.

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

[b]Solid black lines divide the figure into three categories. Listed from top to bottom they are as follows: Weight-for-Age Z-Score, Weight-for-Length Z-Score, Weight Standard Deviation Score.

[c]Within each category, overall population is located above the dashed lines, boys are located below the dashed lines, and girls are below the dotted lines.

[d]Zhang et al. (2022) postnatal weight differences estimated from digitization of their Figure A2 (overall population), A3 (boy infants), and A4 (girl infants); Wang et al. (2016) and Gyllenhammar et al. (2018b) postnatal standardized weight differences estimated from digitization of their Figures 1 and 2, respectively.

Postnatal growth: Weight measures summary

Overall, five of eight studies showed some inverse associations between PFNA and different weight measures in either or both sexes (n = 3) of the overall population (n = 2). This included all three *high* confidence studies and the two *low* confidence studies. In the three studies with inverse sex-specific associations, the mean weight study showed deficits in boys while the two

standardized measure studies showed larger deficits among girls. The inverse associations in the overall population were found in one mean and one standardized weight measure study.

Among the only two studies reporting values for all categories, exposure-response relationships were observed for weight-for-length z-scores in one *high* confidence study and in one *low* confidence study examining mean weight changes, although the latter was seen among boys only. Few patterns across the study results were evident, although the age of examination was younger for the two null studies.

Postnatal growth: Height measures

Six studies examined childhood standardized (n = 4) or mean height (n = 2) measures in relation to PFNA either in both sexes (n = 3) or the overall population combined (n = 6). Two of the four standardized height studies reported data only in the overall population (Gyllenhammar et al., 2018b; Shoaff et al., 2018). One study (Wang et al., 2016) reported data only in the sexes, while one examined the overall population and both sexes (Zhang et al., 2022). Two of six studies examining the overall population were based on mean height changes (Cao et al., 2018; Lee et al., 2018), while one of three sex-specific studies was based on mean height measures.

The *high* confidence study by Wang et al. (2016) detected larger statistically significant standardized height reductions at age 2 years in girls (β = –0.23; 95% CI: –0.51, 0.05) and boys (β = –0.14; 95% CI: –0.49, 0.19) (see Figure 3-21). They also reported average childhood height z -scores from ages 2 to 11 years similar in magnitude for girls (β = –0.21; 95% CI: –0.42, 0.00) and boys (β = –0.15; 95% CI: –0.37, 0.08) (not plotted). The authors saw height reductions that were larger among boys across examination windows (i.e., 2, 5, 8, and 11 years) with results from ages 8 and 11 years being statistically significant (data not shown). The *high* confidence study by Shoaff et al. (2018) reported mixed results with null findings in the overall population from their continuous PFNA analyses, but some height reductions in the categorical analysis. Compared with the tertile 1 referent (≤7 ng/mL), nonstatistically significant and nonmonotonic standardized length-for-age measure deficits measured from 4 weeks to 2 years of age were reported in the highest PFNA tertile (β range: –0.12 to –0.17). Null results were detected for length-for-age z-score per ln-unit PFNA increase in the overall population and both sexes in the *high* confidence Zhang et al. (2022) study, as well as in the *medium* confidence Gyllenhammar et al. (2018b) study for their serial measures of postnatal height (standard deviation scores) at 3, 6, 12, and 18 months of age assessed in the overall population.

The mean height measures also were considered mixed with one study showing increased and another decreased mean height in relation to PFNA exposures (Figure 3-22). Despite limited sensitivity, the *low* confidence study by Lee et al. (2018) detected statistically significant reductions in height (β = –1.12 cm; 95% CI: –1.87, –0.38) measured at age 2 years among the fourth PFNA quartile (≥ 2.19 ng/mL) compared with quartile 1 (< 0.85 ng/mL); this included an exposure-response relationship detected across the quartile categories. Mean height changes from birth to age 2 years were observed across all quartiles but were not statistically significant. They also

reported a decreased mean height deficit (β = −0.48 cm; 95% CI: −1.40, 0.51) at age 2 years but was null from birth to 2 years of age (β = 0.04 cm; 95% CI: −0.37, 0.45) per ln-unit increase. The *low* confidence Cao et al. (2018) study showed mixed categorical results with nonsignificant and imprecise increases in tertile 3 contrasted with decreases in tertile 2 for the overall population and both sexes. For example, large tertile 3 results were seen in the overall population (β = 1.38 cm; 95% CI: −0.51, 3.27), in girls (β = 1.22 cm; 95% CI: −1.83, 4.28), and in boys (β = 1.10 cm; 95% CI: −1.36, 3.56).

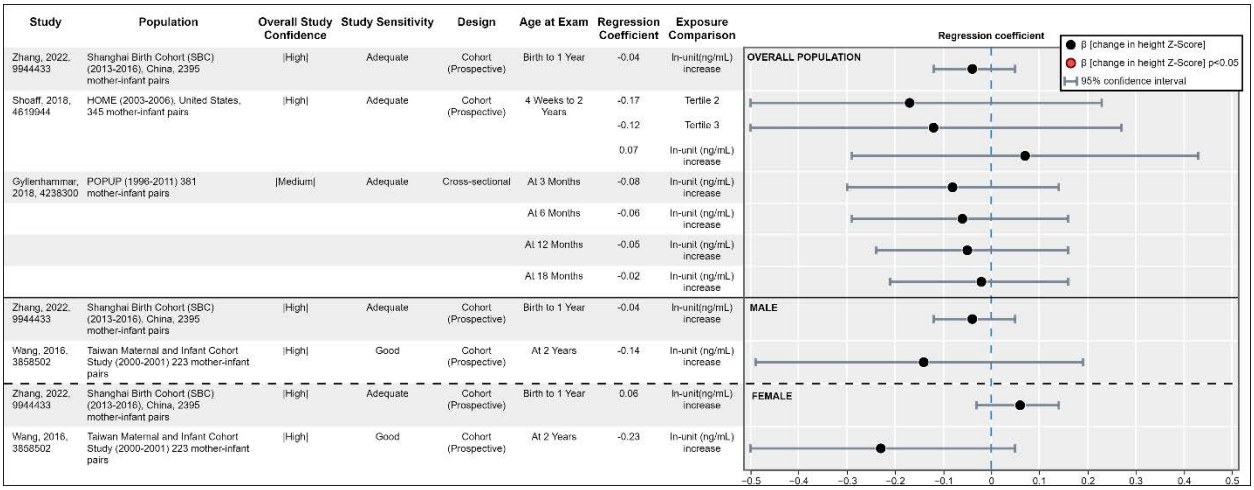


Figure 3-21. PFNA and postnatal growth height standardized measures.[a–d] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Postnatal-Growth-Height-Standardized-9d97/.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Zhang et al. (2022) and Shoaff et al. (2018) examine length-for-age z-score.
[c]Overall population is above the solid black line, while sex-stratified data are below. Within sex-stratified data, boys are above the dashed line, girls below.
[d]Zhang et al. (2022) postnatal height differences estimated from digitization of their Figure A2 (overall population), A3 (boy infants), and A4 (girl infants); Wang et al. (2016) and Gyllenhammar et al. (2018b) standardized postnatal height differences estimated from digitization of their Figures 2 and 3, respectively.

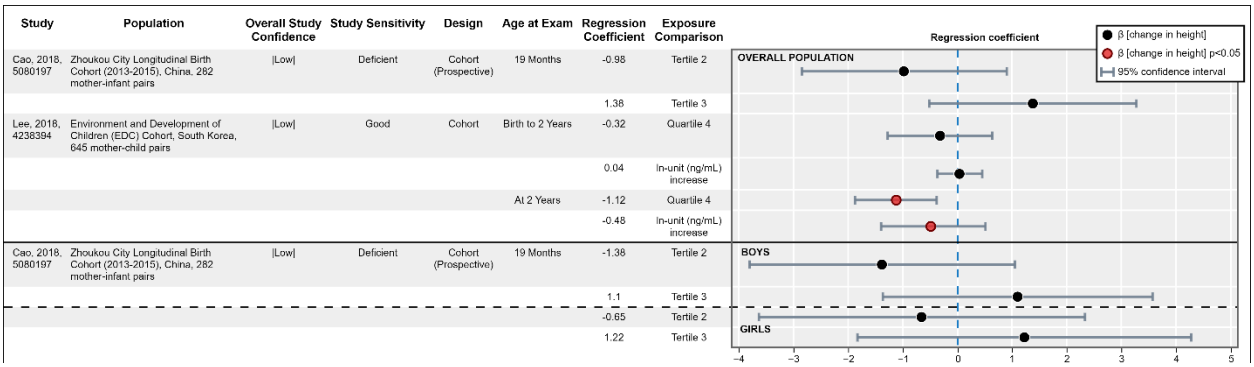


Figure 3-22. PFNA and postnatal growth mean height.[a,b] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Postnatal-Growth-Mean-Height/.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Above the solid black line is overall population data, while below is sex-stratified. Within the sex-stratified data, above the dashed line is boys, below is girls.

Postnatal height summary

Three of six different studies showed some mean or standardized height deficits in early childhood. Three of five of these were null in the overall population and one study showed increased height in relation to PFNA for the overall population and both sexes. Two of the three studies that showed some evidence of inverse associations for standardized height measures were *high* confidence studies, while the mean birth height study was *low* confidence. Few other patterns across the study results were evident, although the age of examination extended to 2 years for these two studies. The *high* confidence study by Wang et al. (2016) detected reductions in average childhood height z-scores (from ages 2 to 11 years) among girls and boys that were comparable in magnitude to that seen for age 2 years only. The average childhood result may, however, be driven by the deficit in birth weight as the age 2 (and at birth) height deficits that were detected did not persist over time. Despite comparable magnitudes observed across the sexes (girls β = –0.23; 95% CI: –0.51, 0.05; boys β = –0.14; 95% CI: –0.49, 0.19) at age 2, the authors reported a statistically significant interaction by sex based on these findings.

Adiposity measures: Overweight status, body mass index, ponderal index and waist circumference

Five studies examined postnatal adiposity-related measures in relation to PFNA exposures (see Table 3-5). This included three *high* confidence studies and one study each being *medium* and *low* confidence. Two studies showed some evidence of increased and decreased adiposity, respectively, while one study Zhang et al. (2022) reported null associations for BMI-for-age z-score in the overall population and both sexes per ln-unit PFNA increase (see Figure 3-23). The *high* confidence study by Starling et al. (2019) reported a slight increase in infant adiposity at age 5 months for each ln-unit increase in PFNA (0.62% fat mass increase; 95% CI: –0.17, 1.41) that

seemed to be driven by results among boys (1.67% fat mass increase; 95% CI: 0.56, 2.78). In contrast, less adiposity as measured by % fat mass was detected among girls (–0.72% fat mass decrease; 95% CI: –1.91, 0.47). The *medium* confidence Jensen et al. (2020a) study examined different measures at 18 months of age and reported larger BMI SDS values in the overall population (β = 0.18; 95% CI: 0.02, 0.34) per ln-unit increase. Results were larger in girls (β = 0.26; 95% CI: 0.03, 0.49) than in boys (β = 0.10; 95% CI: −0.12, 0.33). Similar results for ponderal index SDS values were seen in the overall population (β = 0.24; 95% CI: 0.08, 0.41), in girls (β = 0.36; 95% CI: 0.13, 0.59), and in boys (β = 0.13; 95% CI: −0.10, 0.36). They reported null results for waist circumference in the overall population (β = 0.09; 95% CI: −0.06, 0.24), in girls (β = 0.14; 95% CI: −0.08, 0.35), and in boys (β = 0.04; 95% CI: −0.17, 0.25). Jensen et al. (2020a) also reported that no statistically significant interactions by sex were evident across all endpoints.

Mixed results were seen in the *high* confidence study by Shoaff et al. (2018) for body mass index measured from 4 weeks to 2 years of age in relation to PFNA including null results per ln-unit increase. They did report nonstatistically significant BMI deficits for PFNA tertiles 2 and 3 (β range: −0.09 to −0.16) compared with the tertile 1 referent (≤7 ng/mL). The *low* confidence study by Gross et al. (2020) reported that higher PFNA concentrations (>mean) were associated with lower odds of being overweight at age 18 months (OR = 0.42, 95% CI: 0.17, 1.04) in the overall population. Smaller odds were reported among boys (OR = 0.35; *p*-value = 0.16) than among girls (OR = 0.59; *p*-value = 0.52).

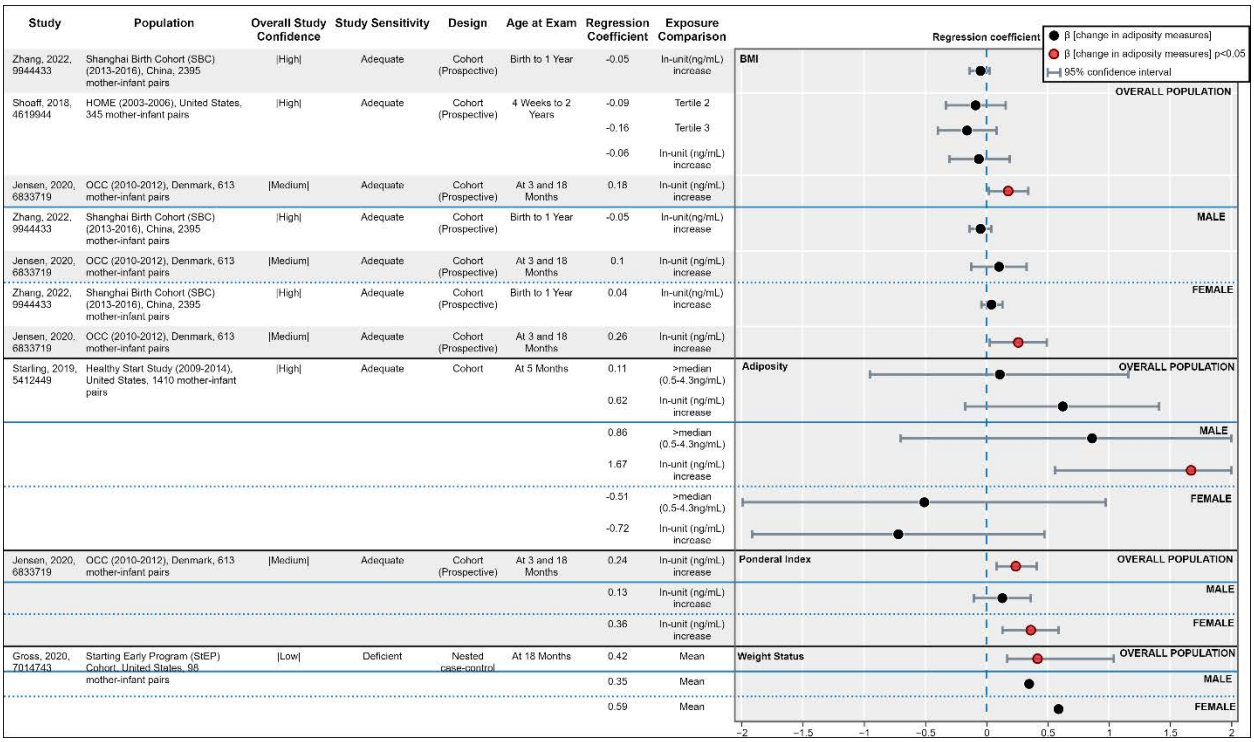


Figure 3-23. PFNA and postnatal growth bmi, adiposity, ponderal index, and weight status.[a–e] See interactive HAWC link: https://hawc.epa.gov/summary/data-

pivot/assessment/100500071/PFNA-and-Postnatal-Growth-BMI-Adiposity-Ponde-14c8/.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Measurement types are separated by the solid black reference lines and are as follows (in descending order): BMI, Adiposity, Ponderal Index, and Weight Status.
[c]Within each category, above the blue solid line are values for overall population, between the two blue dotted lines are values for boys, and below the second dotted line are values for girls.
[d]Zhang et al. (2022) postnatal standardized BMI-for-age differences estimated from digitization of their Figure A2 (overall population), A3 (boy infants), and A4 (girl infants); Gyllenhammar et al. (2018b) standardized postnatal BMI differences estimated from digitization of their Figure 4.
[e]*P*-values only available for sex-specific findings in Gross et al. (2020).

Adiposity measures summary

Only two of five studies showed any suggestion of increase adiposity measures during early childhood, with statistically significant results seen in the overall population and for girls in that study. Few patterns across the study results were evident.

Rapid growth – Background of studies

Four *high* confidence studies (Gao et al., 2022; Starling et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) examined different rapid weight gain measures in relation to PFNA. In the Health Outcomes and Measures of the Environment (HOME) study, Shoaff et al. (2018) examined rapid growth based on weight z-scores in relation to PFNA in the overall population. In the Healthy Start Study, Starling et al. (2019) examined different rapid weight gain measures in relation to PFNA for the overall population and both sexes at 5 months of age. In the Shanghai Birth Cohort, Gao et al. (2022) examined various measures of growth trajectories in the overall population and across sexes for various postnatal growth measures. In the INMA Birth Cohort Study, Manzano-Salgado et al. (2017b) examined rapid growth from birth to 6 months.

Compared with the tertile 1 referent (≤7 ng/mL), Shoaff et al. (2018) detected nonstatistically significant decreased relative risks (β range: 0.75 to 0.85 across PFNA tertiles) for rapid weight gain (defined as an increase in weight z-score >0.67 standard deviations anytime between 4 weeks and 2 years of age) (see Figure 3-24). In contrast, the Starling et al. (2019) study reported null associations for rapid growth based on both weight-for-age (OR = 1.19; 95% CI: 0.73, 1.93) and weight-for-length z-score (OR = 1.14; 95% CI: 0.74, 1.74) per ln-unit PNFA increase. The study by Manzano-Salgado et al. (2017b) was also null for their weight-for-age z-score rapid growth measure (OR = 0.96; 95% CI: 0.79, 1.16).

Per each ln-unit PFNA increase in the Gao et al. (2022) study, most rapid growth relative risks were null based on standardized weight-for-age, weight-for-length, length-for-age, and head circumference measures in the overall population and both sexes at 2 years of age (see Figure 3-25). Compared with the moderate-stable referent, Gao et al. (2022) reported elevated odds of weight-for-age z-score and weight-for-length z-score trajectories for the low-rising (OR = 1.54; 95% CI: 0.91, 2.60; OR = 2.01; 95% CI: 1.15, 5.58, respectively) and low-stable group (OR = 1.36; 95% CI: 0.69, 2.74; OR = 1.51; 95% CI: 0.64, 3.58, respectively). Low-rising was defined, for

example, as those with a low baseline growth trajectory followed by a rapidly increased trend afterward. Among girls, only a decreased odds (OR = 0.40; 95% CI: 0.20, 0.79) for rapid growth based on weight-for-length data was observed in the low-rising trajectory group relative to moderate-stable one. The authors found several inverse associations for PFNA and rapid growth head circumference z-scores including in the high-stable comparison groups among boys (OR = 0.49; 95% CI: 0.22, 1.09). A decreased odds for rapid growth based on head circumference scores was also seen in girls for the low-stable trajectory group (OR = 0.50; 95% CI: 0.28, 0.90). Similar odds were seen among the overall population (OR = 0.53; 95% CI: 0.26, 1.10) and girls (OR = 0.40; 95% CI: 0.14, 1.13) for the low-rising trajectory group. These growth trajectory data are not consistent across sexes and are difficult to interpret, but some results may suggest that some high and low growth trajectories were associated with lower odds of rapid growth in comparison to the moderate-stable growth referent.

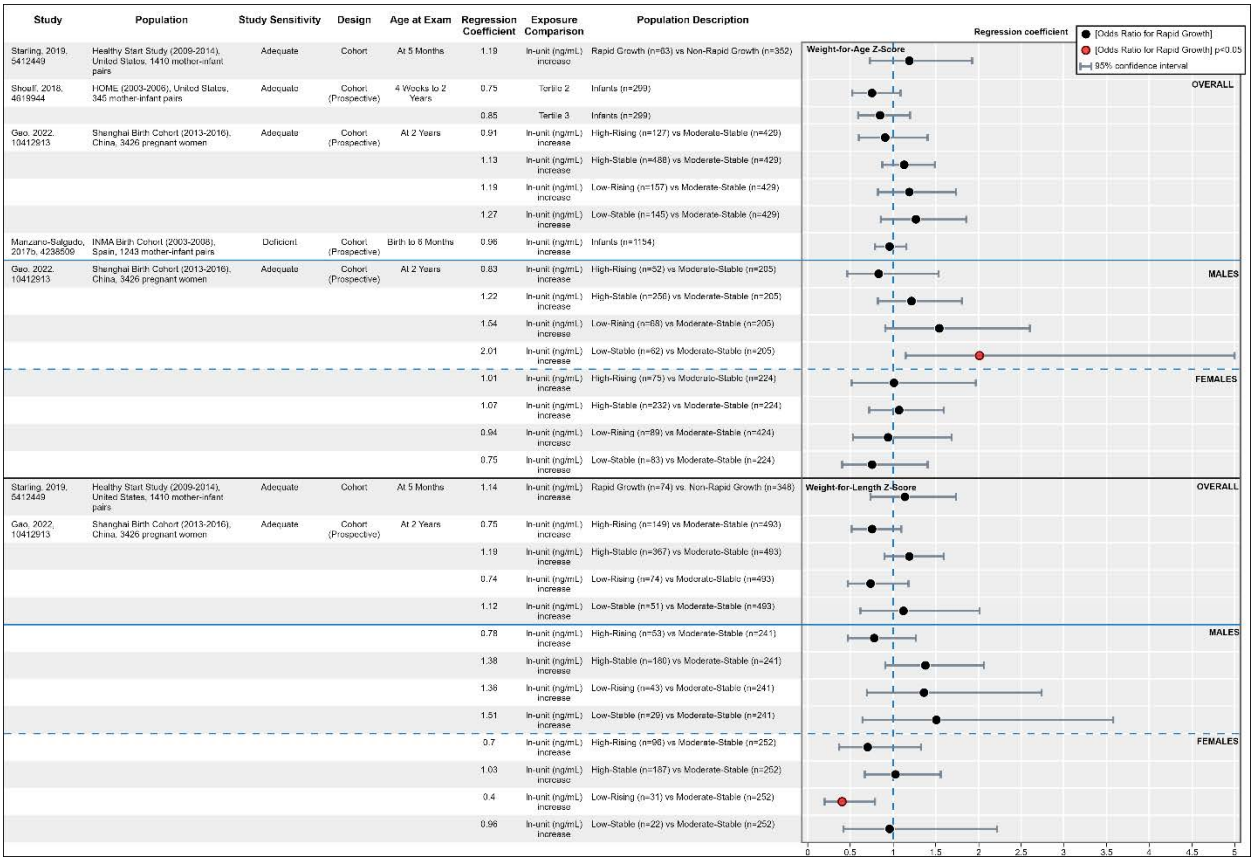


Figure 3-24. PFNA and postnatal growth – rapid growth (weight-for-age and weight-for-length z-score).[a–e] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Postnatal-Growth-Rapid-Growth-Weight-b701/.

[a]All studies were high confidence, with exception of *medium* confidence (Manzano-Salgado et al., 2017b)
[b]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[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: boy infants above the blue dash-dotted line; girls below.

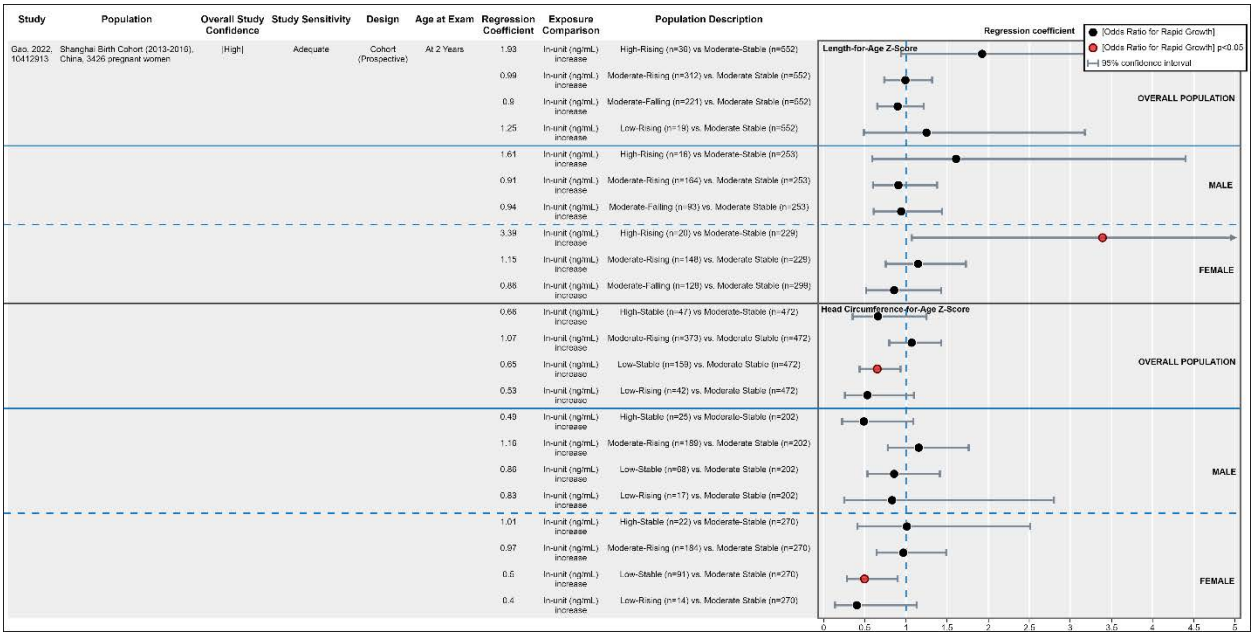


Figure 3-25. PFNA and postnatal growth – rapid growth (length-for-age and head circumference z-score).[a–d] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Postnatal-Growth-Rapid-Growth-Length-1a5f/.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Age at Outcome Measurement: Gao et al. (2022) modeled data (collected at 42 days, 6 months, 12 months, and 24 months).
[c]Length-for-Age Z-Score data above the black reference line; Head Circumference Z-Score below.
[d]Within each outcome group, overall population data are above the solid blue lines and sex-stratified data below blue solid line; boys above blue dotted line; girls below.

Rapid weight gain summary

Only one of the four studies showed any evidence of increased odds for rapid growth with increasing PFNA exposures, although results were not always internally consistent. For example, little evidence of increased odds for rapid growth was shown in the low- and high-rising trajectories groups in Gao et al. (2022). In that study, more evidence of inverse associations was seen among low-stable and some low-rising trajectories. Thus, the overall rapid growth evidence was judged inconclusive on rapid growth given limited associations detected and inconsistent results within and across studies for different rapid growth measures as well as across sexes.

Postnatal growth summary

There was some evidence of association for different postnatal growth measures, namely with the stronger evidence for decreased weight (5 of 8 studies showing inverse associations), with increasing PFNA exposures. More limited evidence, however, was seen for height (2 of 6 studies showing inverse associations), adiposity (2 of 5 studies showing increased adiposity), and rapid growth (1 of 4 studies showing increased rapid growth across various measures). As noted above and in other publications (Perng et al., 2016b), a catch-up period with rapid weight gain is sometimes observed among infants with reduced birth weight. However, four publications examining this hypothesis presented little evidence.

Among the five studies that showed inverse association between PFNA and postnatal weight, three were *high* confidence studies and two were *low* confidence studies. An exposure-response relationship was observed for weight-for-length z-scores in the lone *high* confidence study with categorical data based on the overall population, while one *low* confidence study examining mean weight changes showed some evidence of monotonicity among boys only. An exposure-response relationship was also seen in one of three studies examining postnatal height and PFNA exposures based on categorical data.

Overall, the evidence of inverse associations with postnatal growth was mixed and largely limited to weight, height, and adiposity. Although no patterns were immediately evident across study results, the examination ages for these endpoints varied across studies, which precluded more direct comparisons. Nonetheless, the overall data provide some evidence of associations between both categorical and continuous PFNA exposures and some postnatal growth reductions.

Table 3-5. Summary of 11 epidemiologic studies of PFNA exposure and fetal/neonatal and postnatal growth restriction

Author	Study location/ years	Sample size	Median exposure (range) in ng/mL	Weight	Height	Head circumference	Adiposity	Rapid growth
***High* confidence studies**								
Gao et al. (2022)	China, 2013–2016	1,350	0.54 (0.21, 3.75)					↓ Overall
Manzano-Salgado et al. (2017b)	Spain, 2003–2008	1,154	0.58 (0.05, 11.01)	∅ Overall/ Girls/Boys				∅ Overall
Shoaff et al. (2018)	OH, USA, 2003–2006	345	1.5 (0.1, 32.5)	– Overall	– Overall		– Overall	↓ Overall
Starling et al. (2019)	CO, USA, 2009–2014	415	0.7 (0.2, 2.8)[b]	∅ Overall – Girls + Boys			+ Overall/Boys – Girls	∅ Overall
Wang et al. (2016)	Taiwan, 2000–2001	223	1.55–Boys 1.58–Girls	– Boys + Girls	– Overall/ Girls/Boys			
Zhang et al. (2022)	China, 2013–2016	2,395	0.53 (0, 25.4)	∅ Overall/Boys + Girls	∅ Overall/Boys/Girls	∅ Overall/Boys/Girls	∅ Overall/ Boys/Girls	
***Medium* confidence studies**								
Gyllenhammar et al. (2018b)	Sweden, 1996–2001	381	2.4 (0.32, 26.0)	+ Overall	∅ Overall	+ Overall		
Jensen et al. (2020a)	Denmark, 2010–2012	589	0.66 (0.33, 1.52)[b]				+ Overall/Girls ∅ Boys	
***Low* confidence studies**								

Author	Study location/ years	Sample size	Median exposure (range) in ng/mL	Weight	Height	Head circumference	Adiposity	Rapid growth
Cao et al. (2018)	China, 2013–2015	337	0.09 (0.03, 0.31)[c]	∅ Overall – Boys[a] + Girls	+ Overall/Girls ∅ Boys	– Overall/ Girls ∅ Boys		
Gross et al. (2020)	USA, 2014	98	0.108 (N/A)[d]				↓ Overall/ Girls/Boys	
Lee et al. (2018)	S. Korea, 2012–2013	361	1.43 (0.15, 22.0)	– Overall*	– Overall*[a]			

N/A: not available; ∅ : null association; + : positive association; - : negative association; ↑: increased odds ratio; ¯ : decreased odds ratio.
*Statistically significant results based on $p < 0.05$.
Note: "Adverse effects" are indicated by both increased ORs (↑) for dichotomous outcomes and negative associations (-) for the other outcomes.
/ Denotes multiple groups with the same direction of associations
[a]Exposure-response relationship detected based on categorical data.
[b]No range provided but 5th–95th percentiles included.
[c]No range provided but 10th–90th percentiles included.
[d]Dried Blood spot PFNA sample collected within 48 hours of birth.

Gestational duration

Background of Studies

Eighteen studies examined mean and standardized gestational age differences in relation to PFNA in the overall population (n = 16) or either sex (n = 7) (see Figure 3-26). Two (Hall et al., 2022; Lind et al., 2017a) studies examined only sex-specific associations. Ten studies provided analyses of both preterm delivery and gestational age in relation to PFNA exposures.

Twelve of the 18 gestational duration studies were nested case-control studies or prospective cohort studies (Yang et al., 2022a; Gardener et al., 2021; Hjermitslev et al., 2020; Huo et al., 2020; Gao et al., 2019; Workman et al., 2019; Buck Louis et al., 2018; Meng et al., 2018b; Sagiv et al., 2018; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016), and 6 were either cross-sectional studies or analyses (Hall et al., 2022; Eick et al., 2020; Xu et al., 2019a; Gyllenhammar et al., 2018b; Li et al., 2017; Chen et al., 2012). The epidemiological studies examined here had maternal exposure biomarkers collected either during trimesters 1 (Buck Louis et al., 2018; Lind et al., 2017a; Manzano-Salgado et al., 2017a), 2 (Huo et al., 2020), or 3 (Gardener et al., 2021; Gao et al., 2019) or across multiple trimesters (Eick et al., 2020; Hjermitslev et al., 2020; Workman et al., 2019; Meng et al., 2018b; Sagiv et al., 2018; Bach et al., 2016), or had postpartum maternal or infant samples (Hall et al., 2022; Yang et al., 2022a; Xu et al., 2019a; Gyllenhammar et al., 2018b; Li et al., 2017; Chen et al., 2012).

Nine studies each were classified as having late (defined as trimester 2 exclusive onward) and early biomarker sampling (defined as having at least some trimester 1 maternal sampling). Five of the six cross-sectional studies/analyses had late biomarker sampling. Among the 12 cohort or nested case-control studies, 7 studies had early biomarker sampling (Hjermitslev et al., 2020; Buck Louis et al., 2018; Meng et al., 2018b; Sagiv et al., 2018; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016), while 5 were classified as late (Yang et al., 2022a; Gardener et al., 2021; Huo et al., 2020; Gao et al., 2019; Workman et al., 2019).

Eight of the 18 studies that examined gestational duration in relation to PFNA exposures were *high* confidence (Gardener et al., 2021; Eick et al., 2020; Huo et al., 2020; Buck Louis et al., 2018; Sagiv et al., 2018; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016), 6 were *medium* confidence (Hall et al., 2022; Yang et al., 2022a; Hjermitslev et al., 2020; Gyllenhammar et al., 2018b; Meng et al., 2018b; Chen et al., 2012), and 4 were *low* confidence (Gao et al., 2019; Workman et al., 2019; Xu et al., 2019a; Li et al., 2017). One study (Huo et al., 2020) received a good rating in the study sensitivity domain, while 12 were considered adequate (Gardener et al., 2021; Eick et al., 2020; Hjermitslev et al., 2020; Gao et al., 2019; Buck Louis et al., 2018; Gyllenhammar et al., 2018b; Meng et al., 2018b; Sagiv et al., 2018; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016; Chen et al., 2012), and 5 were deficient (Hall et al., 2022; Yang et al., 2022a; Workman et al., 2019; Xu et al., 2019a; Li et al., 2017).

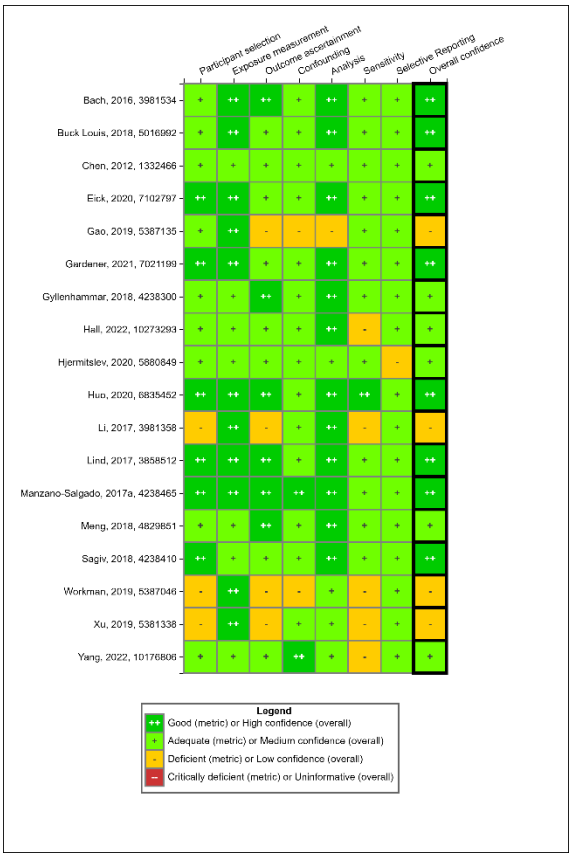


Figure 3-26. Study evaluation heat map of 18 epidemiologic studies of gestational duration and PFNA exposure. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-Gestational-Duration-Measures/.

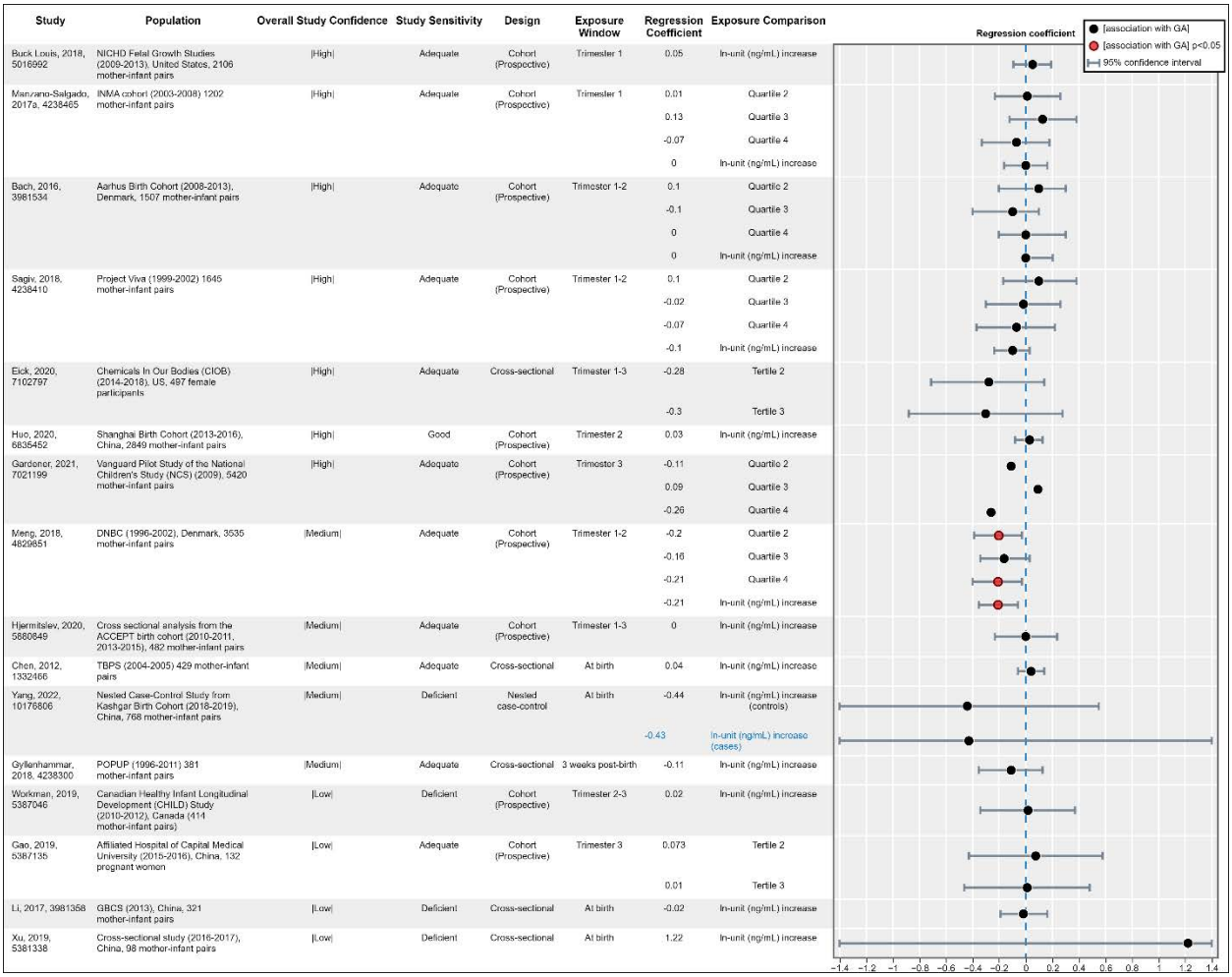
Gestational age – Overall population results

Six of 16 studies showed some inverse associations between PFNA exposure and reduced gestational age in the overall population with all of these occurring in *high* and *medium* confidence studies. Nine of 16 studies examining gestational age in the overall population were null (Hjermitslev et al., 2020; Huo et al., 2020; Gao et al., 2019; Workman et al., 2019; Buck Louis et al., 2018; Li et al., 2017; Manzano-Salgado et al., 2017a; Bach et al., 2016; Chen et al., 2012), while one study showed larger mean gestational age in relation to PFNA (Xu et al., 2019a) (see Figures 3-27; 3-28; Table 3-6).

Six of 12 *high* and *medium* confidence studies showed some inverse associations between PFNA exposure and reduced gestational age in the overall population (see Figure 3-27). The *medium* confidence study by Gyllenhammar et al. (2018b) and the *high* confidence study by Sagiv et al. (2018) showed small nonsignificant decreases in gestational age (β = −0.11 weeks; 95% CI: −0.35, 0.13 and β = −0.10 weeks; 95% CI: −0.24, 0.03, respectively) per ln-unit PFNA increase. The *medium* confidence study by Meng et al. (2018b) reported a statistically significant decrease in gestational age in quartile 4 (β = −0.21 weeks; 95% CI: −0.40, −0.03) and nonmonotonic results

across the quartiles (β range: −0.16 to −0.21 weeks). They also reported a statistically significant decrease similar in magnitude per ln-unit increase (β = −0.21 weeks; 95% CI: −0.35, −0.06). The *high* confidence Gardener et al. (2021) study reported an inverse association similar in magnitude for quartile 4 relative to quartile 1 (Q4 β = −0.26 weeks; 95% CI: not provided) as did the *high* confidence study by Eick et al. (2020) across PFNA tertile 3 (β = −0.30 weeks; 95% CI: −0.88, 0.28) and tertile 2 (β = −0.28 weeks; 95% CI: −0.71, 0.14). The *medium* confidence study in the overall population by Yang et al. (2022a) saw larger and similar mean gestational age differences (in magnitude) among preterm (β = −0.43 weeks; 95% CI: −1.43, 0.55) and term infants (β = −0.44 weeks; 95% CI: −4.23, 3.36).

Although the results were inconsistent across studies, there was some evidence of associations between PFNA exposure and reduced gestational age in the overall population including 6 of 12 *high* and *medium* confidence (and 6 of 16 in total) studies. None of the seven studies with categorical data showed any evidence of exposure-response relationships across different quantiles that would be supportive of the results shown based on continuous exposure comparisons. There was no evidence of any impact of pregnancy hemodynamics on the inverse associations as three of the six studies in the overall population were based on early pregnancy biomarkers. No other patterns were evident across these study results although six of the nine null studies had adequate or good study sensitivity.



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Buck Louis, 2018, 5016992	NICHD Fetal Growth Studies (2009-2013), United States, 2106 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0.05	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0.01	Quartile 2
						0.13	Quartile 3
						-0.07	Quartile 4
						0	ln-unit (ng/mL) increase
Bach, 2016, 3981534	Aarhus Birth Cohort (2008-2013), Denmark, 1507 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1-2	0.1	Quartile 2
						-0.1	Quartile 3
						0	Quartile 4
						0	ln-unit (ng/mL) increase
Sagiv, 2018, 4238410	Project Viva (1999-2002) 1645 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1-2	0.1	Quartile 2
						-0.02	Quartile 3
						-0.07	Quartile 4
						-0.1	ln-unit (ng/mL) increase
Eick, 2020, 7102797	Chemicals In Our Bodies (CIOB) (2014-2018), US, 497 female participants	|High|	Adequate	Cross-sectional	Trimester 1-3	-0.28	Tertile 2
						-0.3	Tertile 3
Huo, 2020, 6835452	Shanghai Birth Cohort (2013-2016), China, 2849 mother-infant pairs	|High|	Good	Cohort (Prospective)	Trimester 2	0.03	ln-unit (ng/mL) increase
Gardener, 2021, 7021199	Vanguard Pilot Study of the National Children's Study (NCS) (2009), 5420 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 3	-0.11	Quartile 2
						0.09	Quartile 3
						-0.26	Quartile 4
Meng, 2018, 4829851	DNBC (1996-2002), Denmark, 3535 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-2	-0.2	Quartile 2
						-0.16	Quartile 3
						-0.21	Quartile 4
						-0.21	ln-unit (ng/mL) increase
Hjermitslev, 2020, 5880849	Cross sectional analysis from the ACCEPT birth cohort (2010-2011, 2013-2015), 482 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-3	0	ln-unit (ng/mL) increase
Chen, 2012, 1332466	TBPS (2004-2005) 429 mother-infant pairs	|Medium|	Adequate	Cross-sectional	At birth	0.04	ln-unit (ng/mL) increase
Yang, 2022, 10176806	Nested Case-Control Study from Kashgar Birth Cohort (2018-2019), China, 768 mother-infant pairs	|Medium|	Deficient	Nested case-control	At birth	-0.44	ln-unit (ng/mL) increase (controls)
						-0.43	ln-unit (ng/mL) increase (cases)
Gyllenhammar, 2018, 4238300	POPUP (1996-2011) 381 mother-infant pairs	|Medium|	Adequate	Cross-sectional	3 weeks post-birth	-0.11	ln-unit (ng/mL) increase
Workman, 2019, 5387046	Canadian Healthy Infant Longitudinal Development (CHILD) Study (2010-2012), Canada (414 mother-infant pairs)	|Low|	Deficient	Cohort (Prospective)	Trimester 2-3	0.02	ln-unit (ng/mL) increase
Gao, 2019, 5387135	Affiliated Hospital of Capital Medical University (2015-2016), China, 132 pregnant women	|Low|	Adequate	Cohort (Prospective)	Trimester 3	0.073	Tertile 2
						0.01	Tertile 3
Li, 2017, 3981358	GBCS (2013), China, 321 mother-infant pairs	|Low|	Deficient	Cross-sectional	At birth	-0.02	ln-unit (ng/mL) increase
Xu, 2019, 5381338	Cross-sectional study (2016-2017), China, 98 mother-infant pairs	|Low|	Deficient	Cross-sectional	At birth	1.22	ln-unit (ng/mL) increase

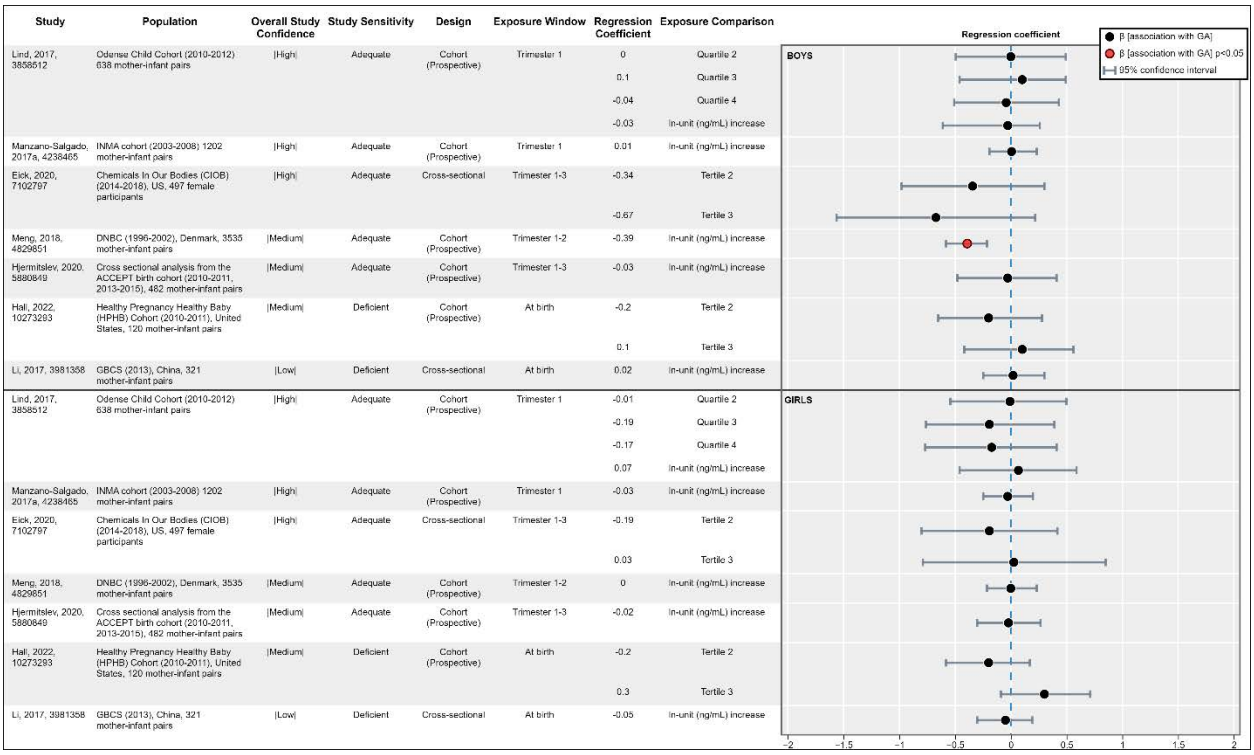
Figure 3-27. PFNA and gestational age in the overall population.[a–g] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Gestational-Age/.

Abbreviations: GA = gestational age.
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Gardener et al. (2021) gestational age differences estimated from digitization of their Figure 4; 95% CIs were not estimable.
[c]Yang et al. (2022a) preterm results in blue are truncated; the complete 95% CI ranges from −4.23 to 3.36.
[d]Yang et al. (2022a) term results are truncated; the complete 95% CI ranges from −1.43 to 0.55.
[e]Xu et al. (2019a) results are truncated; the complete 95% CI ranges from −3.49 to 5.93.
[f]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses as labeled in the design column (e.g., Yang et al. (2022a)).
[g]Sagiv et al. (2018) gestational age differences estimated from digitization of their Figure 1B.

Gestational age – Sex-specific results

Three of seven studies showed some inverse associations between PFNA exposure and reduced gestational age in either sex, including three (two *high* and one *medium*) of six *medium* and *high* confidence studies (see Figure 3-28). The high confidence study by Lind et al. (2017a) showed null results for boys and girls especially for each per ln-unit PFNA increase but did report inverse associations among quartiles 3 and 4 among girls only. The *medium* confidence study by Meng et al.

(2018b) reported a statistically significant decrease in gestational age for boys only (β = −0.39 weeks; 95% CI: −0.58, −0.21). The *high* confidence study by Eick et al. (2020) saw some evidence of an exposure-response relationship for mean gestational age and PFNA tertiles (T2 β: −0.34 weeks; 95% CI: −0.98, 0.30; T3 β: −0.67 weeks; 95% CI: −1.56, 0.22) in boys only. The other two studies that reported quartile exposure data did not report evidence of inverse exposure-response relationships.



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
BOYS							
Lind, 2017, 3858512	Odense Child Cohort (2010-2012) 638 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0	Quartile 2
						0.1	Quartile 3
						-0.04	Quartile 4
						-0.03	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	0.01	ln-unit (ng/mL) increase
Eick, 2020, 7102797	Chemicals In Our Bodies (CIOB) (2014-2018), US, 497 female participants	|High|	Adequate	Cross-sectional	Trimester 1-3	-0.34	Tertile 2
						-0.67	Tertile 3
Meng, 2018, 4829851	DNBC (1996-2002), Denmark, 3535 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-2	-0.39	ln-unit (ng/mL) increase
Hjermitslev, 2020, 5880849	Cross sectional analysis from the ACCEPT birth cohort (2010-2011, 2013-2015), 482 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-3	-0.03	ln-unit (ng/mL) increase
Hall, 2022, 10273293	Healthy Pregnancy Healthy Baby (HPHB) Cohort (2010-2011), United States, 120 mother-infant pairs	|Medium|	Deficient	Cohort (Prospective)	At birth	-0.2	Tertile 2
						0.1	Tertile 3
Li, 2017, 3981358	GBCS (2013), China, 321 mother-infant pairs	|Low|	Deficient	Cross-sectional	At birth	0.02	ln-unit (ng/mL) increase
GIRLS							
Lind, 2017, 3858512	Odense Child Cohort (2010-2012) 638 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	-0.01	Quartile 2
						-0.19	Quartile 3
						-0.17	Quartile 4
						0.07	ln-unit (ng/mL) increase
Manzano-Salgado, 2017a, 4238465	INMA cohort (2003-2008) 1202 mother-infant pairs	|High|	Adequate	Cohort (Prospective)	Trimester 1	-0.03	ln-unit (ng/mL) increase
Eick, 2020, 7102797	Chemicals In Our Bodies (CIOB) (2014-2018), US, 497 female participants	|High|	Adequate	Cross-sectional	Trimester 1-3	-0.19	Tertile 2
						0.03	Tertile 3
Meng, 2018, 4829851	DNBC (1996-2002), Denmark, 3535 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-2	0	ln-unit (ng/mL) increase
Hjermitslev, 2020, 5880849	Cross sectional analysis from the ACCEPT birth cohort (2010-2011, 2013-2015), 482 mother-infant pairs	|Medium|	Adequate	Cohort (Prospective)	Trimester 1-3	-0.02	ln-unit (ng/mL) increase
Hall, 2022, 10273293	Healthy Pregnancy Healthy Baby (HPHB) Cohort (2010-2011), United States, 120 mother-infant pairs	|Medium|	Deficient	Cohort (Prospective)	At birth	-0.2	Tertile 2
						0.3	Tertile 3
Li, 2017, 3981358	GBCS (2013), China, 321 mother-infant pairs	|Low|	Deficient	Cross-sectional	At birth	-0.05	ln-unit (ng/mL) increase

Figure 3-28. PFNA and gestational age (sex-stratified: boys above reference line, girls below).[a–c] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Gestational-Age-Sex-Stratified-Boys--7535/.

Abbreviations: GA = gestational age.
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).
[c]Hall et al. (2022) gestational age differences estimated from digitization of their Figure S3.

Gestational age summary

Although the results were inconsistent across studies, there was some evidence of associations between PFNA exposure and reduced gestational age. Seven (4 *high* and 3 *medium*) of 18 studies in the overall population or either/both sexes showed some inverse associations between PFNA exposure and mean gestational age including 7 of 14 *high* and *medium* confidence studies. Null associations were detected in 9 of the 18 studies in the overall population and/or both

sexes, while 2 other studies showed some evidence of larger gestational age with increasing PFNA exposures. Six of 12 *high* and *medium* confidence (and 6 of 16 in total) studies showed inverse associations in the overall population. Two of the three sex-specific studies that showed some inverse associations were found only in boys. Overall, only one of nine different studies with categorical data showed dose-dependent results that would support the findings based on the continuous exposure data.

There was no evidence of any impact of pregnancy hemodynamics on study results as four of the seven different studies that showed some evidence of inverse associations were based on early pregnancy biomarkers. No other patterns were evident across these study results although six of the nine null studies had adequate or good study sensitivity.

Preterm birth – Background of studies

Two (Huo et al., 2020; Manzano-Salgado et al., 2017a) of the 10 preterm birth (typically defined as <37 gestational weeks) studies reported sex-specific findings in addition to overall population results (see Figures 3-28 and 3-29). Six of the 10 studies that examined preterm birth in relation to PFNA exposures were *high* confidence (Eick et al., 2020; Huo et al., 2020; Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016; Chen et al., 2012) and four were *medium* confidence (Yang et al., 2022a; Hjermitslev et al., 2020; Meng et al., 2018b; Chen et al., 2012) (see Figure 3-28; Table 3-6). Eight studies had adequate sensitivity, while one was good (Huo et al., 2020) and one deficient (Yang et al., 2022a).

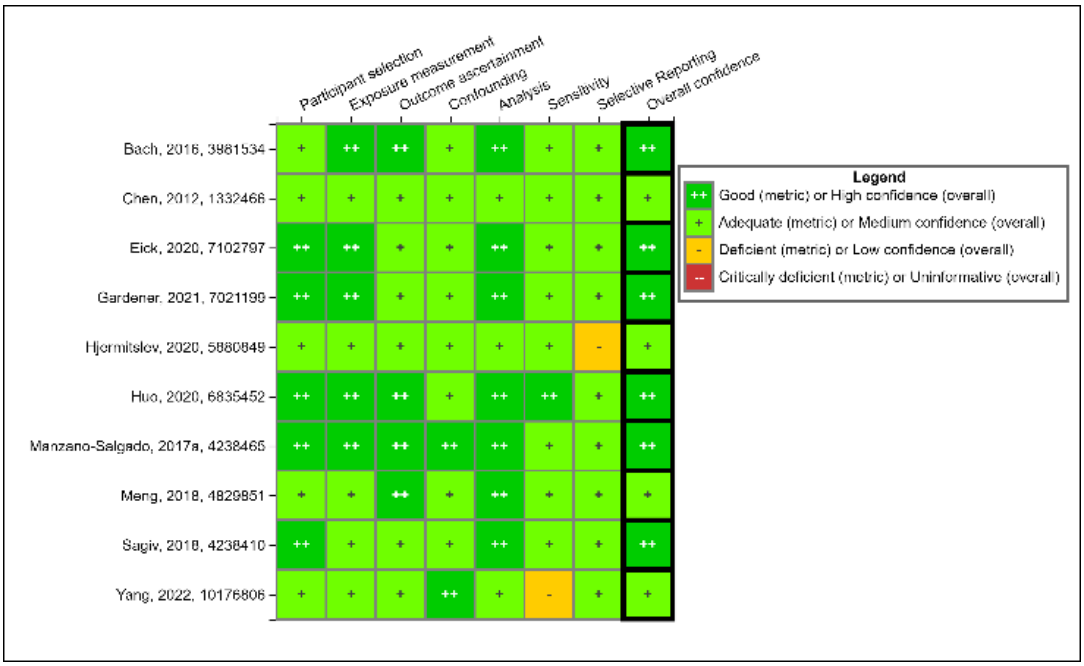


Figure 3-29. Study evaluation heat map of 10 epidemiologic studies of preterm birth and PFNA exposure. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-PTB/.

Preterm birth – Overall population study results

Overall, 4 of 10 studies (all studies were either *medium* or *high* confidence) in the overall population showed some increased risks for preterm birth in relation to PFNA (see Figure 3-30). Null associations were found for preterm birth and PFNA exposures among the overall population in three of the six *high* confidence studies (Huo et al., 2020; Manzano-Salgado et al., 2017a; Bach et al., 2016) and two of four *medium* confidence studies (Yang et al., 2022a; Chen et al., 2012). In contrast, the *medium* confidence study by Hjermitslev et al. (2020) reported a decreased risk of preterm birth (OR = 0.56; 95% CI: 0.21, 1.50) per PFNA ln-unit increase.

Increased risks for preterm birth in relation to PFNA were reported in three *high* confidence studies and one *medium* study. The *high* confidence study by Sagiv et al. (2018) reported an increased OR for preterm birth per ln-unit increase (OR = 1.30; 95% CI: 1.00, 1.61) as well as in the third (OR = 1.4; 95% CI: 0.8, 2.6) and fourth (OR = 1.7; 95% CI: 0.9, 3.0) PFNA quartiles. The *high* confidence Gardener et al. (2021) study reported increased ORs only for quartile 4 relative to quartile 1 (Q4 OR = 2.38; 95% CI: 0.87, 6.49). The *medium* confidence study by Meng et al. (2018b) reported increased ORs for preterm birth of similar magnitude per ln-unit increase (OR = 1.63; 95% CI: 0.86, 2.92) and for quartile 4 (OR = 1.7; 95% CI: 0.8, 3.3). They also found larger effect estimates for very preterm birth defined as either <35 completed gestational weeks (OR = 3.1; 95% CI: 1.2, 9.3) or as <36 completed gestational weeks (OR = 3.3; 95% CI: 1.3, 7.9) (data not plotted). Although the *high* confidence study by Eick et al. (2020) was somewhat limited by small preterm birth cell sizes, increased ORs were seen across tertiles in a monotonic fashion (T2 OR = 1.88; 95% CI: 0.83, 4.30; T3 OR = 2.06; 95% CI: 0.72, 5.89). An exposure-response relationship was not detected in the other five studies examining categorical exposures, although monotonicity was seen among the upper two quartiles for both the Gardener et al. (2021) and Sagiv et al. (2018) studies.

Overall, 4 of 10 studies (3 *high* confidence studies and 1 *medium* study) reported increased risks for preterm birth in relation to PFNA exposures. Few patterns were evident across the study results. For example, four of the five null studies based on the overall population had either adequate or good study sensitivity. Three of the four studies showing increased risk were based on early biomarker sampling, suggesting that pregnancy hemodynamics was an unlikely explanation of these results.

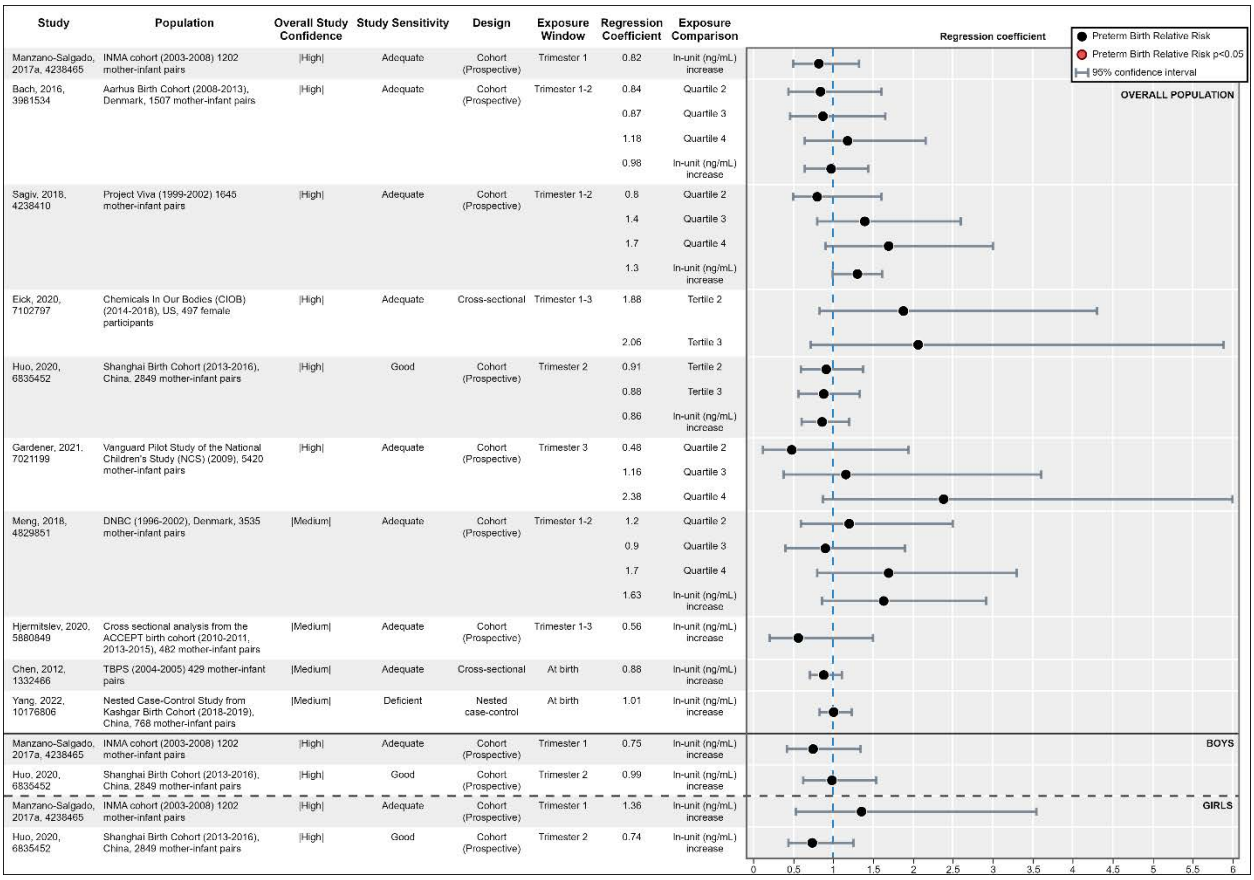


Figure 3-30. PFNA and preterm birth.[a–d] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-Preterm-Birth/.

Abbreviations: PTB = preterm birth.
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Sex specific data below solid black line; newborn boys above dotted line, newborn girls below.
[c]Gardener et al. (2021) Quartile 4 results are truncated: the complete 95% CI ranges from 0.87 to 6.49.
[d]For evaluation of patterns of all study results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (as reflected above in design column).

Preterm birth – Sex-specific results

The results across sexes were mixed with limited evidence of increased odds of preterm birth seen for either sex (see Figure 3-30). Per ln-unit PFNA increase, the *high* confidence Manzano-Salgado et al. (2017a) reported nonsignificant results in the opposite direction for boys (OR = 0.75; 95% CI: 0.42, 1.35) and girls (OR = 1.36; 95% CI: 0.53, 3.14). The *high* confidence Huo et al. (2020) study showed null results for boys (OR = 0.99; 95% CI: 0.63, 1.54) and an inverse association for preterm births among girls (OR = 0.74; 95% CI: 0.44, 1.25) per ln-unit increase.

Gestational duration summary

Overall, there was mixed evidence of associations between PFNA and gestational duration endpoints between and within studies. Seven (4 *high* and 3 *medium* confidence) different studies (of 18) examining either preterm birth or gestational age in relation to PFNA exposures reported some gestational duration impacts in either the overall population or across one of the sexes. Across all groups, 7 of 18 gestational age studies reported inverse associations in relation to PFNA exposures; this included 6 of 16 in the overall population (3 *high* and 3 *medium* confidence). The sex-specific results were more mixed across sexes (3 of 7 studies: 2 *high* and 1 *medium* confidence) with neither sex consistently showing much evidence of associations although statistical power may have been limited in these analyses. There was considerable variability in the magnitude of gestational age differences reported per ln-unit change in the overall population (β range: −0.10 to −0.44 cm). The three studies reporting inverse associations in the overall population showed comparable values based on the highest quantiles (β range: −0.21 to −0.30 cm).

Despite some inconsistency, more evidence was seen for PFNA and preterm birth. For example, 5 (4 *high* and 1 *medium* confidence) of 10 preterm birth studies reported increased ORs and PFNA exposures in either the overall population or either sex. This was limited primarily to 4 of 10 studies in the overall population. Among the four studies with categorical data that showed increased risks, the magnitude of the ORs were consistent in magnitude across the highest quantile (OR range: 1.7 to 2.4). The only evidence of dose-dependence across PFNA quantiles for preterm birth or gestational age was found in one of nine different studies regardless of populations examined. For example, none of seven gestational age studies in the overall population and only one of three sex-specific studies (i.e., in boys only) detected exposure-response relationships. Similarly, only one of six preterm studies showed dose-dependence based on categorical data.

All eight of the studies that reported some evidence of associations between PFNA and gestational duration endpoints were either *medium* (n = 3) or *high* (n = 5) overall confidence with seven considered adequate in the study sensitivity domain. Therefore, there is an overall low risk of bias for this set of studies and endpoints, and few patterns were evident that explained study results. For example, there was no evidence that pregnancy hemodynamic changes related to biomarker sample timing were related to inverse gestational duration associations as half of the preterm birth and gestational age studies in the overall population and all three gestational age studies among the sexes were based on early biomarker sampling. Gestational age estimates can be prone to measurement error if not based on early ultrasound measures, which can reduce study sensitivity to detect an association; thus, more weight is given to the preterm birth findings compared with the largely null results seen for the gestational age analyses. This is due to less impact of binary preterm birth classifications being anticipated as a result of gestational age mismeasurement.

Table 3-6. Summary of nine epidemiologic studies of PFNA exposure and gestational duration measures

Author	Study location/ years	n	Median (ng/mL) exposure	Confidence judgment	Study sensitivity domain judgment	Preterm birth	Gestational age
Bach et al. (2016)	Denmark, 2008–2013	1,533	0.8	*High*	Adequate	∅ All	∅ All
Lind et al. (2017a)	Denmark, 2010–2012	638	0.7	*High*	Adequate		∅ All
Manzano-Salgado et al. (2017a)	Spain, 2003–2008	1,202	0.66	*High*	Adequate	∅ All	∅ All
Sagiv et al. (2018)	USA, 1999–2002	1,645	0.7	*High*	Adequate	↑ All	∅ All
Chen et al. (2012)	Taiwan, 2004–2005	429	2.36	*Medium*	Adequate	∅ All	∅ All
Gyllenhammar et al. (2018b), Swedish Environmental Protection Agency (2017) ‡	Sweden, 1996–2001	381	0.41	*Medium*	Adequate		− All
Meng et al. (2018b)	Denmark, 1996–2002	3,535	0.5	*Medium*	Adequate	↑ All*	− All/Boys* ∅ Girls
Li et al. (2017)	China, 2013	321	0.2	*Low*	Deficient		∅ All
Xu et al. (2019a)	China, 2016–2017	98	0.26	*Low*	Deficient		+ All

*$p < 0.05$; ∅ : null association; + : positive association; − : negative (inverse) association; OR = odds ratio; ↑: increased OR; ↓: decreased OR.

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

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

Birth defects

Two studies examined birth defects in relation to PFNA exposures (see Figure 3-31). The *medium* confidence congenital heart defect study by Ou et al. (2021) reported nonsignificant increased odds for conotruncal defects (OR = 1.62; 95% CI: 0.67, 3.92), septal defects (OR = 1.27; 95% CI: 0.58, 2.80), and total heart defects (OR = 1.52; 95% CI: 0.90, 2.58). Relative to tertile 1, the *low* confidence Cao et al. (2018) study showed evidence of an association between all birth defects and PFNA tertile 3 (OR = 1.56; 95% CI: 0.69, 3.52). There is considerable uncertainty in interpreting results for this broad "all birth defect" groupings that likely decreases study sensitivity given the etiological heterogeneity across different birth defects.

There was some suggestive evidence of associations between PFNA and birth defects based on the two available epidemiological studies with all ORs ranging from 1.3 to 1.6 across different types of defects. The most consistent evidence was in the *medium* confidence study, which showed consistency in the magnitude of associations for different types of heart defects. There was no evidence of exposure-response relationships based on one *low* confidence study with categorical data. Overall, some evidence of associations between PFNA and birth defects was detected; however, there is insufficient information for any specific birth defects to draw further conclusions given the limitations noted above.

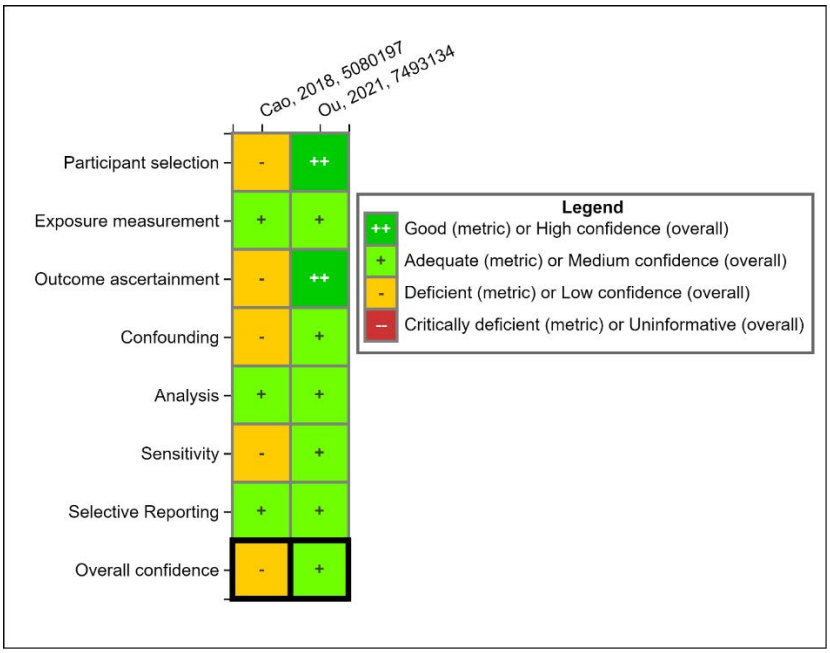


Figure 3-31. Study evaluation heat map of two epidemiological studies of birth defects and PFNA exposure. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-Birth-Defects/.

Fetal loss – Spontaneous abortion

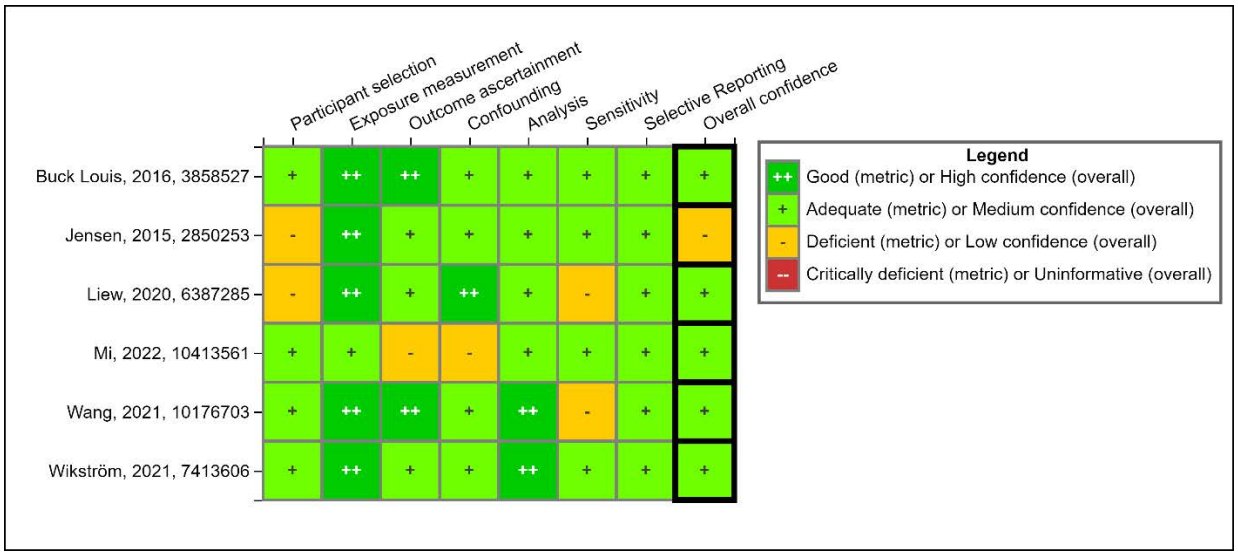


Figure 3-32. Study evaluation heat map of epidemiological studies of six spontaneous abortion and PFNA exposure. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500285/.

Six epidemiological studies examined associations between PFNA exposure and spontaneous abortion, as shown in Figure 3-32. A study examining a cohort of pregnant women enrolled at 8 to 16 weeks gestation (Jensen et al., 2015) was considered *low* confidence primarily due to loss to follow-up and the high risk of incomplete case ascertainment (i.e., not including women with losses that occurred prior to the period of study enrollment, which may bias the results toward or even past the null if there is a true association between PFNA exposure and spontaneous abortion (Radke et al., 2019)). The *medium* confidence case-control study by Liew et al. (2020) identified cases via medical registry and, therefore, also has the potential to not capture early losses. However, this study was not downgraded to *low* confidence as loss to follow-up was not a concern. Four additional studies were considered *medium* confidence. One was a cohort study of couples trying to conceive (Louis et al., 2016), and one was a cohort of women undergoing their first in vitro fertilization embryo transfer treatment cycle (Wang et al., 2021a), both of which would have excellent ascertainment of early losses. In addition, there were two case-control studies of first trimester miscarriages (Mi et al., 2022; Wikström et al., 2021).

The results of these six studies are summarized in Table 3-7. The single *low* confidence study reported a strong positive association with clinical loss, with very large effect estimates in tertiles 2 and 3. It is unlikely that the limitations identified in this study would explain the observed positive associations as bias if any present would be expected to be toward or past the null. However, only one other study reported an increased odds of spontaneous abortion, and that association was not statistically significant. The remaining four studies reported null (Mi et al., 2022; Liew et al., 2020) or inverse associations (Wang et al., 2021a; Louis et al., 2016). Given the

inconsistency in available studies without a clear explanation such as loss timing or study sensitivity, the evidence of an association between PFNA exposure and spontaneous abortion is very limited and highly uncertain.

Table 3-7. Associations between PFNA and spontaneous abortion in epidemiology studies

Reference, study confidence	Population	Median exposure (25th, 75th) in ng/mL or as specified	Spontaneous abortion types included	Effect estimate description	Effect estimate (95% CI)
Wang et al. (2021a), *medium*	Preconception cohort of women undergoing first IVF cycle, China, 305 women	0.2 (0.1–0.2)	Preclinical	RR (95% CI) for log-unit increase	0.27 (0.06, 1.24)
Liew et al. (2020), *medium*	Case-control nested within pregnancy cohort, Denmark; 438 women	0.4 (0.3–0.5)	Clinical, 12–22 wk	OR (95% CI) for quartiles vs. Q1	Q2: 0.9 (0.5, 1.5) Q3: 1.1 (0.6, 1.8) Q4: 1.0 (0.6, 1.8)
Wikström et al. (2021), *medium*	Case-control nested within pregnancy cohort, Sweden; 1,529 women	0.6 (0.4–0.9)	Clinical, first trimester	OR (95% CI) for doubling of exposure	1.25 (0.93, 1.68)
Jensen et al. (2015), *low*	Pregnancy cohort, Denmark; 392 women	0.7 (0.3–0.6)	Clinical, post enrollment at 8–16 wk	OR (95% CI) for tertiles vs. T1	**T2: 10.9 (5.8, 24.8)** **T3: 16.2 (6.9, 38.0)**
Louis et al. (2016), *medium*	Preconception cohort, U.S.; 344 women	0.7 (0.3–0.6)	Total	HR (95% CI) for tertiles vs. T1	T2: 0.74 (0.44, 1.25) **T3: 0.57 (0.34, 0.94)**
Mi et al. (2022), *medium*	Case-control nested within pregnancy cohort, China; 88 women	1.0 (0.5–1.1)	Clinical (9–12 wk)	Median and IQR for cases and controls	Cases: 0.94 (0.72, 1.56) Controls: 1.02 (0.70, 1.24) $p = 0.84$

Abbreviations: OR: odds ratio; HR: hazard ratio; RR: relative risk; T1: tertile 1; T2: tertile 2; T3: tertile 3: IVF: in vitro fertilization. Bold font indicates $p < 0.05$.

Anogenital distance

Various measures of AGD were examined across three *medium* confidence epidemiological studies. In boys, measures can be taken from the center of the anus to the posterior base of the scrotum (anoscrotal distance [ASD]) or from the center of the anus to the cephalad insertion of the penis (anopenile distance [APD]), while in girls, measures can be taken from the center of the anus to the top of the clitoris (ACD) or to the posterior fourchette (AFD).

Three *medium* confidence studies (see Figure 3-33) of birth cohorts in Denmark (Lind et al., 2017a) and China (Tian et al., 2019) and a cross-sectional analysis of a birth cohort from the Faroe Islands (Christensen et al., 2021) examined the association between maternal PFNA exposure and AGD. The timing of AGD measurement varied across studies, from birth (Tian et al., 2019), 2 weeks after expected term date (Christensen et al., 2021), 3 months (Lind et al., 2017a), and 6 and

12 months (Tian et al., 2019). Tian et al. (2019) examined only boys, while the other two studies examined both boys and girls.

For boys, there were discordant results in Lind et al. (2017a) for the two measures of AGD, with a positive association for ASD and an inverse association for APD (neither statistically significant). A previous (independent) study indicated that these measurements are reliable and that the intra- and inter-examiner variability is low and similar across multiple measures (Sathyanarayana et al., 2010); thus, there is not a clear explanation for differences noted in Lind et al. (2017a). Christensen et al. (2021) also reported a positive association with ASD, while Tian et al. (2019) reported inverse associations with both ASD and APD at birth and 6 months ($p > 0.05$), which disappeared by 12 months, possibly due to greater heterogeneity in child size as they grow (Tian et al., 2019).

In girls, Lind et al. (2017a) reported an inverse association in ACD, although there was not a monotonic decrease across quartiles (Q2 versus Q1: β = −0.7 mm; 95% CI: −2.4, 0.9; Q3: β = −2.6 mm; 95% CI: −4.4, −0.9; Q4: −1.2 mm; 95% CI: −3.5, −0.1). A consistent but smaller and nonsignificant inverse association was also observed in the third and fourth quartiles for AFD. In Christensen et al. (2021), no associations were observed with AFD.

Overall, there was inconsistent results in boys and girls from three *medium* confidence studies, which add considerable uncertainty and preclude further weight-of-evidence determinations.

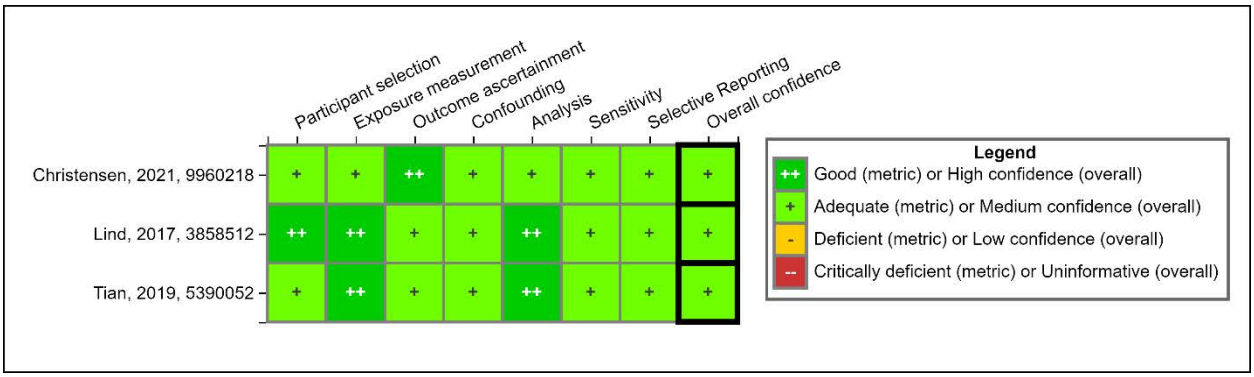


Figure 3-33. Study evaluation heat map of epidemiological studies of anogenital distance and PFNA exposure. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-anogenital-distance-study-evaluation/.

Animal Studies

Five developmental toxicity studies in rodents have examined the effects of gestational exposures to PFNA on prenatal and postnatal development, including two (generally *high* and *medium* confidence for most endpoints) studies in mice (Das et al., 2015; Wolf et al., 2010), one (generally *medium* confidence for most endpoints) study in rats (Rogers et al., 2014), and two

(generally *low* confidence for most endpoints) studies in mice (Zhang et al., 2021; Singh and Singh, 2019c) (see Table 3-8).

Table 3-8. Overall evaluation results of developmental toxicity studies examining the effects of PFNA exposures in rodents[a]

Reference	Species, strain (sex)	Exposure design	Exposure route and dose range	Maternal health	Fetal loss, malformations, and body weight	Postnatal survival	Postnatal body weight	Developmental landmarks
Das et al. (2015)[b]	Mouse, Crl:CD1 Charles River (male/female)	Developmental GD 1–17	Gavage, maternal 0, 1, 3, 5, 10 mg/kg-d[c]	++	++	+	++ +	+
Rogers et al. (2014)[d]	Rat, Crl:CD, Sprague-Dawley, Charles River (male/female)	Developmental GD 1–20	Gavage, maternal 0, 5 mg/kg-d	+	NM	-	+	NM
Singh and Singh (2019c)	Mouse, Parkes (male)	Developmental GD 12–21	Gavage, maternal 0, 2, 5 mg/kg-d	+	NM	NM	-	NM
Wolf et al. (2010)	Mouse, Wild type 129S1/SvlmJ; PPARα-Knockout PPARa$^{tm1Gonz/J}$ (male/female)	Developmental GD 1–18	Gavage, maternal 0, 0.83, 1.1, 1.5, 2 mg/kg-d	++	NM	+	++	+
Zhang et al. (2021)	Mouse, ICR, Orient Bio (female)	Developmental GD 1–18	Gavage, maternal[e] 0, 0.5, 3 mg/kg-d	+	NM	NM	-	-

[a]Dark green (++) = *high* confidence; light green (+) = *medium* confidence; yellow (-) = *low* confidence; red (--) = *uninformative;* NM = not measured. Study evaluation details for all outcomes are available in HAWC.

[b]Das et al. (2015) was judged *medium* confidence for preweaning body weight and *high* confidence for post-weaning body weight.

[c]10 mg/kg-day dose group ceased due to high mortality among treated dams.

[d]Rogers et al. (2014) evaluated effects of PFNA gestational exposures on some cardiovascular and renal endpoints of male offspring that are discussed in Sec. 3.2.8 and 3.2.10. respectively.

[e]Zhang et al. (2021) also co-exposed groups of mouse dams and female offspring to the PPARα inhibitor GW6471 or vasopressin (VAP) to examine mechanistic pathways. These results were generally *low* confidence.

Das et al. (2015) reported severe toxicity [full litter resorption, fetal death] at 10 mg/kg-day PFNA, and discontinued this dose group at GD 13, making 5 mg/kg-day the high dose. At GD 17, a portion of dams were sacrificed for maternal evaluations and fetuses were euthanized, eviscerated, and processed for skeletal or visceral evaluations. Remaining live dams proceeded to delivery after which postnatal survival, body weight, and attainment of developmental landmarks (eye opening, vaginal opening, preputial separation) were evaluated. Prepartum endpoints in fetuses at GD 17 in Das et al. (2015) are discussed in the fetal loss and body weight section, and postpartum survival, birth weight, and other endpoints are discussed under the postnatal headings. Postnatal body weights in Das et al. (2015) were monitored preweaning (PND 1–21) and post-weaning (PND 25–287), and the timing of eye opening and pubertal progression was examined starting on PND 10 and PND 25, respectively. Wolf et al. (2010) exposed pregnant wildtype (129S1/SvlmJ) and PPARα null mice, and evaluated maternal health, postnatal survival, body weight (PND 0–21), and eye opening (starting on PND 11).

The developmental toxicity studies with PFNA in mice by Das et al. (2015) and Wolf et al. (2010) were found to be generally well conducted, except for a lack of evaluator blinding when scoring animals for developmental landmarks and a preference herein for sex-specific reporting for some endpoint evaluations that could differ by sex (e.g., postnatal body weights where males tend to be larger than females). While also found to be generally sound, missing results presentation in the developmental toxicity study in rats by Rogers et al. (2014) resulted in lower confidence conclusions for some endpoints (i.e., offspring survival and condition). The exposure parameters in Zhang et al. (2021) and Singh and Singh (2019c) were considered appropriate for the developmental endpoints examined. However, litter does not appear to have been controlled for in the study design, among other inadequacies in the allocation and observational bias domains, making both studies overall *low* confidence (Zhang et al., 2021; Singh and Singh, 2019c). Zhang et al. (2021) also co-exposed mouse dams and female offspring to the PPARα inhibitor GW6471 or vasopressin to discern potential modes of action, but results were also judged to be *low* confidence based on generally similar deficiencies to those noted in animals that were not co-exposed to GW6471 or vasopressin. The maternal health and pregnancy endpoints in both Zhang et al. (2021) and Singh and Singh (2019c) were found to be *medium* confidence.

Das et al. (2015) and Wolf et al. (2010) also evaluated effects of PFNA gestational exposures on liver weight and gene expression of mouse dams and offspring. Rogers et al. (2014) evaluated effects on nephron counts of male rat offspring and blood pressure in offspring of both sexes. The evidence to inform organ/system-specific effects of PFNA following developmental exposures is discussed principally in the individual hazard sections and cross-referenced here (i.e., liver weights, blood pressure, and nephron counts in developmentally exposed offspring are discussed in Section 3.2.3, Section 3.2.9, and Section 3.2.10, respectively). Effects on the timing of pubertal progression (preputial separation, vaginal opening) are discussed here and summarized in the male and female reproductive toxicity sections.

Maternal health and pregnancy endpoints

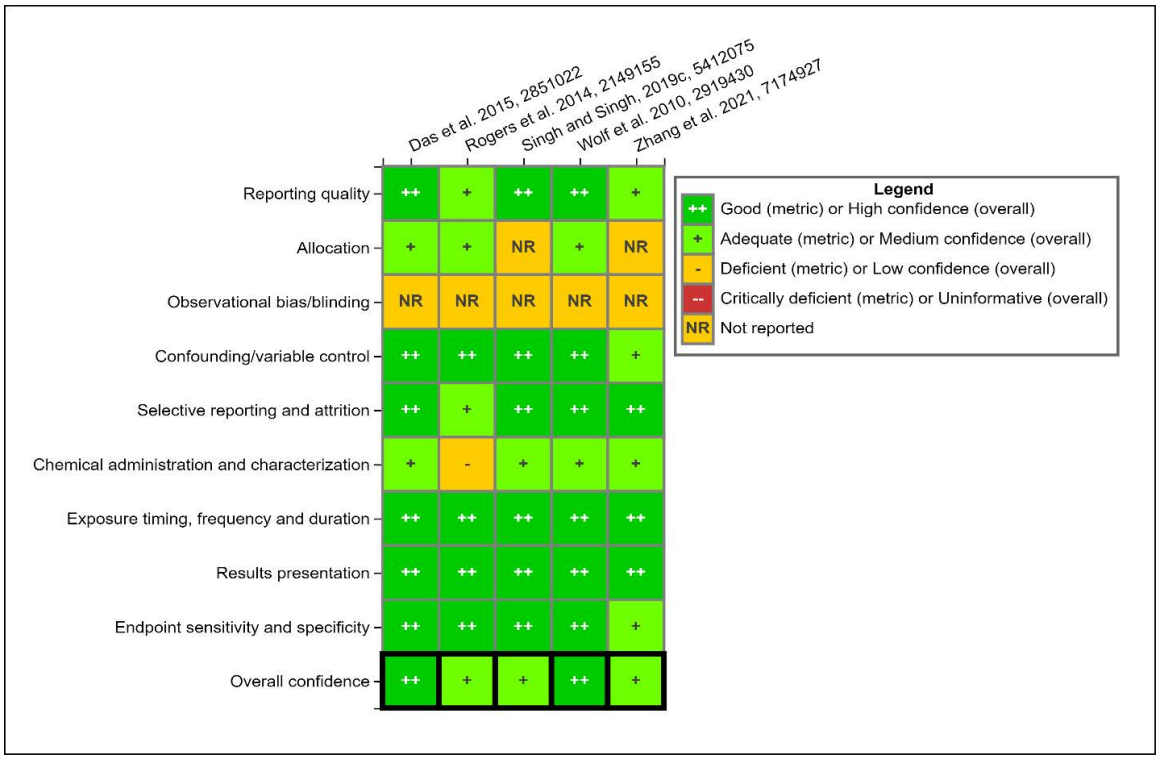


Figure 3-34. Study evaluation heatmap of animal developmental toxicity studies examining PFNA effects on maternal health and pregnancy endpoints. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Maternal-Health-Endpoints/.

Effects of PFNA exposure on maternal health and pregnancy endpoints were evaluated in mouse and rat dams in all five developmental toxicity studies (Zhang et al., 2021; Singh and Singh, 2019c; Das et al., 2015; Rogers et al., 2014; Wolf et al., 2010). The studies were found to be *high* or *medium* confidence for these endpoints with no major concerns, although some domains were downgraded (e.g., allocation domain due to missing explanation of randomization procedures when assigning dams to treatment groups; see Figure 3-34).

Pregnancy rates (i.e., percent of plugged mice with uterine implants), number of uterine implants, and total litter sizes of live and dead fetuses of CD-1 and 129S1/SvlmJ mice (and PPARα null mice) were unaffected by PFNA gestational exposures up to 5 mg/kg-day (Das et al., 2015) or 2 mg/kg-day (Wolf et al., 2010). There was a nonstatististically significant increase of 35% litter loss in wild type mouse dams at 2 mg/kg-day PFNA, compared with 14.3% litter loss in controls (Wolf et al., 2010). Increases in litter loss could reflect effects of exposure on either the dams and their ability to maintain pregnancy (especially if litter loss occurs earlier during pregnancy) or the developing offspring, although the currently available evidence does not allow for an interpretaion at this level of specificity. No elevations in litter loss were observed in PPARα null dams (Wolf et al.,

2010) or in CD-1 mouse dams (Das et al., 2015). However, there was a dose-dependent declining trend in pregnancy rates in the PPARα knockout animals (Wolf et al., 2010). Singh and Singh (2019a) reported no significant differences in birth rate, number of pups per dam, or the ratio of male to female pups in pregnant Parkes mice exposed to PFNA.

Maternal body weight gain results varied among studies; differences were difficult to interpret and may be associated with study design, dose, strain and/or species. Statistically significant increases in maternal body weight gain ranging from 24% to 34% and 21% to 41% were reported in CD-1 mice during late gestation (GD 11–17) at 3 and 5 mg/kg-day PFNA, respectively (Das et al., 2015). Zhang et al. (2021) also reported modest but statistically significant increases (12%) in body weight gain of pregnant ICR mice exposed to 3 mg/kg-day PFNA, which was not observed in mouse dams co-administered the PPARα inhibitor GW6471. There were no effects on body weight or body weight gain of wild type or PPARα null mouse dams exposed through pregnancy at lower doses up to 2.0 mg/kg-day PFNA (Wolf et al., 2010). However Singh and Singh (2019a) reported no statistically significant differences in maternal body weights of pregnant Parkes mice exposed to 2 or 5 mg/kg-day PFNA at GD 12 and at the end of the pregnancy term. In contrast to mice, there were significant deficits in body weight gain throughout the pregnancies of SD rats exposed to 5 mg/kg-day PFNA, ranging from 10% to 28% reductions from GD 4 to 19 (Rogers et al., 2014). Studies reported no evidence of maternal deaths or other overt toxicity in dams of either species (at up to 5 mg/kg-day PFNA) during pregnancy.

Dose-dependent increases in absolute and relative liver weight were also reported in both strains of pregnant and nonpregnant mice at ≥1 mg/kg-day, as well as nonpregnant PPARα null mice at ≥1.1 mg/kg-day (Das et al., 2015; Wolf et al., 2010). There was no effect on liver weights of PPARα null dams (Wolf et al., 2010). Similarly, statistically significant increases in absolute and relative liver weights were reported in CD-1 mouse fetuses (at GD 17) (Das et al., 2015). Liver weight increases in pregnant mice and their offspring are consistent with results from short-term exposure studies in adult rodents and are discussed in more detail in Section 3.2.3.

Fetal loss, malformations, and weight at late gestation

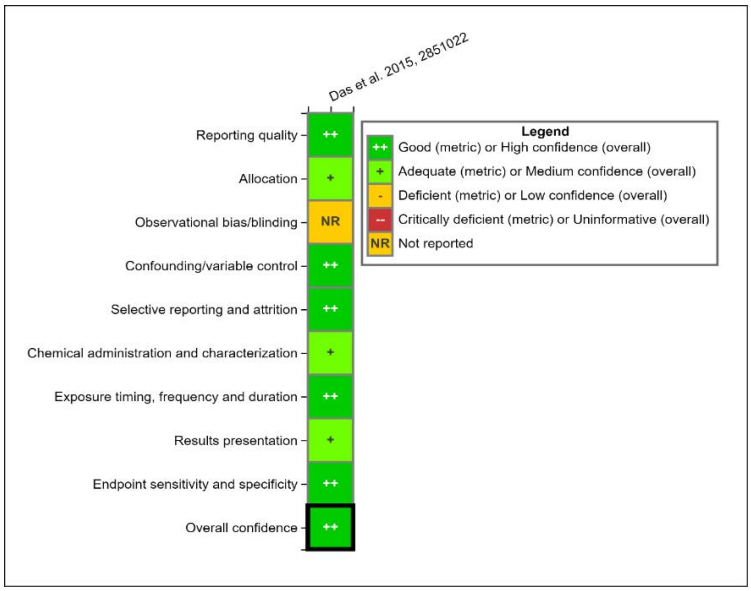


Figure 3-35. Study evaluation heatmap of animal studies examining PFNA effects on fetal loss, malformations, and body weight at late gestation (GD 17).
See interactive HAWC link:
https://hawc.epa.gov/summary/visual/assessment/100500071/Fetal-loss-and-body-weight/.

PFNA effects on fetal survival, weight, and visceral and skeletal variations were examined in CD-1 mice at late gestation (GD 17) (Das et al., 2015) with results judged to be overall *high* confidence with no notable concerns (see Figure 3-35). Survival and body weights of CD-1 mouse fetuses were reported to be unaffected at any dose tested up to 5 mg/kg-day PFNA, and there were no teratological malformations reported (Das et al., 2015). Fetal survival and fetal weight endpoints during gestation were not examined in the Wolf et al. (2010) or Rogers et al. (2014) studies. Pup survival and weights measured at birth or later are discussed under the postnatal survival and body weight sections.

Postnatal survival

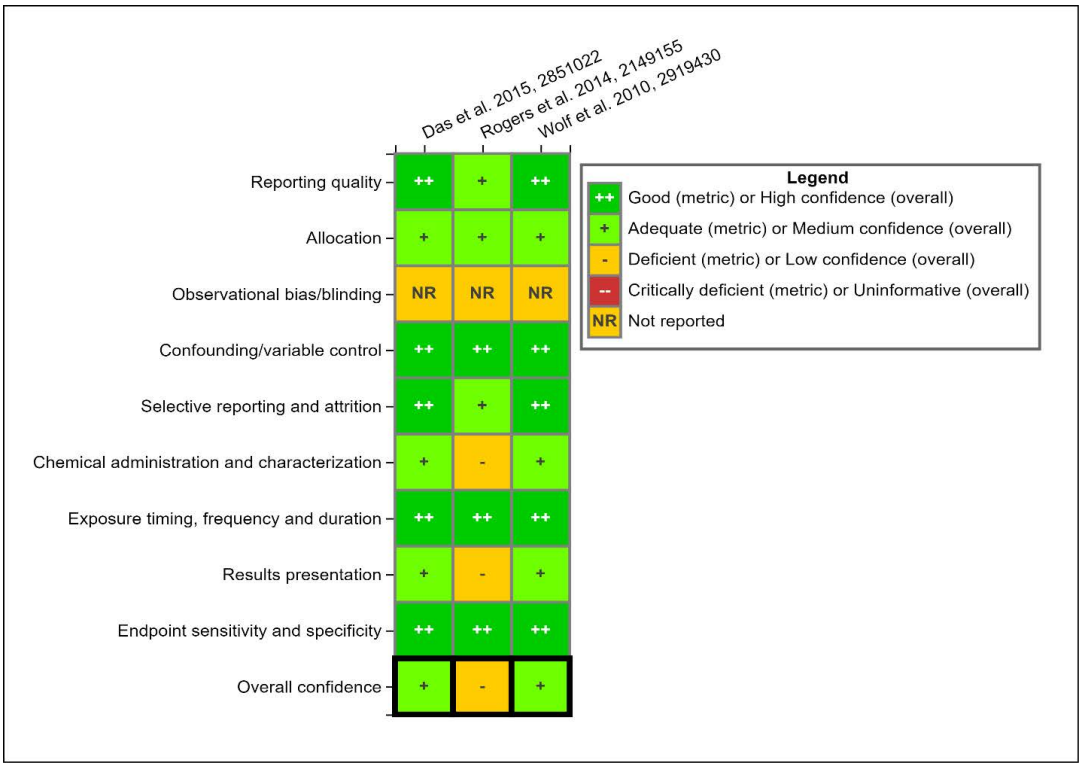


Figure 3-36. Study evaluation heatmap of animal studies examining PFNA effects on postnatal pup survival. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Postnatal-survival/.

Effects of PFNA gestational exposures on postnatal survival of preweaning neonates were examined in mice and rats (Das et al., 2015; Rogers et al., 2014; Wolf et al., 2010). Results in mice for this outcome were judged to be *medium* confidence with minor limitations noted (see Figure 3-36). Results in rats were *low* confidence for this endpoint in Rogers et al. (2014) largely due to a lack of published results on offspring survival (authors indicated results were null but no other details were provided).

At the high dose of 5 mg/kg-day, PFNA significantly reduced the survival of CD-1 mice by 12% at day of birth (PND 0) with most of the decline in percent survival occurring in the first half of the lactational term up to about PND 10 (68% mortality) and 79% loss by PND 21 (see Table 3-9). Postnatal survival was unaffected by PFNA at 1 or 3 mg/kg (Das et al., 2015). Similarly, statistically significant reductions in numbers of live wild type mouse pups born per litter were reported at 1.1 and 2 mg/kg-day PFNA by Wolf et al. (2010), with 46% and 54% reductions, respectively, compared to controls. This study also reported large reductions in postnatal survival of wild type mouse neonates over the weaning term at 1.1 and 2 mg/kg-day (63% and 68% reductions, respectively, by PND 21). However, although decreased survival in wild type mice in both studies

exhibited a clear time dependence (larger effects at later postnatal ages), the nonstatistically significant decreases (19%–25% compared with controls) in survival for the 1.5 mg/kg-day PFNA dose group complicates the interpretation of dose-dependence. Although slight decreases in numbers of live PPARα null mice born per litter and preweaning pup survival were observed and were larger at later postnatal ages and higher PFNA doses, with effect magnitudes of potential concern at 2 mg/kg-day (decreases in survival relative to controls of up to 12%), these changes were not statistically significant. In addition, the changes were far smaller than the statistically significant reductions observed in PPARα wild type mice, suggesting a potential role for PPARα activation in neonatal development and survival (see "Mechanistic and Supplemental Information," below).

Table 3-9. PFNA effects on survival of preweaning neonates (males/females combined) shown as mean litter values (statistical unit) of percent of pups alive per litter with the percent decrease relative to controls in parenthesis[a]

Dose (mg/kg-d)	Postnatal age (d)								
	0	**1**	**2**	**3**	**7**	**10**	**14**	**17**	**21**
CD-1 mouse offspring Das et al. (2015)[b]; *medium* confidence									
0	87.1	87.6	86.4	86.4	86.0	85.3	83.7	83.7	83.7
1	87.9	88.6	87.7	87.7	87.7	87.7	85.9	85.9	85.9
3	89.6	88.9	86.3	86.3	85.7	85.7	84.5	84.5	84.5
*5	*74.8 (-12)*	*66.6 (−24)*	*60.8 (−30)*	*57.3 (−34)*	*36.4 (−58)*	*27.5 (−68)*	*19.1 (−77)*	*18.3 (−78)*	*17.3 (−79)*
129S1/SvImJ mouse offspring Wolf et al. (2010)[b]; *medium* confidence									
0	-	76.1	73.7	73.7	73.7	73.7	72.9	72.9	72.1
0.83	-	64.6 (−15)	63.5 (−14)	63.5 (−14)	61.3 (−17)	61.3 (−17)	61.3 (−16)	61.3 (−16)	61.3 (−15)
*1.1	-	*40.1 (−47)*	*29.4 (−60)*	*29.4 (−60)*	*28.2 (−62)*	*26.5 (−64)*	*26.5 (−64)*	*26.5 (−64)*	*26.5 (−63)*
1.5	-	62.0 (−19)	58.6 (−21)	57.0 (−23)	57.0 (−23)	55.5 (−25)	55.5 (−24)	55.5 (−24)	55.5 (−23)
**2	-	*37.7 (−51)*	*29.7 (−60)*	*29.7 (−60)*	*26.7 (−64)*	*22.8 (−69)*	*22.8 (−69)*	*22.8 (−69)*	*22.8 (−69)*
PPARα null (129S1/SvImJ) mouse offspring Wolf et al. (2010)[b]; *medium* confidence									
0	-	90.3	90.3	89.6	89.6	89.6	89.6	89.6	89.6
0.83	-	95.4	95.4	95.4	95.4	95.4	95.4	95.4	95.4
1.1	-	89.4	88.5 (−2)	88.5 (−1)	86.9 (−3)	86.9 (−3)	86.9 (−3)	86.9 (−3)	86.9 (−2)
1.5	-	84.3	84.3 (−7)	84.3 (−6)	84.3 (−6)	84.3 (−6)	84.3 (−6)	84.3 (−6)	84.3 (−7)
2	-	95.2	81.8 (−9)	81.8 (−9)	79.8 (−11)	78.6 (−12)	78.6 (−12)	78.6 (−12)	78.6 (−12)

[a]Italicized values denote PFNA dose level with statistically significant differences reported between control and treatment groups (*$p < 0.05$; **$p < 0.01$).

[b](Das et al., 2015) and (Wolf et al., 2010) reported day 0 as the day of birth. (Das et al., 2015) reported the percent survival of live pups per litter as a percentage of uterine implantation from PND 0 to PND 21, whereas (Wolf et al., 2010) normalized the percent survival of live pups by litter to PND 0 (denoted with a dash [-]). Rogers et al. (2014) reported that PFNA did not affect survival of SD rats but did not show quantitative data to support this finding, and litters were cross-fostered to control dams to preclude postnatal exposures by lactational transfer.

Postnatal body weight

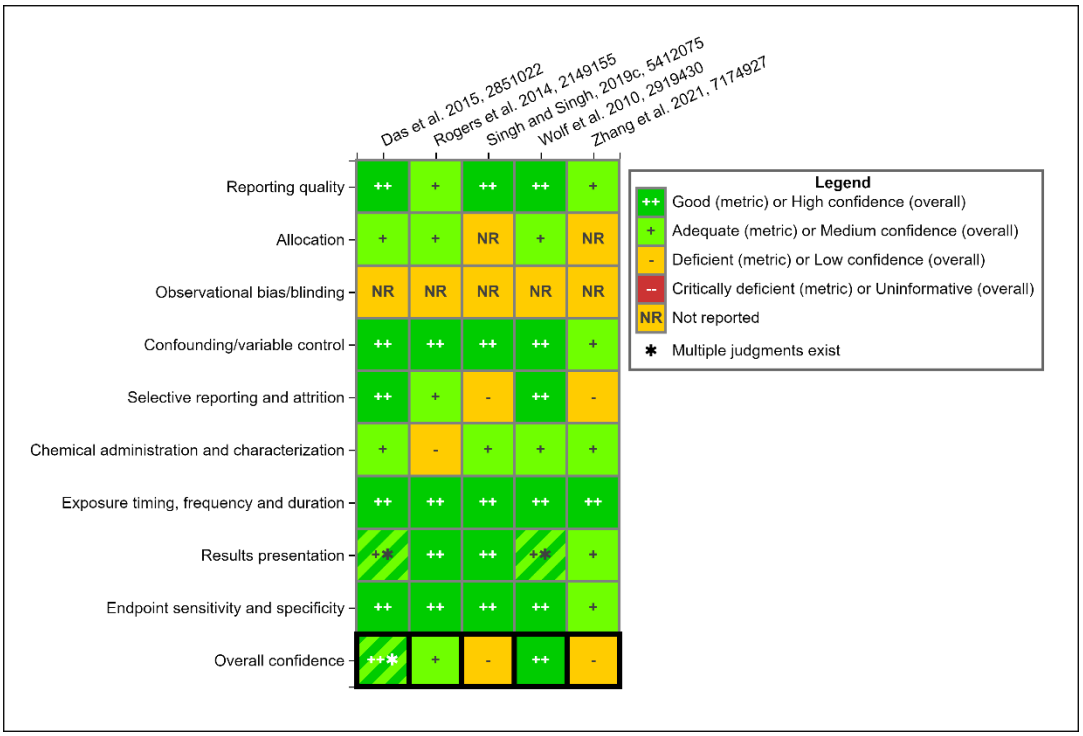


Figure 3-37. Study evaluation heatmap of animal studies examining PFNA effects on body weight of pre- and post-weaning pups.[a] See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Postnatal-body-weight/.

[a]Das et al. (2015) judged to be *medium* confidence for preweaning body weight and high confidence for post-weaning body weight.

Postnatal body weights (including birth weights) were evaluated in preweaning rodents in all the developmental toxicity studies with PFNA (Zhang et al., 2021; Singh and Singh, 2019a; Das et al., 2015; Rogers et al., 2014; Wolf et al., 2010), with one study also monitoring body weight of mice post-weaning into adulthood (Das et al., 2015). The pre- and post-weaning body weight measurements in 129S1/SvlmJ mice was found to be *high* confidence overall (Wolf et al., 2010), and results in CD-1 mice (Das et al., 2015) were judged to be *medium* confidence based on a preference for sex-specific reporting in preweaning offspring due to potential sex-related differences in body weight (see Figure 3-37). A lack of reporting on controlling for litter in Singh and Singh (2019a) and Zhang et al. (2021), and missing reporting details on randomization procedures employed in dam and litter/pup allocations to treatment groups made these studies *low* confidence for this endpoint. The study in SD rats was found to be *medium* confidence for this endpoint with only minor limitations (e.g., lack of reporting on source/purity of PFNA) (Rogers et al., 2014).

Mouse birth weights were unaffected by gestational PFNA exposure in all mouse strains tested, as well as in PPARα null newborns (Das et al., 2015; Wolf et al., 2010), including the *low* confidence studies (Zhang et al., 2021; Singh and Singh, 2019a). During the preweaning period, Das et al. (2015) observed dose-dependent reductions in body weight gain of CD-1 mouse pups (sexes combined) with statistically significant deficits at 3 and 5 mg/kg-day PFNA (22%–25% and 36%–61%, respectively) that extended to weaning at PND 21 (see Table 3-10). Consistent with results in CD-1 mice, statistically significant reductions in body weight gain were also observed in preweaning male and female wild type mouse neonates at 2 mg/kg-day PFNA in Wolf et al. (2010) (see Table 3-10). Sexes were combined in CD-1 mice, but sex-specific differences in body weight gain were observed in the 129S1/SvlmJ mouse strain. Body weight gain in males was significantly reduced by 18%–37% from PND 7 to 14, after which it was not significantly different from controls. In females, the onset and magnitude (15%–36%) of reductions in body weight gain at PND 7 were similar to observations in males, but unlike in males the declines in females extended to weaning at PND 21 where weight gain was significantly reduced by 21% relative to controls. Body weight gain of preweaning PPARα null mice was unaffected at PFNA treatments up to 2 mg/kg-day (Wolf et al., 2010). Rogers et al. (2014) also reported statistically significant reductions in birth weights of rat pups exposed gestationally to 5 mg/kg-day PFNA (males = 12%; females = 13%; see Table 3-10). The potential influence in this study of the reduced weight gain in dams during pregnancy on birth weights is an unknown. No statistically significant reductions in pup body weight gain were observed at weaning (PND 21) in rat offspring that were cross-fostered to control dams (so incurred no lactational exposure). However, despite the absence of lactational exposures, statistically significant deficits in weight gain were reported up to PND 11 in males and PND 7 in females (Rogers et al., 2014). The cross-fostering in the rat study makes it difficult to draw inferences to results in mouse offspring that are presumed to have been lactationally exposed.

Another data gap in the mouse developmental testing relates to whether PFNA affects differentiation of the mammary gland in exposed dams; if so, this could play a role in the reduced weight gain by impairing lactation. Developmental toxicity testing in mice with the structurally similar PFOA compound shows altered mammary gland differentiation in exposed dams, leading to preweaning growth deficits in offspring (White et al., 2009). Thus, while mammary gland and lactational endpoints were not evaluated in the animal bioassays with PFNA, it is plausible that impaired mammary gland development in dams could also play a role in the PFNA-induced deficits in preweaning body weight gain, currently an unknown for PFNA. In alignment with the *high* and *medium* confidence findings of reduced offspring body weight gain, the *low* confidence results in ICR mice by Zhang et al. (2021) also reported dose-dependent reductions in preweaning body weight gain of female offspring with a similar age of onset (PND 7) and dose to female 129S1/SvlmJ mice. The other *low* confidence developmental toxicity study by Singh and Singh (2019c) that examined effects of gestational exposures to male mouse offspring did not evaluate offspring body weight gain beyond PND 1. This study reported no statistically significant effects on male body

weight gain at PND 1 at doses up to 5 mg/kg-day, which is consistent with male 129S1/SvlmJ mice at PND 1 where there were also no effects observed.

The developmental study in CD-1 mice monitored post-weaning body weight gain of male and female mice into adulthood from PND 24 to 287 and reported significant PFNA-induced decrements that exhibited some sex dependence (Das et al., 2015). In male mice, dose-dependent treatment-related effects were reported and persisted to 41 weeks of age with no catch-up in growth (see Figure 3-38). Statistically significant reductions in body weight of males over the post-weaning period ranged from 53% at PND 24 to 15% at PND 287 in the 5 mg/kg-day dosing group. Body weight deficits in females at 5 mg/kg-day were generally smaller over the post-weaning period than those measured in males, and the reductions relative to controls were no longer statistically significant by 7 weeks of age (reductions of: 41% at PND 24, 13% at PND 49; 8% at PND 287). The female body weights at 5 mg/kg-day continued to lag control animals by ≥10% until about PND 77 (body weight differences of 10% or more are considered potentially biologically significant (U.S. EPA, 2012a)).

Table 3-10. Effects of PFNA gestational exposures on body weight gain of preweaning mouse and rat litters displayed as percent change relative to controls (Das et al., 2015; Rogers et al., 2014; Wolf et al., 2010)[a]

Dose (mg/kg-d)	Postnatal age (d)								
	0	1	2	3	7	10	14	17	21
CD-1 mice, males/females combined Das et al. (2015); *medium* confidence									
1	–	−6.4	−7.5	−9.0	−9.4	−10	−10	−14	−14
3	–	−7.9	−9.2	−13	*−24**	*−23**	*−22**	*−22**	*−25**
5	–	−17	−24	*−36**	*−61**	*−60**	*−53**	*−47**	*−51**
Male 129S1/SvlmJ mouse offspring Wolf et al. (2010); *high* confidence									
0.83	0	−1.9	−3.0	−10	−10	−15	−5.5	−1.3	−1.2
1.1	0	+2.0	+4.9	−12	+0.1	−9.1	+9.7	+14	+7.3
1.5	+3	+4.0	+2.6	−1.8	−9.6	−9.4	−0.7	+5.1	+0.5
2	+10	+2.8	−4.4	−6.8	*−37***	*−33***	*−18**	−12	−13
Female 129S1/SvlmJ mouse offspring Wolf et al. (2010); *high* confidence									
0.83	+3	−4.1	−6.1	−15	−9.4	−11	−3.5	+1.6	−2.5
1.1	+6	−2.7	−3.5	−14	−13	−4.7	+6.1	+11	+6.6
1.5	+4	−3.7	−1.8	−5.3	−16	−10	−3.0	+2.6	−3.0
2	+3	−1.9	−13	−25	*−36****	*−31****	*−26****	*−15**	*−21***
Male PPARα null (129S1/SvlmJ) mouse offspring Wolf et al. (2010); *high* confidence									
0.83	+3	−0.9	−2.8	−4.0	−8.1	−5.6	−2.5	−1.2	−2.5
1.1	+1	−2.2	−5.2	−3.2	−4.9	−3.2	−4.9	−0.1	−6.2
1.5	−3	−0.9	−2.6	−1.5	−3.8	−5.6	−5.7	−11	−6.4
2	+1	−2.6	−2.6	−4.4	−10	−6.0	−6.4	−6.2	−7.4
Female PPARα null (129S1/SvlmJ) offspring Wolf et al. (2010); *high* confidence									
0.83	+5	−0.3	−1.9	−5.9	−8.5	−6.2	−4.3	−2.5	−6.0
1.1	+1	−2.2	−4.4	−5.9	−7.0	−5.0	−6.5	−2.5	−6.1
1.5	−4	−4.4	−5.7	−6.0	−7.5	−6.7	−6.8	−10	−8.2
2	+8	+0.4	−2.4	−7.8	−9.3	−6.9	−6.1	−5.5	−5.4
Male SD rat offspring Rogers et al. (2014); *medium* confidence									
5	*−13**	–	–	–	*−11**	–	*−8**	–	−7
Female SD rat offspring Rogers et al. (2014): *medium* confidence									
5	*−12**	–	–	–	*−10**	–	−5	–	−6

[a]Italicized values denote statistically significant differences reported between vehicle control and treatment groups (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). A plus sign indicates a percent increase and a negative sign indicates a percent reduction relative to controls. Rogers et al. (2014) supplied quantitative litter data at PND 0, 7, 14, and 21 with litters cross-fostered to control dams to preclude lactational transfer.

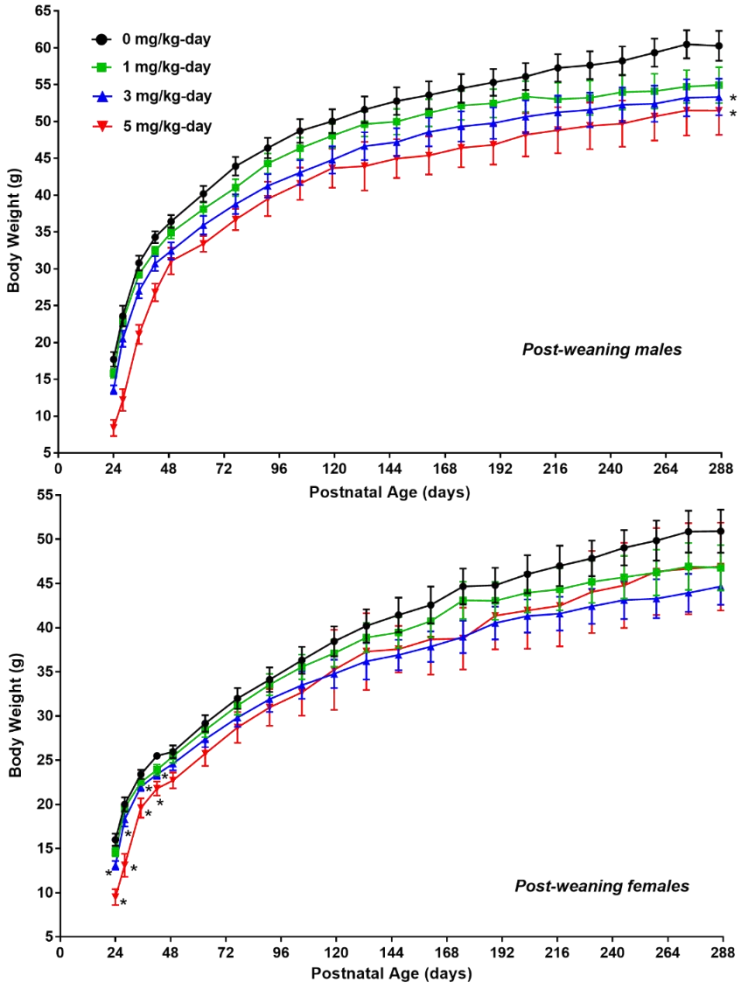


Figure 3-38. PFNA effects on body weight gain of post-weaning male and female mice (PND 24–287) exposed from GD 1 to 17 (Das et al., 2015).[a]
Underlying data available at: https://hawc.epa.gov/ani/animal-group/100500511/ (females); https://hawc.epa.gov/ani/animal-group/100500510/ (males).

[a]Data points represent the mean ± SE of body weight by sex with litter as the statistical unit. After weaning, pups in each litter were weighed as a group by sex with the mean body weight derived for that sex that was then used to derive the mean ± SE by sex across litters by treatment group. Statistically significant differences (*$p < 0.05$) reported between control and treatment groups at ≥3 mg/kg-day in post-weaning males up to PND 287 and in post-weaning females up to PND 49.

Postnatal developmental landmarks

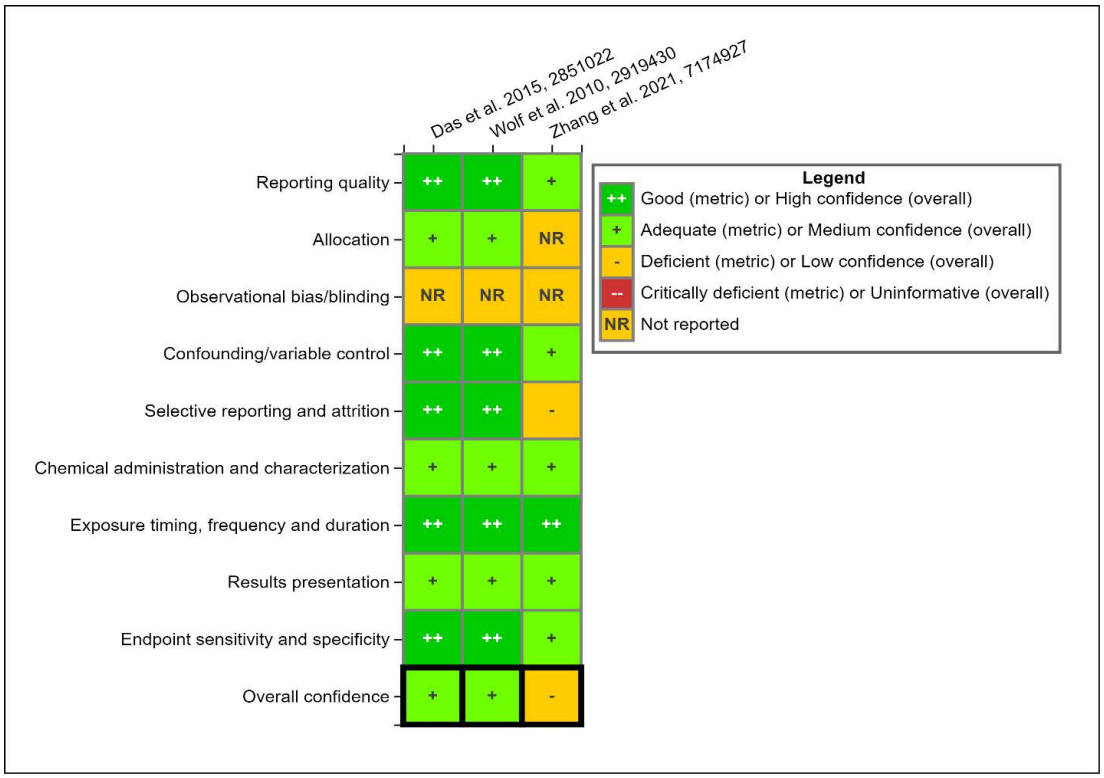


Figure 3-39. Study evaluation heatmap of animal studies examining PFNA effects on postnatal developmental markers in preweaning pups. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Postnatal-developmental-landmarks/.

Two studies in mice examined effects of PFNA gestational exposures on developmental milestones related to the timing of eye opening (Das et al., 2015; Wolf et al., 2010), and one of these studies also evaluated the timing of preputial separation and vaginal opening (Das et al., 2015). The evaluations of these endpoints were judged to be *medium* confidence with limitations that included a lack of investigator blinding when scoring animals and not reporting the timing of eye opening by sex as this landmark may be affected by postnatal body weight that is sex dependent (males tend to be larger than females; see Figure 3-39). A third study by Zhang et al. (2021) also evaluated the timing of vaginal opening in gestationally exposed ICR mice. This study was found to be *low* confidence for this endpoint due to inadequacies in accounting for litter in the analyses, among other limitations (e.g., lack of investigator blinding when scoring animals, no reporting on randomization when allocating dams/pups to dose groups).

Dose-dependent delays in eye opening were reported in CD-1 mouse neonates with delays of approximately 2 and 5 days observed at 3 and 5 mg/kg-day PFNA, respectively (see Table 3-11). Consistent with these findings, statistically significant delays in eye opening were also observed in

129S1/SvlmJ mouse offspring following maternal PFNA exposures (Wolf et al., 2010). The mean day of eye opening in wild type mice was delayed by approximately 2 days (from PND 13.7 in controls to PND 15.8) at 2 mg/kg-day PFNA (see Table 3-12). Eye opening in PPARα null mice was not significantly affected at any of the doses tested (see additional discussion below). Statistically significant delays in preputial separation of approximately 2 and 5 days and vaginal opening of approximately 3 and 7 days were reported at 3 and 5 mg/kg-day PFNA, respectively (see Table 3-11, (Das et al., 2015)). The delays in vaginal opening align with the *low* confidence findings by Zhang et al. (2021) that also reported statistically significant delays in vaginal opening of approximately 2 days in gestationally exposed ICR mouse pups at 3 mg/kg-day PFNA.

Table 3-11. PFNA effects on landmarks of developmental progression in gestationally exposed mouse neonates, including eye opening, vaginal opening, and preputial separation on PND 15, 30, and 28, respectively (Das et al., 2015)[a]

Dose (mg/kg-d)	Eye opening			Vaginal opening			Preputial separation		
	Mean (d)	SE	Delay (d)	Mean (d)	SE	Delay (d)	Mean (d)	SE	Delay (d)
0	15.4	0.15		29.9	0.58		28.5	0.29	
1	15.8	0.21	0.4	31.2	0.50	1.3	28.9	0.31	0.4
3	*17.3**	*0.31*	*1.9*	*32.9**	*0.40*	*3.0*	*30.7**	*0.21*	*2.1*
5	*20.3**	*0.65*	*4.8*	*36.5**	*0.62*	*6.6*	*33.8**	*0.49*	*5.3*

[a]Italicized values denote statistically significant differences reported between control and treatment litters (statistical unit; *$p < 0.05$).

Table 3-12. PFNA effects on 129S1/SvlmJ and PPARα null mouse neonates eye opening from PND 13 to 19; Mean percent of neonates with both eyes open (Wolf et al., 2010)[a]

Dose (mg/kg-d)	Wild type mice							PPARα null mice						
	Postnatal age (d)							Postnatal age (d)						
	13	14	15	16	17	18	19	13	14	15	16	17	18	19
0	63	73	89	96	98	99	99	42	75	92	96	96	96	98
0.83	31	77	93	100	100	100	100	30	63	84	96	98	100	99
1.1	45	82	93	100	100	100	100	29	66	88	91	93	94	94
1.5	31	88	90	90	91	93	97	46	85	94	99	99	99	100
2	*4.8***	*26***	*38****	*76**	86	93	100	33	72	80	85	90	94	95

[a]Italicized values denote significant differences reported between control and treatment litters (statistical unit; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Mechanistic and Supplemental Information

Potential mechanisms related to developmental effects reported in the epidemiological evidence base are not well understood. Analyses in epidemiological studies have shown little support for known fetal growth restriction risk factors (e.g., thyroid hormones or maternal glucose levels associated with gestational diabetes mellitus) as a mediator of PFNA-related birth weight decreases (Xiao et al., 2019; Starling et al., 2017; Valvi et al., 2017). Considering the experimental animal evidence, based primarily on the Wolf et al. (2010) study in wild type and PPARα null mice, the data suggest that activation of PPARα plays a role in PFNA-induced developmental effects. In comparison to wild type mice, PPARα null mouse pups were less affected by PFNA exposure as evidenced by a reduction in or complete lack of significant effects on postnatal survival, body weight gain, and eye opening (see Tables 3-9, 3-10, 3-11, 3-12). The *low* confidence study by Zhang et al. (2021) was also consistent with Wolf et al. (2010). In this study, the significant delays in vaginal opening of mouse pups exposed gestationally to 3 mg/kg-day PFNA showed recovery to control levels with co-administration of the PPARα inhibitor GW6427 from PND 1 to 14 (this endpoint was not evaluated in pups born to dams co-administered GW6427 during pregnancy). The suggested PPARα dependence of developmental effects in rodent offspring has also been reported with the structurally similar PFOA compound (Abbott et al., 2012; Lau et al., 2006). While still not well characterized, the putative functioning of PPARα in the early life of rodents is thought to be similar to adults; fetal expression of PPARα occurs later in rodent gestation (GD 13.5) and generally predominates in cells with high catabolic rates of fatty acid and peroxisomal metabolism, such as hepatocytes and cardiomyocytes (Abbott et al., 2010; Abbott, 2009; Michalik et al., 2002).

Beyond the in vivo mechanistic testing, in vitro testing with bovine embryos also reported declines in developmental competence at 10 µg/mL PFNA, as well as impaired blastocyst development 7 and 8 days after fertilization (Hallberg et al., 2019). Additionally, the mechanistic evidence for PFNA-related developmental effects includes testing in other well-known non-mammalian models of early vertebrate development, including embryonic zebrafish (*Danio rerio*) and frogs (*Xenopus laevis*). Several studies with PFNA employing standard embryotoxicity screening in aqueously exposed larval zebrafish report dose-dependent mortality, delayed hatching, and morphological impairments (Gong et al., 2022; Rainieri et al., 2017; Jantzen et al., 2016; Liu et al., 2015; Ulhaq et al., 2013a; Zheng et al., 2012), behavioral abnormalities (Liu et al., 2023; Rericha et al., 2021; Jantzen et al., 2016; Ulhaq et al., 2013b), and oxidative stress (Rainieri et al., 2017; Liu et al., 2015; Yang et al., 2014). ToxCast screening assays in embryonic zebrafish with PFNA likewise report one assay with modest activity on hatching success, survival, and morphology, as well as two additional assays with some bioactivity of pleiotropic neuronal and tissue development (see Appendix C.2 for active hits and a link to null assays). While not generally targeted in the zebrafish studies with PFNA, the developmental testing by Liu et al. (2015) reported no significant upregulation in expression of PPARα (*pparaa* and *pparab*) in larval zebrafish exposed to PFNA at 100 µMol/L (46 mg/L) from 1 to 96 hours post fertilization. PFNA-induced mortality

and developmental malformations have also been reported in embryonic *Xenopus laevis* but molecular mechanisms were not evaluated in this study (Kim et al., 2013).

The human relevance of findings from the experimental animal evidence pointing to a role for PPARα activation in PFNA-induced developmental effects is not well studied. However, the PPAR isoforms (PPARα, PPARβ/δ, PPARγ) are evolutionarily conserved and widely expressed in developing humans, rodents, amphibians, birds, and teleosts but with differing and overlapping spatiotemporal expression patterns (reviewed in Michalik et al. (2002), Abbott (2009), Ibabe et al. (2005), Bertrand et al. (2007), and Desvergne and Wahli (1999)). This evidence supports the broad functioning of PPAR transcription factors in vertebrate development as major regulators of tissue differentiation and organ development, although there continue to be unknowns as to PPARα functioning in these processes. Thus, taken together, the small mechanistic evidence base informing PFNA-induced developmental effects in mammalian and non-mammalian species is somewhat aligned but remains to be more fully characterized. In particular, the in vivo animal data suggest that the involvement of PPARα could lead to exaggerated responses for some endpoints as compared with what might be expected in humans (based on the inference that rodent PPARα is generally more responsive than human PPARα). However, no studies employing humanized PPARα animal models have examined these PFNA-induced effects, and both PPARα-dependent and PPARα -independent pathways have been determined to contribute to the effects of other PFAS (this is poorly studied for PFNA). Therefore, the possibility remains that non-PPARα pathways that might be only a minor contributor to rodent responses could be major contributors to responses in humans.

Evidence Integration

The human epidemiological evidence base provides *robust* evidence of associations between PFNA exposures and adverse developmental health effects, primarily fetal growth restriction but to a lesser degree birth length and postnatal growth endpoints. The evidence base was judged to provide *robust* evidence of an effect based primarily on consistent and relatively large magnitude reductions in birth weight. However, there are residual uncertainties related to some potential sources of bias by sample timing and uncertainty regarding potential impact of PFAS co-exposures (see meta-analysis, Appendix C.1). While there was some attenuation in EPA's meta-analytical results in early sampled studies, the evidence was *robust* in showing inverse associations across all sampling periods and study confidence levels, indicating there are demonstrated birth weight deficits as PFNA exposure levels increase. Some residual uncertainty remains regarding potential bias due to potential confounding by exposure to other co-occurring PFAS. As detailed in Appendix C.1, PFDA had the strongest and most consistent correlations across all epidemiological studies with PFNA. Yet, subsequent evaluations in Appendix C.1 did not show a consistent direction or impact of confounding from PFAS co-exposures across the available studies. The PFNA evidence base for fetal growth restriction and developmental epidemiological effects is also considerably stronger than that seen for PFDA (e.g., contrasting the *robust* human evidence conclusion in this

assessment, the draft IRIS assessment for PFDA judged that the human studies on developmental effects provided only *slight* evidence) (U.S. EPA, 2023a). Thus, the potential impact of PFAS co-exposures did not substantially reduce confidence in the evidence base. In short, these residual sources of uncertainty (i.e., PFAS co-exposures and PFNA exposure sampling timing) are not judged to explain the consistent results seen across the birth weight endpoints detailed in the synthesis or the meta-analysis.

The fetal growth restriction endpoints provide the strongest evidence for adverse developmental effects among the available studies including many high-quality and sensitive studies constituting the evidence base. The vast majority of 32 studies showed some deficits across various birth weight measures in relation to PFNA. This included 16 of 20 *high* (8 of 11) or *medium* (8 of 9) confidence studies that showed some evidence of mean birth weight deficits in the overall population. Although the majority of studies with sex-specific analyses reported mean birth weight reductions (10 of 12) including, a clear interpretation of potential sex differences could not be drawn based solely on the epidemiological literature. Among the 20 birth weight studies (and 26 fetal growth studies in total) that were either *high* or *medium* confidence, there was minimal concern for risk of bias across this large evidence base. Given the prospective nature of most of the studies, temporality was established, since exposure measures were collected before outcomes were determined. Sensitivity of both the exposure and certainty of the outcome measures was also a strength of this evidence base, as birth weight is a precise and accurately recorded health endpoint.

Consistent with the categorical and continuous mean birth weight results, the meta-analysis shown in Appendix C provides additional supportive evidence of an adverse effect on birth weight from maternal exposure to PFNA. Mean birth weight deficits were evident irrespective of timing of exposure measurement, with a statistically significant decreased mean birth weight summary estimate of 32.9 g per ln-unit increase in PFNA (95% CI: −47.0, −18.7). This overall result from 27 studies was similar when studies were restricted to just the 12 *high* confidence studies (β = −28.0 g; 95% CI: −49.0, −6.9) and when the 22 *medium* and *high* confidence studies were combined (β = −32.9 g; 95% CI: −48.0, −17.8). Statistically significant results were also observed when restricted to studies with early pregnancy sampling periods such as predominately trimester one (β = −22.0 g; 95% CI: −40.1, −4.0), although larger deficits were seen in later periods such as mid- and late pregnancy (β = −48.4 g; 95% CI: −67.7, −29.0) and postpartum (β = −42.9 g; 95% CI: −88.0, 2.2). The strength of the association is reflected by the magnitude of the observed birth weight decrements in individual studies, with three of these overall population studies reporting birth weight deficits in excess of 100 grams per unit increase or across PFNA exposure categories. So, while the 33 g deficit per ln-unit increase may seem modest, these differences need to be extrapolated across the full exposure range reported across studies. For example, PFNA exposure levels ranged from 0.2 to 2.3 ng/mL with maximum values of 0.81 to 22 ng/mL (median of the maximums = 4.5 ng/mL).

Overall, the magnitude of birth weight deficits in the overall population or either sex ranged from 15 to 150 grams per ln-unit PFNA increase. Some consistency in magnitude of associations was demonstrated for a majority of studies in the overall population as 12 of 18 studies ranged from –40 to –62 g per ln-unit increase. Birth weight deficits can increase the number of infants at higher risk for comorbidities and mortality, especially during the first year of life, and can increase risk for adverse health outcomes later in life (Perng et al., 2016b; De Boo and Harding, 2006). These population-level changes may have a large public health impact when mean birth weight deficits shift the birth weight distribution to include more infants in the low-birth-weight category. Additional factors that increased confidence in this judgment included the detection of exposure-response relationships in four fetal growth restriction studies based on categorical analyses, including three analyses specific to girls. Although the sex-specific differences are generally unclear, the evidence of a biological gradient is consistent with the numerous other studies demonstrating developmental reductions with increasing PFNA exposures. The consistent and strong evidence for decreases in birth weight in PFNA-exposed humans is further supported by coherent evidence for inverse associations between PFNA exposure and postnatal growth (childhood weight and height) and to a lesser degree birth length. Six of 13 studies detected adverse associations between increasing PFNA exposures and reduced birth length with 4 of these being either *high* or *medium* confidence. The postnatal growth data also showed some consistency across most studies including some evidence of exposure-response relationships. Four of six studies, including two of three *high* confidence studies, reported deficits in childhood weight and height in relation to PFNA. All three studies with categorical data demonstrated monotonicity (one *high* and two *low* confidence studies) in either postnatal height or weight deficits providing additional dose-dependence support for the primary results based on continuous exposure metric differences.

In support of the human evidence, three *high* and *medium* confidence animal studies with PFNA in two strains of mice and one rat strain provide *moderate* evidence of developmental toxicity in offspring exposed to PFNA. Gestational exposures to PFNA caused consistent, dose-dependent, and coherent reductions in the survival and body weights of mice at birth (survival only) and postnatally, generally at PFNA dose levels ≥2 mg/kg-day, as well as delays in developmental milestones (eye opening, vaginal opening, preputial separation). The number of live pups born per litter was significantly reduced in both mouse strains tested with 21% and 31% of CD-1 and 129S1/SvlmJ mouse pups surviving to weaning at 5 mg/kg-day and 2 mg/kg-day, respectively, although mortality in 129S1/SvlmJ mice did not follow a clear dose gradient.

Dose-dependent reductions in postnatal body weight were also observed in both mice and rats; at weaning, sex-combined CD-1 mice weighed ~75% and 50% of concurrent controls at 3 and 5 mg/kg-day doses, respectively. Compared with controls, body weight deficits of 129S1/SvlmJ mice ranged from 15% to 36% at 2 mg/kg-day during the preweaning period. Birth weights of SD rats were also significantly reduced at 5 mg/kg-day PFNA (and were of similar magnitude among males and females; 12%–13%). The reduced body weight in rats recovered by weaning and was

possibly influenced by the lack of lactational exposure after birth as preweaning offspring were cross-fostered to control dams. A related unknown for PFNA is whether altered mammary gland differentiation in nursing dams could have played a role in reducing offspring body weight during the preweaning term, as has been observed with the structurally similar PFOA (White et al., 2009). Developmental delays in both strains of mice provided further evidence of early lifestage effects. Dose-dependent delays in eye opening were observed in both CD-1 and 129S1/SvlmJ mice at ≥2 mg/kg-day PFNA, along with dose-dependent delays in attaining other developmental milestones (preputial separation and vaginal opening) in CD-1 mice at ≥3 mg/kg-day PFNA. The adversity and human relevance of these developmental delays have been previously described (U.S. EPA, 2022a, 2021b), and are likewise interpreted as adverse and human relevant herein.

In contrast to wild type mice, PPARα null mice were generally unaffected by PFNA gestational exposures for the endpoints examined (i.e., postnatal survival, body weight gain, timing of eye opening) except for significant liver weight increases at 2 mg/kg-day PFNA (see Section 3.2.3, "Liver weight"). While this suggests a role for PPARα in the MOA of PFNA-induced developmental toxicity in rodents, it is not well studied and the relative contribution of PPARα dependent versus independent mechanisms remains unknown. PPARα activation pathways are also suggested in the developmental toxicity pathway of PFOA (Abbott, 2009; Rosen et al., 2009; Lau et al., 2006). While PPARα is shown to be well conserved in vertebrate development, there is less understanding of PFNA-induced responses of these pathways in human development with no studies in humanized PPARα animal models.

The cross-stream coherence of findings among PFNA-exposed humans and rodents, particularly the growth restriction endpoints, lends support to the human relevance of the findings in mice and rats, and reduces uncertainties in the individual evidence streams that strengthen the evidence integration judgments. For example, the growth restriction findings in rodents (i.e., deficits in body weight and delays in attaining developmental landmarks) helps further mitigate residual uncertainties regarding the fetal and postnatal growth restriction observed in the epidemiology studies (e.g., some uncertainty due to sample timing and PFAS co-exposures).

Taken together, given the strength of the fetal and postnatal growth restriction data in humans, with support from rodent bioassays, the ***evidence demonstrates*** that PFNA causes developmental growth impairments in humans given sufficient exposure conditions (see Table 3-13).[11] This determination is based on *robust* evidence of decreased birth weights in studies of exposed humans and median PFNA values during pregnancy ranging from 0.2 to 2.3 ng/mL, with support from generally coherent epidemiological findings for other fetal and postnatal growth restriction endpoints (e.g., birth length, postnatal weight and height). In further support, cross-stream coherence is provided by *moderate* animal evidence for PFNA-induced developmental effects from gestationally exposed rodent offspring generally at PFNA doses ≥2 mg/kg-day.

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

Table 3-13. Evidence profile table for developmental effects

Summary of human, animal, and mechanistic evidence					Inferences across evidence streams
Evidence from studies of exposed humans-fetal growth restriction (see Section 3.2.2. Developmental Effects, Human Studies)					⊕⊕⊕ ***Evidence demonstrates***
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream summary**	
Fetal growth restriction (Mean birth weight (BWT)/*z-score*s and related dichotomized measures) Fifteen *high* confidence, 11 *medium* confidence, 9 *low* confidence studies	• 27 of 35 mean or standardized BWT studies, including 24 of 32 mean BWT studies showed some deficits either in the overall population (19 of 27), or among boys (7 of 12) or girls (7 of 11) • Nearly half of studies reported some statistically significant BWT deficits • Decreased BWT observed in studies with PFNA median values ranging from 0.2 to 2.3 ng/mL • Meta-analysis (per ln-unit increase) showed consistent pooled mean BWT deficits for all 27 studies (β = −32.9 g; 95% CI: −47.0, −18.7) and across *medium* and *high* confidence (β range: −28 to −39 g) studies; results *robust* to sampling timing strata with deficits also seen for earlier pregnancy	• *Consistent evidence* with 16 of 20 *medium* (8 of 9) or *high* (8 of 11) confidence studies showing some evidence of mean BWT deficits in overall population, all with adequate and study sensitivity scores • *Consistency* in magnitude of associations demonstrated for majority of studies in overall population (12 of 18 studies ranged from –40 to –62 g per ln-unit increase) • *Consistency* further supported by BWT deficits in a meta-analysis, irrespective of sample timing and study confidence, with decreases seen for all studies as well	• 4 of 14 different studies in either sex or overall population (including 2 of 10 studies in overall population) with categorical data showed an *exposure-response relationship* • Uncertainty whether co-exposure to highly correlated PFAS (e.g., PFDA) may lead to confounding; the associations for PFDA and BWT are less consistent than PFNA but comparable in magnitude, and thus would not likely fully explain the associations seen here	⊕⊕⊕ *Robust* Based on consistent evidence of neonatal and postnatal growth restriction, most notably for weight indices which are the most sensitive endpoints Although there was less consistency seen in other endpoints, there was some coherence across the other fetal growth endpoints	*Primary basis:* *Robust,* consistent BWT deficits in human studies and in EPA's meta-analysis, with median PFNA exposures ranging from 0.2 to 2.3 ng/mL. The epidemiological evidence is supported by *moderate* animal evidence from 3 *high/medium* confidence studies in mice and rats treated gestationally generally at doses ≥2 mg/kg-d *Human relevance:* Evidence in animals is presumed relevant to humans based on biological similarities in birth and early developmental processes in mammalian models *Cross-stream coherence:* Impaired fetal growth is observed in both humans

Summary of human, animal, and mechanistic evidence					Inferences across evidence streams
	sampling periods (β = −22.0 g; 95% CI: −4.1, −4.). • 6 of 8 studies of small for gestational age (SGA), low birth weight (LBW), or very LBW showed some increased risks in relation to PFNA	as for high or *medium* and *high* confidence studies alone • Some of the mean BWT deficits and odds ratios for SGA, LBW and very LBW were *large in magnitude* • *26 high* and *medium* confidence studies available with accurate outcome measures • Fetal growth restriction results were statistically *robust* across sample timing strata as demonstrated in quantitative meta-analysis and qualitatively for other endpoints			and mice, reducing uncertainties within each evidence stream in isolation *Summary of potential susceptible populations and lifestages:* Fetus, infants, children
<u>Fetal growth restriction (Birth length)</u> Seven *high* confidence, 6 *medium* confidence, 7 *low* confidence studies	• 12 of 20 studies reported inverse associations between PFNA and birth length • 7 of 17 studies reported inverse associations between PFNA exposures and birth length in the overall population (i.e., 6 of 11 *medium* and *high* studies in total)	• 6 of 7 studies in the overall population showing adverse effects were *high* or *medium* confidence • The four *medium* and *high* studies of the overall population had inverse mean birth length	• Uncertainty whether co-exposure to highly correlated PFAS (e.g., PFDA) may lead to confounding; however, as described for birth weight, this would not likely fully explain the associations seen for PFNA and other fetal growth measures		

Summary of human, animal, and mechanistic evidence					Inferences across evidence streams
		associations similar in magnitude	• Evidence of exposure-response relationships based on categorical PFNA data in 2 of 4 studies • 5 of the 7 studies showing inverse association in overall population were based on late biomarker sampling suggesting potential impact of pregnancy hemodynamics • Unexplained inconsistency		
<u>Fetal growth restriction (Head circumference)</u> Six *high* confidence, 7 *medium* confidence, 4 *low* confidence studies	• 8 of 17 studies reported an inverse association between PFNA and head circumference • 4 (3 *high*; 1 *medium*) of 13 studies reported an inverse association between PFNA and head circumference in the overall population (i.e., 4 of 10 *medium* and *high* studies in total)	• 13 of 17 studies, were *high* or *medium* confidence with all 13 having good or adequate study sensitivity • Evidence of exposure-response relationships based on categorical data in 2 of 4 studies. • 5 of 8 studies with inverse associations were based on early biomarker sampling suggesting limited impact of pregnancy hemodynamics	• Unexplained inconsistency • Lack of evidence of exposure-response relationships based on categorical PFNA data		

Summary of human, animal, and mechanistic evidence					Inferences across evidence streams
Evidence from studies of exposed humans-postnatal growth (**see Section 3.2.2. Developmental Effects, Human Studies**)					
<u>Postnatal growth (Weight measures)</u> Five *high*, 1 *medium*, 2 *low* confidence studies	• 5 of 8 studies (including 3 of 5 *high* and both *low* confidence) studies showed some evidence of postnatal weight reductions	• *Exposure-response relationships* observed both postnatal weight studies using PFNA categorical data • Consistency in inverse association in majority of studies	• Uncertainty as to whether co-exposures to highly correlated PFAS (e.g., PFDA) in some of these studies would be confounders (see details above)	⊕⊙⊙ *Slight* Based on inconsistent evidence (including the higher quality studies) across endpoints. However, evidence was stronger for weight change measures which also demonstrated some exposure-response relationships	
<u>Postnatal growth (Height measures)</u> Three *high*, 1 *medium*, 2 *low* confidence studies	• 3 of 6 studies (including 2 of 4 *high* and 1 or 2 *low* confidence) studies showed some evidence of postnatal height reductions	*Exposure-response relationships* observed in one of the two height studies with categorical data	• Uncertainty as to whether co-exposures to highly correlated PFAS (e.g., PFDA) in some of these studies would be confounders (see details above)		
<u>Postnatal growth (Adiposity measures)</u> Three *high*, 1 *medium*, One *low* confidence studies	• Two studies each showed some evidence of increased and decreased adiposity	• No factors noted	• Uncertainty as to whether co-exposures to highly correlated PFAS (e.g., PFDA) in some of these studies would be confounders (see details above) • Unexplained inconsistency		

Summary of human, animal, and mechanistic evidence					Inferences across evidence streams
<u>Postnatal growth (Rapid weight gain)</u> Four *high* confidence studies	• One of four studies some evidence of increased odds for rapid growth	• No factors noted	• Uncertainty as to whether co-exposures to highly correlated PFAS (e.g., PFDA) in some of these studies would be confounders (see details above) • Unexplained inconsistency		
Evidence from studies of exposed humans-preterm birth (**see Section 3.2.2. Developmental Effects, Human Studies**)					
<u>Preterm birth</u> Six *high* confidence and 4 *medium* confidence studies	• 4 (1 *medium* and 3*high* confidence) of 10 studies reported some increased risks of preterm birth	• *Consistent* effect magnitude of moderate size in both continuous and categorical results (all ORs of 1.4–1.7) • 3 of 4 studies with increased risks were based on early biomarker sampling, suggesting limited impact of pregnancy hemodynamics	• Uncertainty as to whether co-exposure to highly correlated PFAS like PFDA may lead to confounding • Lack of *exposure-response* relationships based on categorical PFNA data • Unexplained inconsistency	⊕⊙⊙ *Slight* Based on inconsistent and limited evidence (based on evidence in 8 of 18 different gestational duration studies) in either sex or the overall population	
<u>Gestational age</u> Eight *high* confidence, 6 *medium* confidence, 4 *low* confidence studies	• 7 of 18 studies showed some evidence of inverse associations with gestational age including 6 of 16 studies in the overall population (3 each *high* and *medium* confidence)	• 6 of 7 studies with inverse associations were either *high* or *medium* confidence	• Unexplained inconsistency, with most studies not finding effects. • Lack of expected exposure-response relationships based on categorical PFNA data		

Summary of human, animal, and mechanistic evidence					Inferences across evidence streams
Evidence from studies of exposed humans-other effects (see Section 3.2.2. Developmental Effects, Human Studies)					
<u>Birth defects</u> One *medium* confidence and 1 *low* confidence study	• Both studies showed nonsignificant but increased odds ranging from 1.3 to 1.6 for all different types of defects examined	• Consistency in relative risk reported in the *medium* confidence study for various heart defects	• Broad groupings such as "all birth defects" in *low* confidence study examined in one study lack specificity for drawing further conclusions	⊕⊙⊙ *Slight* Based on limited evidence in one medium confidence study which showed consistent associations for heart defects	
<u>Anogenital distance</u> Three *medium* confidence studies	• Associations in both directions in boys • Inverse association in 1 of 2 studies in girls	• No factors noted	• Unexplained inconsistency	⊕⊙⊙ *Slight* Based on inconsistent and limited evidence in either sex	
<u>Fetal loss – spontaneous abortion</u> Five *medium* confidence and 1 *low* confidence studies	• 1 *low* and 1 *medium* confidence study showed some evidence of positive associations, while other studies reported null or inverse associations	• No factors noted	• Unexplained inconsistency	⊙⊙⊙ *Indeterminate* Based on inconsistent evidence	

Summary of human, animal, and mechanistic evidence					Inferences across evidence streams
Evidence from in vivo animal studies (see Section 3.2.2. Developmental Effects, Animal Studies)					
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream summary**	
<u>Fetal loss, malformations, and weight at late gestation</u> One *high* confidence study • CD-1 mice (GD 1–17)	• No effects on fetal (GD 17) survival or body weight; no skeletal or visceral malformations	• No factors *noted*	• No factors *noted*	⊕⊕⊙ *Moderate* Consistent, generally dose-dependent, and coherent reductions in preweaning neonatal survival, pre- and post-weaning body weight, and developmental milestones in two strains of mice, and reduced birth weights in rats. There were some differences in sensitivity across species, sex, and offspring lifestage gradients for some endpoints	
<u>Postnatal survival</u> Two *medium* confidence studies • CD-1 mice (GD 1–17) • 129S1/SvlmJ wild type, PPARα null mice (GD 1–18) One *low* confidence study • SD rats (GD 1–20)	• Significant reductions in number of live pups born and preweaning survival of CD-1 mice at 5 mg/kg-d and 129S1/SvlmJ mice at 2 mg/kg-d	• *Consistent* reductions in postnatal survival in two mouse strains *in medium* confidence endpoints • *Magnitude* of effect, as high as 79% in CD-1 mice and 69% in wild type mice at weaning PND 21 • *Coherence* with reduced body *weight* and delayed developmental landmarks	• Lack of expected dose-*dependence* in 129S1/SvlmJ mice		
<u>Postnatal body weight</u>	• Reductions in preweaning body weights in three strains of gestationally exposed mice in 2	• *Dose-dependent* reductions in preweaning body weight gain in 3	• Some unexplained inconsistency across strains in the persistence of		

Summary of human, animal, and mechanistic evidence					Inferences across evidence streams
Three *high/medium* confidence studies • CD-1 mice (GD 1–17) • 129S1/SvlmJ wild type, PPARα null mice (GD 1–18) • SD rats (GD 1–20) Two *low* confidence studies • Parkes mice (GD 12–21) • ICR mice (GD 1–18)	*high/medium* confidence studies, 1 *low* confidence study, generally at ≥2 mg/kg-day; mouse birth weights unaffected • Persistent post-weaning deficits in body weight gain in male CD-1 mice into adulthood (41 wk of age); smaller deficits post-weaning in female CD-1 mice that were no longer statistically significant by 7 wk of age and no longer biologically significant (≤10% difference relative to controls) by 11 wk • Persistent preweaning deficits in body weight gain of female 129S1/SvlmJ mice up to PND 21 (weaning), statistically significant reductions in male body weight gain up to PND 17 • Significant reductions in cross-fostered male and female rat birth weights at gestational exposures of 5 mg/kg-d that persisted in males to PND 11 and in females to PND 7; null effects by weaning PND 21	mouse strains with consistent ages of onset (PND 7) • *Dose-dependent* reductions in body weight gain of post-weaning males and females, persisting well into adulthood in males and 6 wk of age in females. • *Coherence* with reduced survival and delayed development • *Magnitude* of effects as high as 51% at weaning PND 21 (sexes combined) and 15% in adult males at 41 wk • *High* and *medium* confidence endpoints	postnatal body weight decreases across sexes		

Summary of human, animal, and mechanistic evidence					Inferences across evidence streams
<u>Postnatal attainment of developmental landmarks</u> Two *medium* confidence studies • CD-1 mice (GD 1–17) • 129S1/SvlmJ wild type, PPARα null mice (GD 1–18) One *low* confidence study • ICR mice (GD 1–18)	• Delays in eye opening in 2 strains of mice exposed gestationally at ≥2 mg/kg-d. • Delays in vaginal opening and preputial separation in 2 strains of mice exposed gestationally at ≥3 mg/kg-d • Time to eye opening unaffected in PPARα null mice exposed gestationally at up to 2 mg/kg-d	• *Consistent* delays in postnatal age of eye opening in 2 mouse strains and as observed with other PFAS • *Dose-dependent* delays in postnatal age of eye opening, preputial separation, and vaginal opening in CD-1 mice • *High* and *medium* confidence studies • *Coherence* with reduced postnatal survival and body weight gain • Concerning *magnitude* of effect, with delays in eye opening and preputial separation of 2–5 d and in vaginal opening of 3–7 d	• No factors noted		

Summary of human, animal, and mechanistic evidence			Inferences across evidence streams
Mechanistic evidence and supplemental information (see subsection above)			
Biological events or pathways	**Summary of key findings and interpretation**	**Evidence stream judgment**	
Molecular events – PPARα and CAR/PXR activation	PPARα null mouse neonates generally unaffected by PFNA gestational exposures (null effects on postnatal survival, body weight, eye opening); Gene expression profiles in fetal and offspring livers of CD-1 mice indicate PPARα activation, as well as to a lesser extent CAR/PXR activation.	The mechanistic evidence base remains to be better characterized but indicates a role for PPARα in PFNA developmental effects	

1

3.2.3. Hepatic Effects

This section describes potential effects of PFNA exposure on the liver. Available epidemiological studies include evaluations of liver enzymes and hepatobiliary markers in the serum, while animal studies include these endpoints in addition to organ weight, histopathology, and levels of triglyceride, cholesterol, and glycogen in the liver. This section has some overlap with the evidence synthesis and integration summaries for other health outcomes where studies evaluated potential PFNA effects on developmental and cardiometabolic endpoints, including liver weight in gestationally exposed mouse offspring and serum lipids in humans and rodents (see Sections 3.2.2 and 3.2.9).

Human Studies

There are 18 human studies of hepatic effects. Sixteen epidemiology studies (19 publications) report on the relationship between PFNA exposure and serum markers of hepatobiliary injury (i.e., ALT, AST, GGT, and/or total bilirubin). Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are considered reliable markers of hepatocellular function/injury, with ALT considered more specific and sensitive (Boone et al., 2005). Alkaline phosphatase (ALP), bilirubin, and γ-glutamyl transferase (GGT) are routinely used to evaluate hepatobiliary toxicity (Hall et al., 2012; EMEA, 2008; Boone et al., 2005); elevation of these markers is an indication of potential liver injury. Two studies examined clinical liver disease, specifically non-alcoholic fatty liver disease.

Serum markers of hepatobiliary injury

For serum markers, based on evaluation of the 16 available studies, 12 were classified as *medium* confidence, three were *low* confidence, and 1 (Jiang et al., 2014) was considered *uninformative* due to lack of consideration of potential confounding and excluded from further analysis (see Figure 3-40). Nine of the informative studies were cross-sectional studies in adults. Of these, three by Lin et al. (2010), Gleason et al. (2015), and Jain and Ducatman (2019d) were analyses of different sets of NHANES study years (1999–2004, 2007–2010, and 2011–2014, respectively). Omoike et al. (2020) examined NHANES waves from 2005 to 2012; because of the overlapping population with other NHANES studies, this paper was not considered a separate study. The remaining cross-sectional studies were in the general population in Canada (Cakmak et al., 2022), Korea (Kim et al., 2023), residents near a fluoropolymer plant (Yao et al., 2020), pregnant women (Liao et al., 2023), and primarily government employees in China (Liu et al., 2022; Nian et al., 2019). Cross-sectional studies were considered appropriate for this outcome as there is not an expectation of reverse causation; additionally, the long half-life of PFNA increases the likelihood of the current exposure being representative of an etiologically relevant period and there is potential for a short-term response in liver enzymes. These studies were all considered *medium* confidence, except Yao et al. (2020), which had concerns for selection bias as limited information was provided

on selection procedures. In addition, there was a cohort of elderly adults (Salihovic et al., 2018) and a cohort of workers at a polymer production factory (Mora et al., 2018), the latter of which was considered *low* confidence due concerns for potential confounding (such as occupational co-exposures) and exposure measurement, which was based on work history alone. In children and adolescents, in addition to the NHANES 2007–2010 analysis in Gleason et al. (2015) that included adolescents but did not provide stratified estimates, Attanasio (2019) examined NHANES data from 2013 to 2016 in adolescents. There were also two birth cohorts with follow-up into childhood (Stratakis et al., 2020; Mora et al., 2018)., both considered *medium* confidence. One cross-sectional study of obese children (Khalil et al., 2018) was *low* confidence due to concerns for potential confounding, exposure misclassification, and selection bias. Across the studies, liver enzymes were analyzed appropriately in serum. Analysis of PFNA in serum or plasma samples was considered appropriate in all studies.

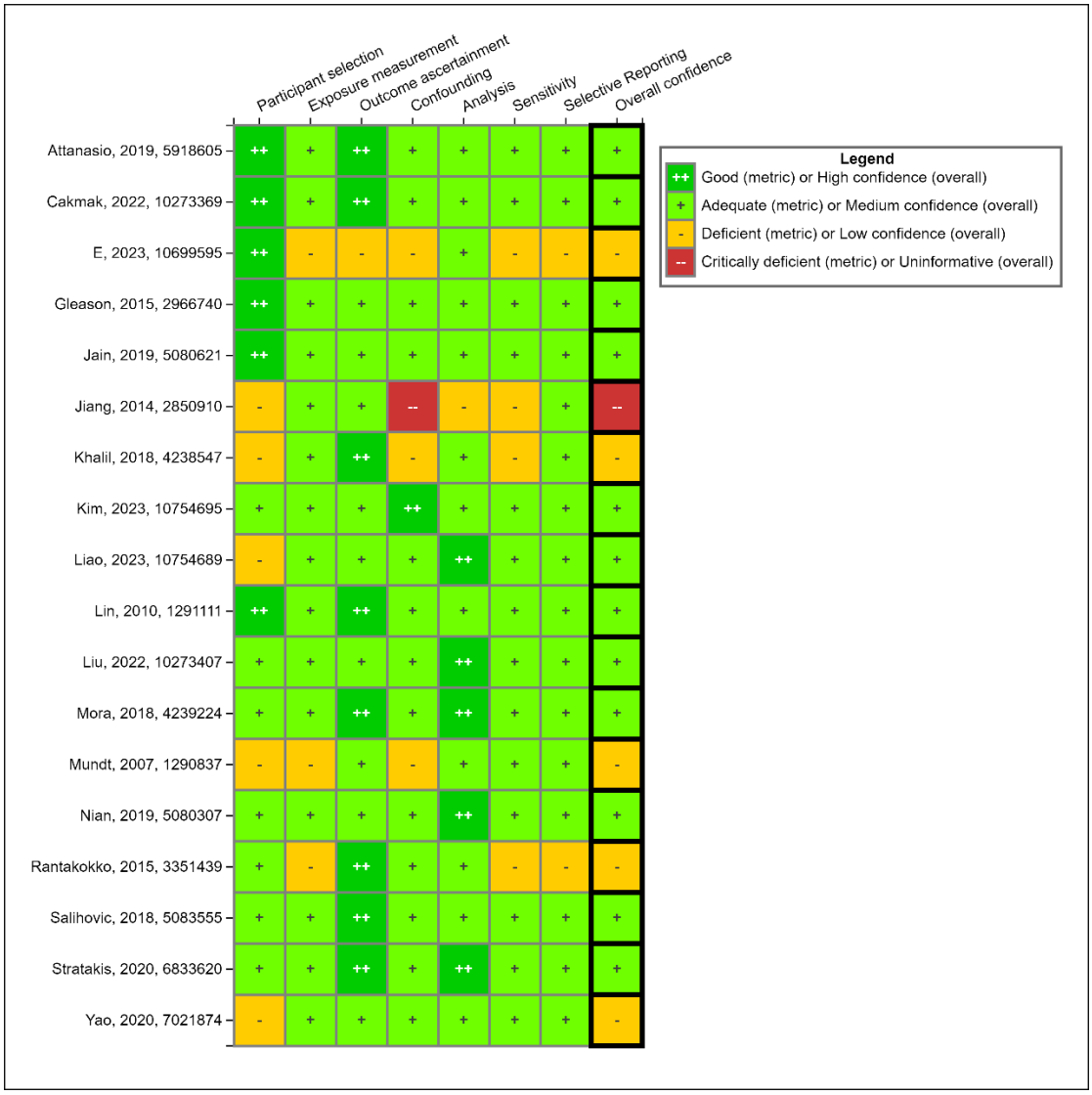


Figure 3-40. Study evaluation heat map of human epidemiology studies of hepatic effects. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500133/.

The results for the 10 *medium* confidence studies are presented in Table 3-14. All nine *medium* confidence studies in adults reported small positive associations between PFNA exposure and ALT, with statistical significance in all but three studies, which also were the studies with lowest exposure levels, and thus may have had more limited sensitivity. In Jain and Ducatman (2019d), the positive association was only observed in obese participants. While effect estimates were small in most studies, a percent difference of 20% was observed for the 95th percentile (2.9 ng/mL) compared with the 25th percentile in Liu et al. (2022), and an exposure-response gradient across quantiles was present. The three other studies that reported results as a percent change observed differences of 3.6, 8.6, and 6.2% (Kim et al., 2023; Cakmak et al., 2022; Nian et al., 2019), respectively, similar to the 6.4% in the 50th versus 25th percentiles in Liu et al. (2022).

Statistically significant positive associations with GGT were observed in six studies (Kim et al., 2023; Liao et al., 2023; Cakmak et al., 2022; Liu et al., 2022; Jain and Ducatman, 2019d; Nian et al., 2019). Positive associations were also observed in six studies in adults for AST (statistically significant in five, positive association only observed in obese participants for Jain and Ducatman (2019d)). Most studies also reported positive associations with total bilirubin, while associations with ALP were less consistent. One *low* confidence study reported positive associations with ALT, AST, and GGT (statistically significant for GGT) (Yao et al., 2020), while the *low* confidence study of occupational exposure reported no association between PFNA exposure and liver enzymes (Mundt et al., 2007).

In adolescents, Attanasio (2019) reported mixed associations, with a positive association with ALT and AST in girls but an inverse association in boys. In children, one *medium* confidence cohort study in children reported an inverse association in ALT with childhood PFNA exposure (Mora et al., 2018). The second cohort study in children (Stratakis et al., 2020) did not report results for individual liver enzymes but defined liver injury risk as having any liver enzyme concentration above the 90th percentile for the study population. They found increased odds of liver injury risk with higher PFNA exposure. The *low* confidence study of obese children reported no association between PFNA and liver enzymes (Khalil et al., 2018).

It is possible that the observed associations (primarily in adults) could be due to confounding by co-occurring PFAS. In the studies that reported correlations across PFAS, the correlations between PFNA and PFDA, PFOA, and PFOS were moderate to high. Correlations with PFDA were highest, ranging from 0.44 to 0.89, while PFOA and PFOS were generally less than 0.6 (with the exception of 0.86 for PFOS in Liu et al. (2022)). Most of the studies did not perform multipollutant modeling, but five studies did present mixture results using various methods. In each study, the analyses were not designed to identify the association for PFNA with and without adjustment for other PFAS, but rather to examine the effect of a mixture of PFAS. However, weights for each PFAS in the mixture provide an indication of which PFAS(s) were most influential on the association with liver enzymes. In two studies, PFNA and PFOA had the greatest contribution/weight in the mixture (Borghese et al., 2022; Stratakis et al., 2020), and in a third, PFNA was the strongest driver for ALT and GGT (Kim et al., 2023). In contrast, in Liu et al. (2022), PFOS was the dominant component to the combined effect, but the correlation with PFOS was notably higher than in other studies which may make it difficult to disentangle the effects. In Liao et al. (2023), PFBS and PFHxS were the top contributors, depending on the specific liver enzyme. While the possibility of confounding across PFAS is difficult to rule out, it is unlikely to explain the observed associations given that PFNA was a top contributor across several PFAS in three of five studies. In addition, a strong association with hepatic effects was observed in animal studies (see following section), which would not be subject to this source of confounding. This increases the likelihood that the association observed in epidemiology studies is a true effect.

Non-alcoholic fatty liver disease

The two cross-sectional studies of non-alcoholic fatty liver disease (NAFLD) were both evaluated as *low* confidence due to concerns that exposure measured concurrent with this chronic outcome does not represent an etiologically relevant period. Rantakokko et al. (2015) used histological findings from biopsies obtained during elective gastric bypass operation and reported an inverse association with PFNA exposure (OR 0.64, 95% CI <0.01, 0.53 for 2–4 foci versus none per 200× field). E et al. (2023), using data from NHANES, analyzed a surrogate for NAFLD that included several variables including liver enzymes, waist circumference, insulin, and glucose (authors report that the area under the receiver operating characteristic curve was 0.78 in predicting ultrasound-diagnosed NAFLD). This study reported a positive association in women but not men, with the strongest association in premenopausal women (OR 2.3, 95% CI 1.22, 4.39 in quartile 4 versus quartile 1). Sex stratification was not performed in Rantakokko et al. (2015), so it is not possible to assess consistency in these sex differences across studies.

Given the consistency of the positive direction of association for ALT, AST, and GGT across the studies in adults there is evidence that PFNA exposure may be associated with altered liver function. There is some uncertainty as to the biological significance of the observed changes due to the small magnitude of effect in most studies, particularly given that the two available studies of more functional hepatic endpoints (e.g., histopathology) are *low* confidence and the functional results are inconsistent across studies. However, abnormally increased serum ALT indicates impaired liver functioning and even small increases can be predictive of liver disease (U.S. EPA, 2022c; Valenti, 2021; Park et al., 2019).

Table 3-14. Associations between PFNA and liver enzymes in *medium* confidence epidemiology studies

Reference	Population	Median exposure (IQR) or as specified	Effect estimate	ALT	AST	ALP	GGT	Total bilirubin
Adults								
Cakmak et al. (2022) Borghese et al. (2022)	Cross-sectional (2007–2017); Canada; 3,021 adults	GM 0.6	% change (95% CI) for GM change	3.6 (−1.7, 9.3)	1.0 (−1.2, 3.2)	2.3 (−1.1, 5.7)	**11.4 (0.8, 23.1)***	0.00 (−11.1, 12.5)
	1,404 adults		% change (95% CI) for doubling	NR	**6.4 (3.8, 9.0)***	**3.2 (0.3, 6.3)***	**13.8 (4.2, 24.3)***	NR
Liao et al. (2023)	Cross-sectional analysis within cohort (2015–2019); Canada, 420 pregnant women	0.6 (0.4–1.0)	β (*p*-value) for tertiles vs. T1	T2: 2.08 (−0.90, 5.05) T3: 1.06 (−1.93, 4.05)	T2: 0.84 (−1.34, 3.03) T3: 0.002 (−2.19, 2.19)	NR	**T2: 1.23 (−0.39, 2.85)** **T3: 1.68 (0.06, 3.30)***	T2: 0.37 (−0.93, 1.67) T3: −0.06 (−1.36, 1.24)
Lin et al. (2010)	NHANES cross-sectional (1999–2004), U.S.; 2,216 adults	mean (SE) 0.7 (1.1) (women)	β (SE) for log-unit increase	0.8 (0.5), $p = 0.1$	NR	NR	0.0 (0.03), $p = 0.9$	0.5 (0.3), $p = 0.05$
Salihovic et al. (2018)	Cohort (2001–2014); Sweden; 1,002 elderly adults	0.7 (0.5–1.0)	β (*p*-value) for ln-unit increase	**0.04 (0.03,0.06)***	NR	**0.1 (0.1,0.2)***	0.04 (−0.01,0.1)	**−1.6 (−1.9,−1.2)***
Liu et al. (2022)	Cross-sectional (2018–2019);	1.1 (0.7–1.7)	% change (95% CI)	**50th: 6.42 (4.43, 8.46)***	**50th: 2.07 (0.80, 3.35)***	50th: −0.39 (−1.46, 0.69)	**50th: 6.60 (4.24, 9.01)***	**50th: 2.55 (1.12, 4.00)***

Reference	Population	Median exposure (IQR) or as specified	Effect estimate	ALT	AST	ALP	GGT	Total bilirubin
	China; 1,303 adults		vs. 25th percentile	**75th: 11.96 (8.09, 15.97)*** **95th: 20.20 (11.40, 29.70)***	**75th: 5.65 (3.22, 8.14)*** **95th: 12.48 (6.97, 18.28)***	75th: −1.63 (−3.59, 0.37) 95th: −4.06 (−8.13, 0.20)	**75th: 14.58 (9.90, 19.45)*** **95th: 28.82 (17.75, 40.94)***	**75th: 5.46 (2.75, 8.25)*** **95th: 10.33 (4.29, 16.73)***
Gleason et al. (2015)	NHANES cross-sectional (2007–2010), U.S.; 4,333 adolescents and adults (12+ yr)	1.4 (1.0–2.1)	β (95% CI) for ln-unit increase	**0.04 (0.02,0.07)***	**0.02 (0.01,0.03)***	−0.01 (−0.03,0.02)	0.01 (−0.01,0.03)	**0.03 (0.01,0.05)***
Jain and Ducatman (2019d)	NHANES cross-sectional (2011–2014), U.S.; 2,883 adults	0.8	β (*p*-value) for log-unit increase	Nonobese **3.30 (0.6)** Obese **0.07 (<0.01)***	Nonobese 0.003 (0.9) Obese 0.03 (0.1)	Nonobese −0.008 (0.7) Obese 0.006 (0.7)	Nonobese 0.04 (0.3) **Obese 0.08 (0.02)***	**Nonobese 0.05 (0.01)*** Obese 0.05 (0.09)
Kim et al. (2023)	Cross-sectional (2015–2017), Korea; 1,404 adults	2.0 (1.4–2.9)	% change (95% CI) for doubling	**8.6 (5.1, 12.3)***	**4.6 (2.3, 7.0)***	NR	**11.1 (5.9, 16.5)***	**NR**
Nian et al. (2019)	Cross-sectional (2015–2016); China; 1,605 adults	2.0 (1.1–3.1)	% change (95% CI) for ln-unit increase	**6.2 (3.1,9.4)***	**2.5 (0.5,4.5)***	−1.5 (−3.2,0.2)	**3.8 (0.5,7.0)***	**2.5 (0.1,4.8)***

Reference	Population	Median exposure (IQR) or as specified	Effect estimate	ALT	AST	ALP	GGT	Total bilirubin
Children and adolescents								
Mora et al. (2018)	Project Viva cohort (1999–2002), U.S.; 682 children (7–8 yr)	prenatal 0.6 (0.5–0.9)	β (95% CI) for IQR increase	−0.1 (−0.9,0.6)	NR	NR	NR	NR
		child 1.5 (1.1–2.3)		**−0.3 (−0.5, −0.1)***	NR	NR	NR	NR
Attanasio (2019)	NHANES cross-sectional (2013–2016); 354 boys and 305 girls (12–19 yr)	GM (SE) boys 0.6 (0.03) girls 0.5 (0.03)	β (95% CI) for quartiles vs. Q1	boys Q2: −0.13 (−0.28, 0.03) Q3: −0.14 (−0.30, 0.02) Q4: −0.15 (−0.29, −0.004) *p*-trend 0.017 girls Q2: 0.04 (−0.07, 0.14) Q3: 0.08 (−0.03, 0.18) Q4: 0.16 (0.06, 0.27) *p*-trend 0.02	boys Q2: −0.09 (−0.18, 0.00) Q3: −0.03 (−0.12, 0.06) Q4: −0.12 (−0.20, −0.04) *p*-trend 0.04 girls Q2: 0.01 (−0.08, 0.10) Q3: 0.08 (−0.01, 0.17) Q4: 0.10 (0.004, 0.20) *p*-trend 0.06	NR	boys Q2: −0.02 (−0.16, 0.11) Q3: −0.07 (−0.18, 0.04) Q4: −0.09 (−0.24, 0.06) *p*-trend 0.32 girls Q2: 0.04 (−0.07, 0.15) Q3: 0.08 (−0.07, 0.23) Q4: 0.02 (−0.09, 0.12) *p*-trend 0.60	boys Q2: 0.12 (0.00, 0.25) Q3: 0.09 (−0.06, 0.23) Q4: 0.02 (−0.11, 0.14) *p*-trend 0.07 girls Q2: 0.06 (−0.02, 0.14) Q3: 0.03 (−0.07, 0.13) Q4: 0.10 (0.02, 0.18) *p*-trend 0.09

*$p < 0.05$; NR = not reported.

Animal Studies

Eleven short-term exposure studies (28 days or less) have evaluated the effects of PFNA on hepatic endpoints in differing strains of adult rats and mice (NTP, 2018; Das et al., 2017; Rosen et al., 2017; Hadrup et al., 2016; Fang et al., 2015; Wang et al., 2015a; Fang et al., 2012a, b; Fang et al., 2012c; Kinney et al., 1989; Kennedy, 1987). There were no chronic or subchronic exposure studies, making the 28-day by NTP (2018) the longest exposure duration. Additionally, most studies administered PFNA by gavage, except for a *low* confidence study by Kinney et al. (1989) that was an acute 4-hour inhalation exposure and an *uninformative* study by Kennedy (1987) that was a 14-day dietary exposure (see Table 3-15).

A majority of studies were found to be *high* or *medium* confidence for the examined hepatic endpoints, and two studies were judged to be *low* confidence due to deficiencies in methods reporting, results presentation, and endpoint sensitivity (Fang et al., 2012b; Kinney et al., 1989). Another two studies were considered *uninformative* and are not considered further; Fang et al. (2015) lacked appropriate negative controls (non-diabetically induced rats) and Kennedy (1987) was found to have deficiencies in variable control, including potential age differences across treatment groups that could influence results, missing food consumption reporting, and high attrition. Two overall *high/medium* confidence developmental toxicity studies evaluated PFNA-induced liver weight changes in mouse dams and their offspring, and nonpregnant females (Das et al., 2015; Wolf et al., 2010). Additionally, a third developmental toxicity study by Zhang et al. (2021) also evaluated PFNA effects on liver weight and was *medium* confidence for maternal liver weight but *low* confidence for offspring liver weight due to several deficiencies, most notably that it was unclear whether offspring results were evaluated by litter. Controlling for litter in evaluations is important as the dam, and not the conceptus, is the individual treated during gestation and pups within a litter tend to respond more similarly. The developmental toxicity study by Wolf et al. (2010) also examined PFNA effects in PPARα null mice to evaluate toxicity mechanisms, as did two 7-day gavage exposure studies by Das et al. (2017) and Rosen et al. (2017).

Due to high mortality in the NTP 28-day study at the two highest doses (5 and 10 mg/kg-day in male rats; 12.5 and 25 mg/kg-day in female rats), the evidence synthesis only considers dose levels up to 6.25 mg/kg-day in females and 2.5 mg/kg-day in males except for the histopathology data where observations were noted at the time of death (see Section 3.2.1, "Adult rodent mortality"). It is also possible that PFNA-induced body weight loss reported in this, and other studies could be a factor affecting some of the liver endpoints evaluated (i.e., liver weight and lipids). The NTP 28-day study reported weight loss in males of 17% and 44% at 1.25 and 2.5 mg/kg-day, respectively, and some of the 14-day studies in male rats also noted reduced body weight at 5 mg/kg-day (generally, 21%–31%) (Hadrup et al., 2016; Wang et al., 2015a; Fang et al., 2012c). While effects of body weight loss on liver histopathology, serum liver enzymes, and hepatobiliary components are less clear, studies in feed-restricted rats show metabolically induced declines in liver weight and lipids with reductions in body weight (Linden et al., 2016; Moriyama et

al., 2008; Laws et al., 2007; Hubert et al., 2000; Keenan et al., 1994; Levin et al., 1993; Chatamra et al., 1984; Oishi et al., 1979). Thus, it is possible that the treatment-related body weight loss in males could partially mask potential direct effects of PFNA-mediated increases in liver weight and lipids (Linden et al., 2016; Moriyama et al., 2008; Laws et al., 2007; Hubert et al., 2000; Keenan et al., 1994; Levin et al., 1993; Chatamra et al., 1984; Oishi et al., 1979). Taken together, hepatic effects observed in male rats at doses ≤1.25 mg/kg-day in the NTP study and at PFNA doses <5 mg/kg-day in the 14-day studies are considered most reliable with potentially reduced sensitivity to detect the magnitude of direct effects of PFNA on liver weight and lipids. Hepatic effects in male rats with concurrent, substantial body weight loss at 2.5 mg/kg-day in the 28-day study and at 5 mg/kg-day in the 14-day studies are discussed to provide context but are considered inconclusive. No weight loss or other indicators of overt toxicity were reported in the 7-day studies (Das et al., 2017; Rosen et al., 2017).

Another important consideration for the PFNA evidence base of hepatic effects relates to sex- and species-specific differences in pharmacokinetics among experimental rodent models that may influence susceptibilities (see Section 3.1). Serum half-lives of PFNA are longer in male rats than female rats, presumably due to slower renal clearance in male rats, and bioaccumulation in the liver of both rats and mice shows greater uptake capacity in males (Tatum-Gibbs et al., 2011; Ohmori et al., 2003). However, PFNA appears to be more persistent in mice compared with rats and with less pronounced sex differences (i.e., elimination in female mice is just slightly faster than in male mice) (Tatum-Gibbs et al., 2011).

Table 3-15. Overall evaluation results of hepatotoxicity studies examining the effects of PFNA exposures in rodents[a]

Reference	Species, strain (lifestage/sex)	Exposure design	Exposure route and dose range	Organ weight	Histopathology	Clinical chemistry
Das et al. (2015)[b]	Mouse, Crl:CD1 Charles River (dam/offspring)	Developmental GD 1–17	Gavage, maternal 0, 1, 3, 5, 10 mg/kg-d[c]	++ +	NM	NM
Das et al. (2017)	Mouse, SV129 wild type; PPARα null (adult male)	Short-term 7 d	Gavage 0, 10 mg/kg-d	+	-	NM
Fang et al. (2012b)	Rat, Sprague-Dawley (adult male)	Short-term 14 d	Gavage, 0, 0.2, 1, 5 mg/kg-d	NM	-	NM
Fang et al. (2012a)[d]	Rat, Sprague-Dawley (Adult male)	Short-term 14 d	Gavage 0, 0.2, 1, 5 mg/kg-d	NM	NM	NM
Fang et al. (2012c)	Rat, Sprague-Dawley (Adult male)	Short-term 14 d	Gavage 0, 0.2, 1, 5 mg/kg-d	+	NM	+

Reference	Species, strain (lifestage/sex)	Exposure design	Exposure route and dose range	Organ weight	Histopathology	Clinical chemistry
Fang et al. (2015)	Rat, Sprague-Dawley (Adult male, diabetically induced)	Short-term 7 d	Gavage 0, 0.2, 1, 5 mg/kg-d	NM	NM	--
Hadrup et al. (2016)	Rat, Wistar-Hannover (Adult male)	Short-term 14 d	Gavage 0, 0.0125, 0.25, 5 mg/kg-d	++	+	NM
Kennedy (1987)	Mouse, Crl:CD1 Charles River (adult male and female)	Short-term 14 d	Dietary 0, 3, 10, 30, 300, 3,000 ppm	--	NM	NM
Kinney et al. (1989)	Rat, Crl:CdBr Charles River (adult male)	Acute 4 hr	Inhalation 0, 67, 590 mg/m^3	-	NM	NM
NTP (2018)[e]	Rat, Sprague-Dawley (Harlan) (adult male and female)	Short-term 28 d	Gavage; 0, 0.625, 1.25, 2.5, 5, 10 mg/kg-d (m); 0, 1.56, 3.12, 6.25, 12.5, 25 mg/kg-d (f)	++	++	++
Pfohl et al. (2021)	Mice, C57BL/6J (adult male)	Subchronic 12 wk	Dietary 0.24 mg/kg/day (approximately) with either a low or high-fat diet	-	-	
Rosen et al. (2017)	Mouse, 129S1/SvlmJ; PPARα null 29S4/SvJae-*Ppara*^{tm1Gonz} (adult male)	Short-term 7 d	Gavage 0, 1, 3 mg/kg-d	+	NM	NM
Wang et al. (2015a)	Mouse, BALB/c (Adult male)	Short-term 14 d	Gavage 0, 0.2, 1, 5 mg/kg-d	+	NM	+
Wolf et al. (2010)	Mouse, 29S1/SvlmJ; PPARα null PPARa^{tm1Gonz} (dam/offspring)	Developmental GD 1–18	Gavage, maternal 0, 0.83, 1.1, 1.5, 2 mg/kg-d	++ +	NM	NM
Zhang et al. (2021)[f]	Mouse, ICR (dam/female offspring)	Developmental GD 1–18	Gavage, maternal 0, 0.5, 3 mg/kg-d	+ -	NM	NM

[a]Dark green (++) = *high* confidence; Light green (+) = *medium* confidence; yellow (-) = *low* confidence; red (--) = *uninformative*; NM = Not measured. Study evaluation details for all outcomes are available in HAWC.

[b]Maternal and prenatal fetal liver weights were *high* confidence, and postnatal offspring liver weights were *medium* confidence.

[c]10 mg/kg-d dose group ceased due to high mortality among treated dams.

[d]No evaluation of heatmap endpoints; rather in vivo evaluation of mechanistic endpoints.

[e]High mortality at 5 and 10 mg/kg-d in males and 12.5 and 25 mg/kg-d, making 2.5 and 6.25 mg/kg-d high dose in males and females, respectively, except for the histopathology that was evaluated at time of death.

[f]Maternal liver weights were overall *medium* confidence; postnatal offspring liver weights were overall *low* confidence.

Liver weight

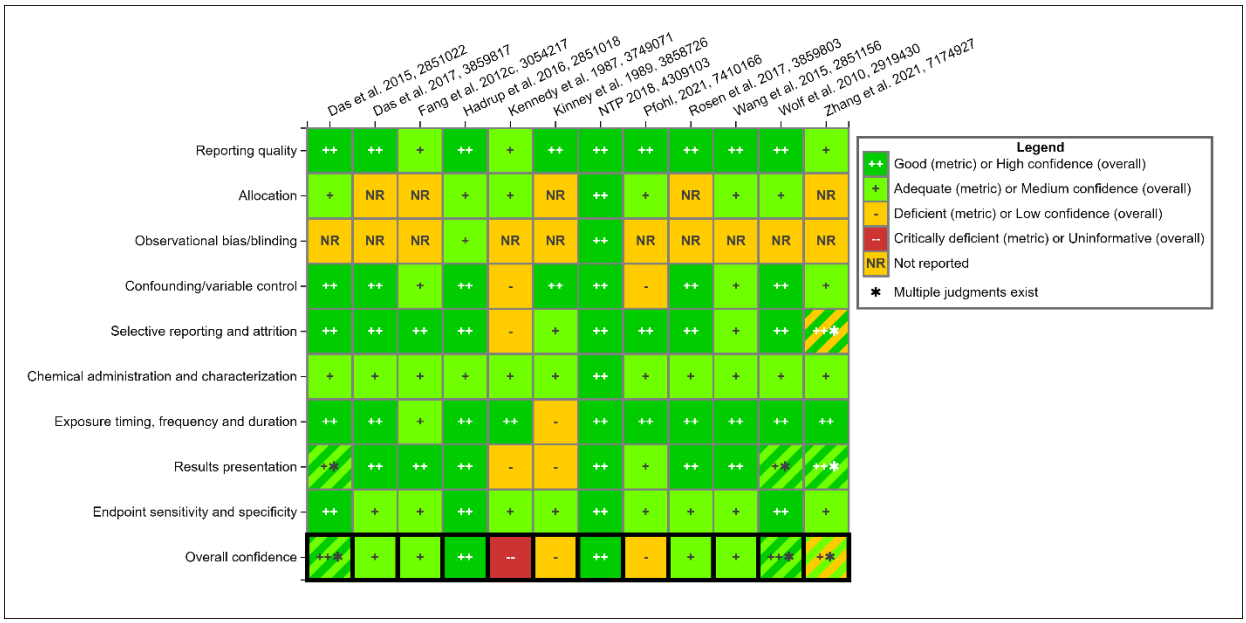


Figure 3-41. Heat map of study quality evaluations of in vivo animal studies that examined PFNA effects on liver weight.[a] See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Liver-weight-animals/.

[a]In Das et al. (2015), maternal and prenatal liver weights were *high* confidence, and postnatal offspring liver weights were *medium* confidence. In Wolf et al. (2010), maternal liver weights were *high* confidence and postnatal offspring liver weights were *medium* confidence. In Zhang et al. (2021), postnatal offspring liver weights were overall *low* confidence and deficient and adequate for selective reporting and results presentation domains, respectively, with an overall rating of *low* confidence for this endpoint in offspring and *medium* confidence for maternal endpoints.

PFNA effects on liver weight of adult male and female rats and mice have been evaluated in 11 informative studies; specifically, 7 short-term oral exposure studies of 28-days or less (NTP, 2018; Das et al., 2017; Rosen et al., 2017; Hadrup et al., 2016; Wang et al., 2015a; Fang et al., 2012c; Kennedy, 1987), one subchronic dietary exposure study (Pfohl et al., 2021), three developmental toxicity studies evaluating oral gestational exposures (Zhang et al., 2021; Das et al., 2015; Wolf et al., 2010), and one *uninformative* acute inhalation study (Kinney et al., 1989). Zhang et al. (2021) evaluated absolute liver weights alone, and not relative weights, in dams and offspring. The liver weight data in eight studies were judged to be *high* and *medium* confidence with minor limitations (see Figure 3-41). Maternal and fetal liver weight data in two of the developmental toxicity studies were *high* confidence, whereas postnatal liver weight data in these studies were considered *medium* confidence because they did not include sex-specific reporting of results, which is preferred for postnatal measures primarily due to substantial weight differences across sex (Das et al., 2015; Wolf et al., 2010). The offspring liver weights reported in mice by Zhang et al. (2021) were considered *low* confidence due to deficiencies in the selective reporting and allocation domain as it did not appear that the evaluations were conducted by litter (see Section 3.2.2, "Animal Studies").

The 4-hour inhalation exposure study by Kinney et al. (1989) was judged to be *low* confidence due to deficiencies in results presentation of group variability and negative control comparisons, and the insensitivity of the acute exposure duration for eliciting hepatic effects. The subchronic dietary study by Pfohl et al. (2021) was *low* confidence due to the lack of an appropriate negative control of mice on a standard diet (only low-fat and high-fat diets were tested) and inadequate reporting of dietary exposures (only an approximate dose across all the treatment groups was provided).

Pronounced and generally consistent dose-dependent increases in absolute and relative liver weights were observed across multiple PFNA studies, rodent models, sexes, and lifestages at doses that did not elicit overt toxicity (see Figures 3-42 to 3-46). In general, relative liver-to-body weight is recommended instead of absolute liver weight to minimize variations given liver weight is shown to be proportional to body weight (Hall et al., 2012; Bailey et al., 2004). Results based on absolute liver weight mostly tracked those for relative liver weight. Both are presented graphically for completeness, with the discussion focusing on relative liver weight as the preferred endpoint.

Adult male rodents

NTP (2018) reported statistically significant and dose-dependent increases in relative liver weights of adult male SD rats (see Figure 3-42) exposed to PFNA at 0.625, 1.25, and 2.5 mg/kg-day (relative weight increases by 23%, 60%, and 86%, respectively) after 28 days. Results of the 14-day exposure studies generally aligned with the 28-day study findings. In adult male SD rats, significant relative liver weight increases were reported at 1 mg/kg-day (31%) and 5 mg/kg-day (81%) (Fang et al., 2012c). In adolescent (postpubertal) male Wistar rats relative liver weight increases were reported only at the high, and overtly toxic, dose of 5 mg/kg-day (33%) which decreased body weight by 29% (Hadrup et al., 2016). A 14-day exposure in adult male BALB/c mice reported significant increases in liver weight of 17%–144% at ≥0.2 mg/kg-day PFNA. This suggested an increased sensitivity of PFNA-induced hepatic effects in mice.

Seven-day exposures to PFNA caused increased relative liver weights in adult male 129S1/SvlmJ mice dosed at 1 and 3 mg/kg-day (an increase of 56% and 94%, respectively) and PPARα null mice receiving 3 mg/kg-day PFNA (relative liver weights increased by 47%; Figure 3-42) without significant changes in body weight (Rosen et al., 2017). Likewise, Das et al. (2017) reported increased relative liver weight in adult male SV129 mice (128% increase) and PPARα null mice (126% increase) receiving a high dose of 10 mg/kg-day for 7 days without evidence of body weight decreases. The high doses in both 7-day studies would be expected to result in overt toxicity (i.e., decreased body weight) if the exposure duration was longer given other studies with PFNA. The results in the *low* confidence study by Pfohl et al. (2021) also indicated increases in liver weight (132% and 118% respectively) in mice fed a low or high-fat diet for 90 days. The *low* confidence inhalation exposure study that examined liver weight five and 12 days after a single 4-hour treatment to either 57 or 590 mg/m^3 PFNA particulate also reported increases up to 50% in relative liver weight (Kinney et al., 1989). Thus, taken together, liver weights are consistently increased in adult male rodents, with limited evidence of greater sensitivity in male

mice and an indication that both PPARα dependent and independent pathways are involved in mediating increases.

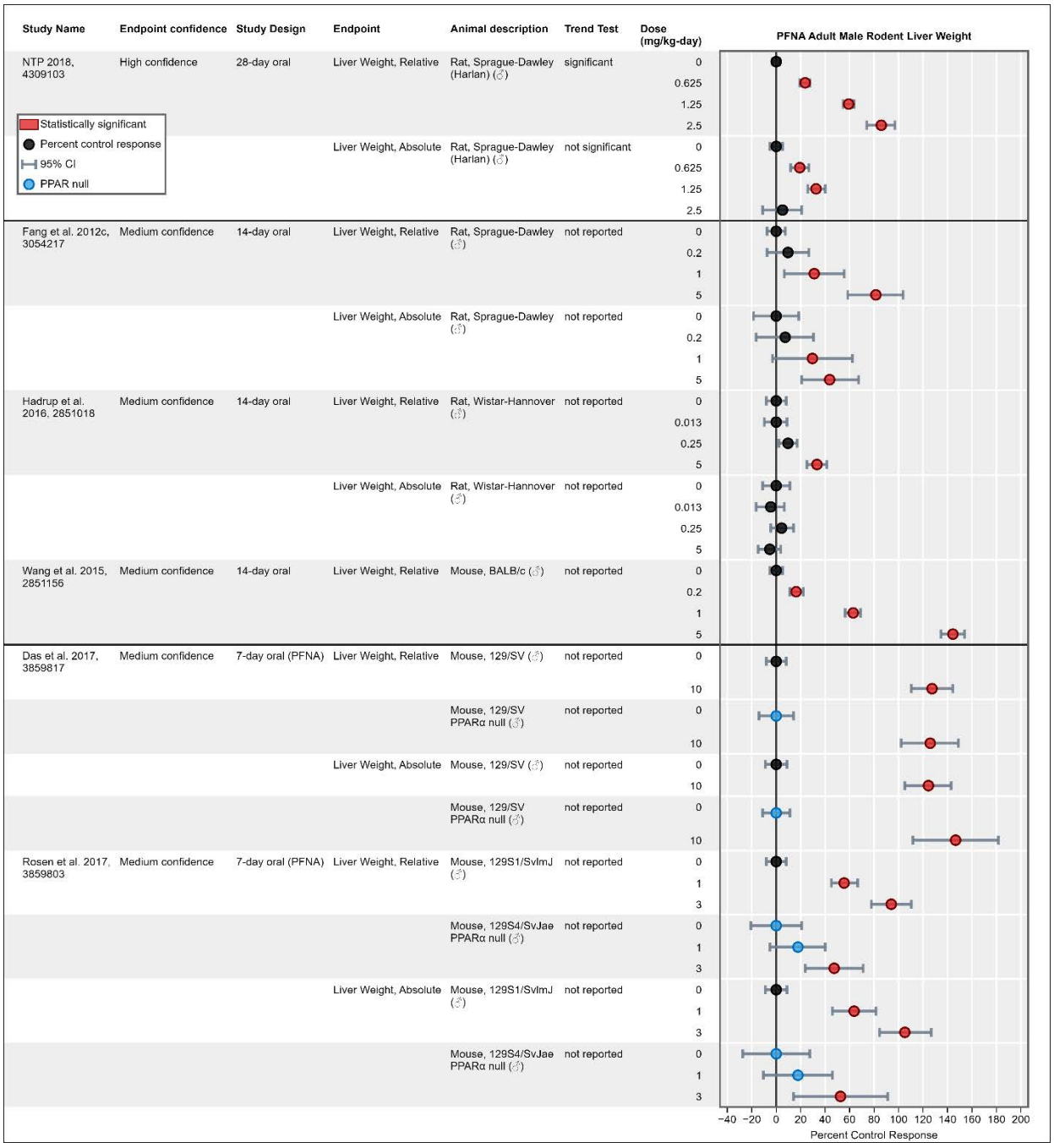


Figure 3-42. PFNA effects on adult male liver weights displayed in *high* and *medium* confidence studies as percent control responses.[a] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-adult-male-rodent-liver-weight/.

[a]Wang et al. (2015a) did not report absolute liver weights.

Adult female rodents

The NTP 28-day study observed significant dose-dependent increases in relative liver weights of adult female SD rats exposed to PFNA at 1.56, 3.12, and 6.25 mg/kg-day (relative weight increases by 21%, 35%, and 47%, respectively; Figure 3-43). Developmental toxicity studies conducted with PFNA also reported increased relative liver weights in PFNA-exposed nonpregnant mice, dams and their gestationally exposed offspring, including in nonpregnant PPARα null females (Das et al., 2015; Wolf et al., 2010).

In 29S1/SvlmJ mouse dams, dose-dependent increases in relative liver weights were reported at weaning (PND 21 at 1.1, 1.5, and 2 mg/kg-day PFNA corresponding to statistically significant increases of 31%, 44%, and 65%) (Wolf et al., 2010). Nonpregnant 29S1/SvlmJ female mice also showed signficant dose-dependent increases in relative liver weights at day 42 (equivalent to PND 21) at all doses tested (increases of 73%, 99%, 116%, and 139% corresponding with doses of 0.83, 1.1, 1.5, and 2.0 mg/kg-day). Consistent with Wolf et al. (2010), the Das et al. (2015) developmental study showed liver weight increases among gestationally exposed dams at the end of pregnancy (GD 17) and in nonpregnant mice at day 17. In pregnant mice, the increase in relative liver weight was dose-dependent (relative weight increases of 37%, 75%, and 115% at 1, 3 and 5 mg/kg-day, respectively). The significant increase in relative liver weight in dams extended to terminal sacrifice at PND 28 (up to 195% relative weight). In nonpregnant mice at day 17, liver weight increases were also statistically significant with increases of 74% at 1 mg/kg-day and 153% at 3 mg/kg-day at day 17 (Das et al., 2015). At 5 mg/kg-day, nonpregnant mouse sample sizes were reduced to n = 2; thus, 3 mg/kg-day is the highest dose considered reliable. It is difficult to directly compare PFNA-induced liver weight increases between pregnant and nonpregnant animals due to gestational liver enlargement. Nonetheless, even with the expected increase in liver size in pregnant animals, Das et al. (2015) reported relative liver weights of pregnant mice to be about half that of nonpregnant mice at equivalent doses at GD 17. Although not examined directly, the difference may be attributable to placental transfer of PFNA to the fetal compartment, as fetal livers accumulated PFNA at levels consistent with maternal serum, and serum and hepatic accumulation of PFNA was substantially lower in pregnant mice than nonpregnant mice at term. Zhang et al. (2021), which only reported absolute liver weight, also showed a significant increase in mouse dams on GD 18 at 3 mg/kg-day.

Liver weight results differed between pregnant and nonpregnant PPARα null females (Wolf et al., 2010). Specifically, while 29S1/SvlmJ female mouse liver weights (both pregnant and nonpregnant) were increased, pregnant PPARα null female liver weight was unaffected by PFNA (see Figures 3-43 and 3-44). In nonpregnant PPARα null mice, relative liver weights were significantly elevated at 1.1, 1.5, and 2.0 mg/kg-day PFNA (increases of 25%, 51%, and 62%, respectively), supporting PPARα-independent pathways in liver enlargement. The underlying reasons for differences in liver weight responses in pregnant wild type and PPARα null females are unknown at this time.

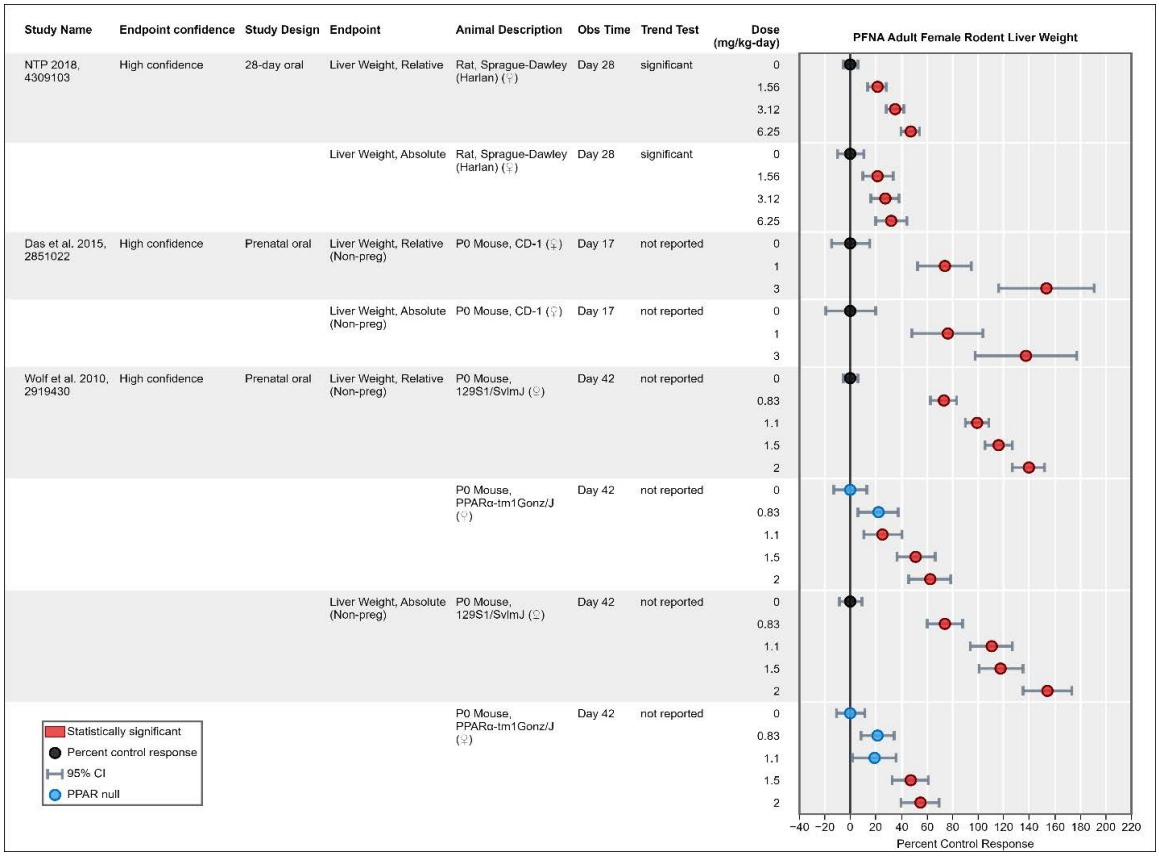


Figure 3-43. PFNA effects on adult nonpregnant female liver weights in *high* confidence studies displayed as percent control responses.[a] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-Adult-Female-Rodent-Liver-Weight-Non-Pregnant/.

[a]Liver weight in nonpregnant females in Das et al. (2015) is not included at 5 mg/kg-d due to small sample size (n = 2).

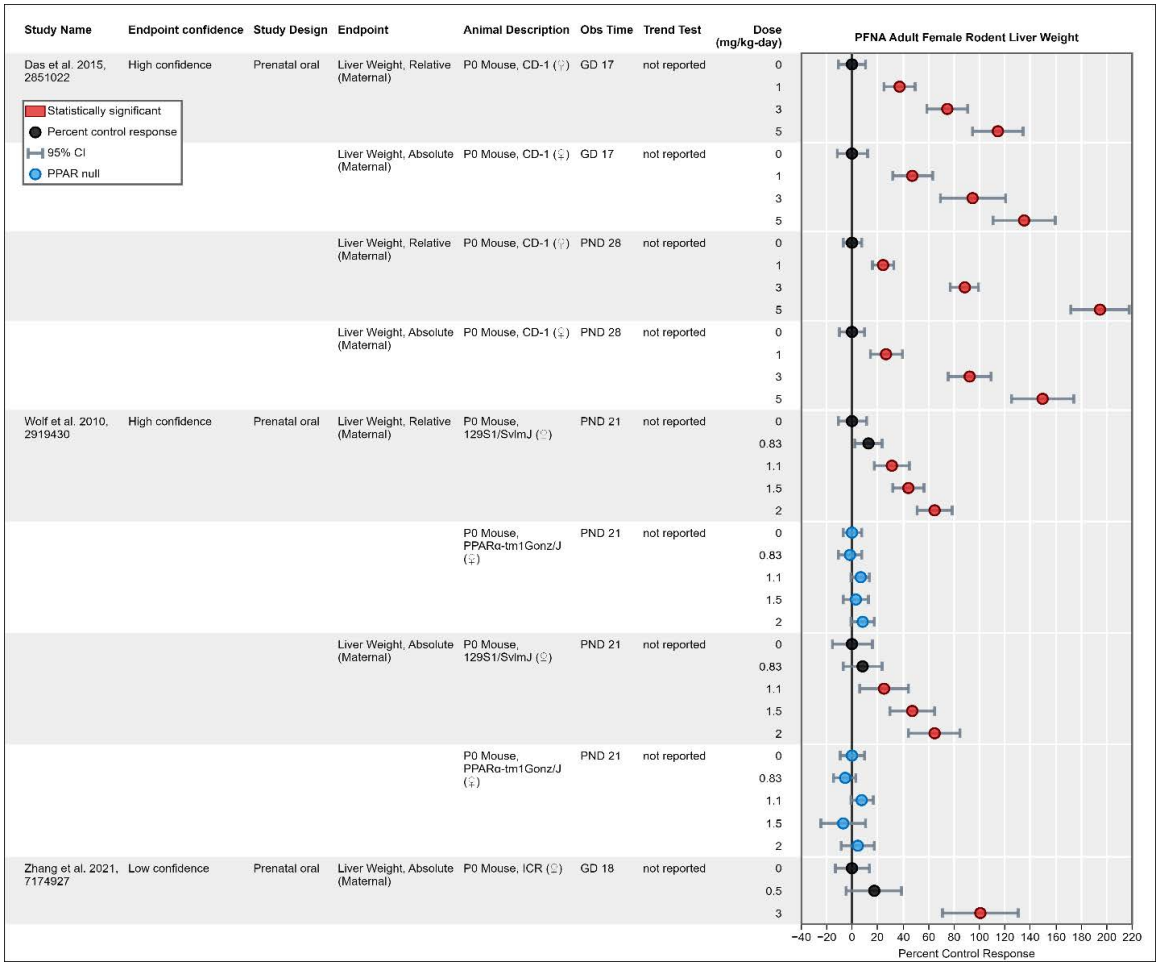


Figure 3-44. PFNA effects on adult maternal liver weights in *high* and *medium* confidence studies displayed as percent control responses.[a] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-Adult-Female-Rodent-Liver-Weight-Maternal/.

[a]Das et al. (2015) reported systemic maternal toxicity at the high dose of 10 mg/kg-d with all animals euthanized on GD 13, making the 5 mg/kg-d dose the highest tested. Zhang et al. (2021) did not report relative liver weights.

Prenatal/postnatal mice

Consistent with observations in pregnant dams, Das et al. (2015) reported statistically significant increases in relative liver weight ranging from 20% to 23% in gestationally exposed mouse fetuses (GD 17, sexes combined) at all PFNA doses tested (1, 3, and 5 mg/kg-day) with no effects on fetal weight or overt toxicity in dams. This hepatomegaly continued through the preweaning period with significant dose-dependent increases in relative liver weight in mouse pups at PND 1 (32% at 1 mg/kg-day; 53% at 3 and 5 mg/kg-day), 10 (38% at 1 mg/kg-day; 82% at 3 mg/kg-day; 144% at 5 mg/kg-day) and 24 (25% at 1 mg/kg-day; 48% at 3 mg/kg-day; 71% at 5 mg/kg-day) (see Figure 3-45). Increases in relative liver weight were observed at the lowest dose of 1 mg/kg-day, where there were small and nonsignificant reductions in offspring body weight

gain (see Section 3.2.2, Table 3-10). Changes at higher doses are more difficult to interpret given the statistically significant reductions in body weight gain at 3 and 5 mg/kg-day that could have exacerbated the observed increases in relative liver weights (i.e., PFNA-induced liver enlargement co-occurring with reduced body weight gain). The lack of changes in absolute liver weights is likewise difficult to interpret because any PFNA-induced increases in liver weight could be offset by decreases in organ weight expected due to the observed body weight loss. However, increased relative liver weights also appeared to be commensurate with an increase in PFNA accumulation in offspring livers and serum by maternal exposure pathways (i.e., placental and lactational transfer). At PND 42, offspring exposed to 3 and 5 mg/kg-day continued to have significantly elevated relative liver weights. By PND 70 liver weights returned to within the range of vehicle controls, concomitant with declines in PFNA concentrations in serum and liver (Das et al., 2015). Thus, it can be reasonably postulated that the recovery of treatment-related liver enlargements in offspring appeared possibly attributable to cessation of exposure from the dam, rather than related to an adaptive response in offspring with continued exposures. It is difficult to decipher the potential role of body weight reductions in relative liver weight of offspring post-weaning because post-weaning body weight is reported by sex and was not reported at PND 70. It is also the case that the reduced PFNA concentrations through the preweaning period and post-weaning could have potentially played a role in ameliorating treatment-related decreases in body weight that further complicates interpreting the relative liver weight results.

The developmental study by Wolf et al. (2010) also observed statistically significant liver enlargement in gestationally exposed, 29S1/SvlmJ mouse pups (sexes combined) at PND 21 at all doses tested (0.83, 1.1, 1.5, and 2.0 mg/kg-day corresponding to increases of 42%, 59%, 62%, and 73% respectively). Similar to findings by Das et al. (2015), serum concentrations of PFNA were elevated in parallel with the observed liver enlargement as well as body weight reductions at 2 mg/kg-day that complicate interpretation at that PFNA dose (see Figure 3-46). Relative liver weights of PPARα null pups were only significantly increased at 2 mg/kg-day PFNA (although with no change in body weight gain). Thus, while the knockout pups exhibited the same effect as 29S1/SvlmJ pups, the response was attenuated, indicating a contributing role for both PPARα-dependent and independent pathways. The *low* confidence findings in ICR mice generally aligned with results in Das et al. (2015) and Wolf et al. (2010), and reported statistically significant increases in absolute liver weights of offspring up to weaning PND 21 at 0.05 and 3 mg/kg-day PFNA (Zhang et al., 2021).

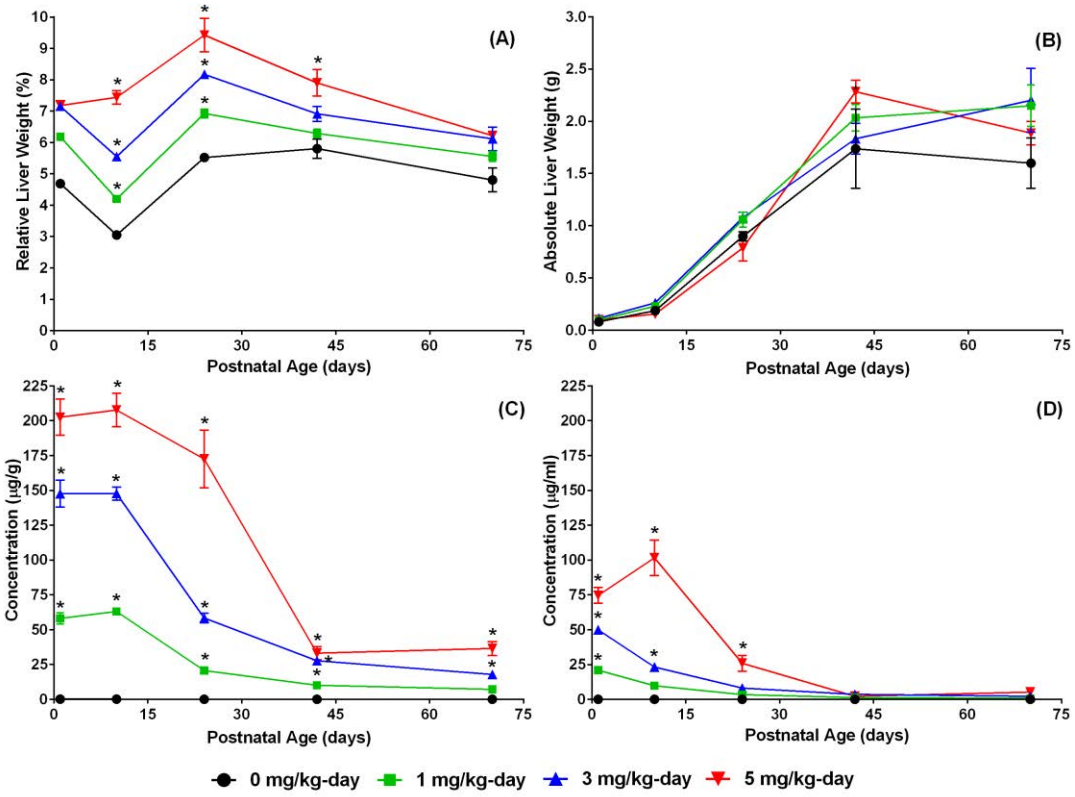


Figure 3-45. PFNA effects on relative (A) and absolute (B) liver weights of prenatally exposed CD-1 mice (sexes combined) at PND 1, 10, 24, 42, and 70[a], and associated liver (C) and serum (D) concentrations of PFNA (Das et al., 2015). *$p < 0.05$. Underlying data available at: https://hawc.epa.gov/ani/animal-group/100500513/.

[a]Reduced sample sizes in 5 mg/kg-d PFNA at PND 70 (n = 2 litters).

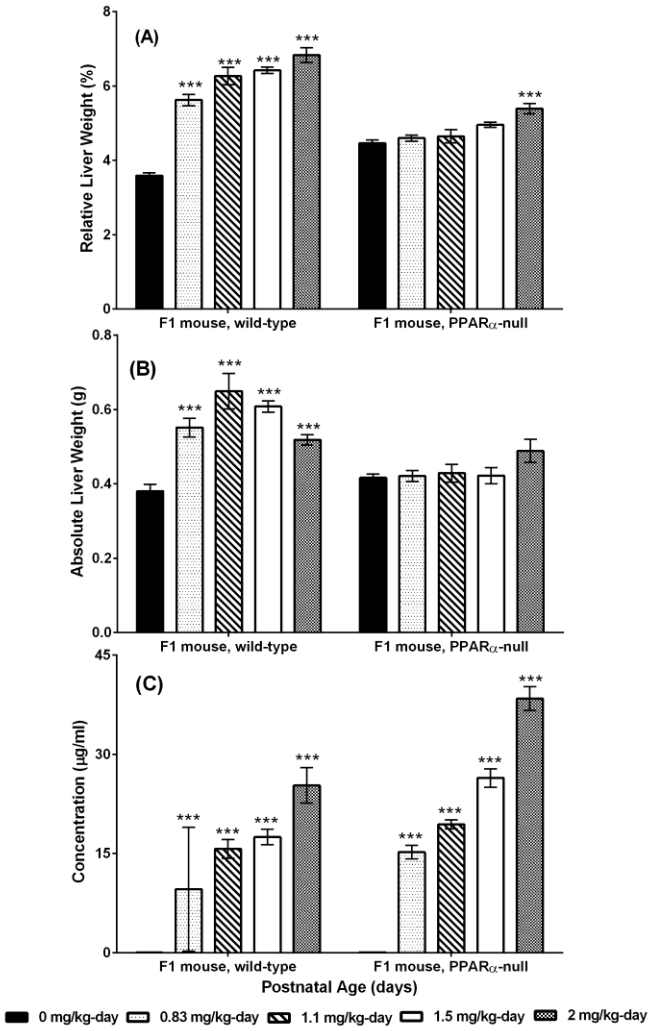


Figure 3-46. PFNA effects on relative (A) and absolute (B) liver weights of wild type and PPARα null mice (sexes combined) at weaning (PND 21), and associated PFNA concentrations in serum (C) (Wolf et al., 2010). Underlying data available at: https://hawc.epa.gov/ani/endpoint/100505518/, https://hawc.epa.gov/ani/endpoint/100505517/, https://hawc.epa.gov/ani/endpoint/100505535/, https://hawc.epa.gov/ani/endpoint/100505536/.

Histopathology

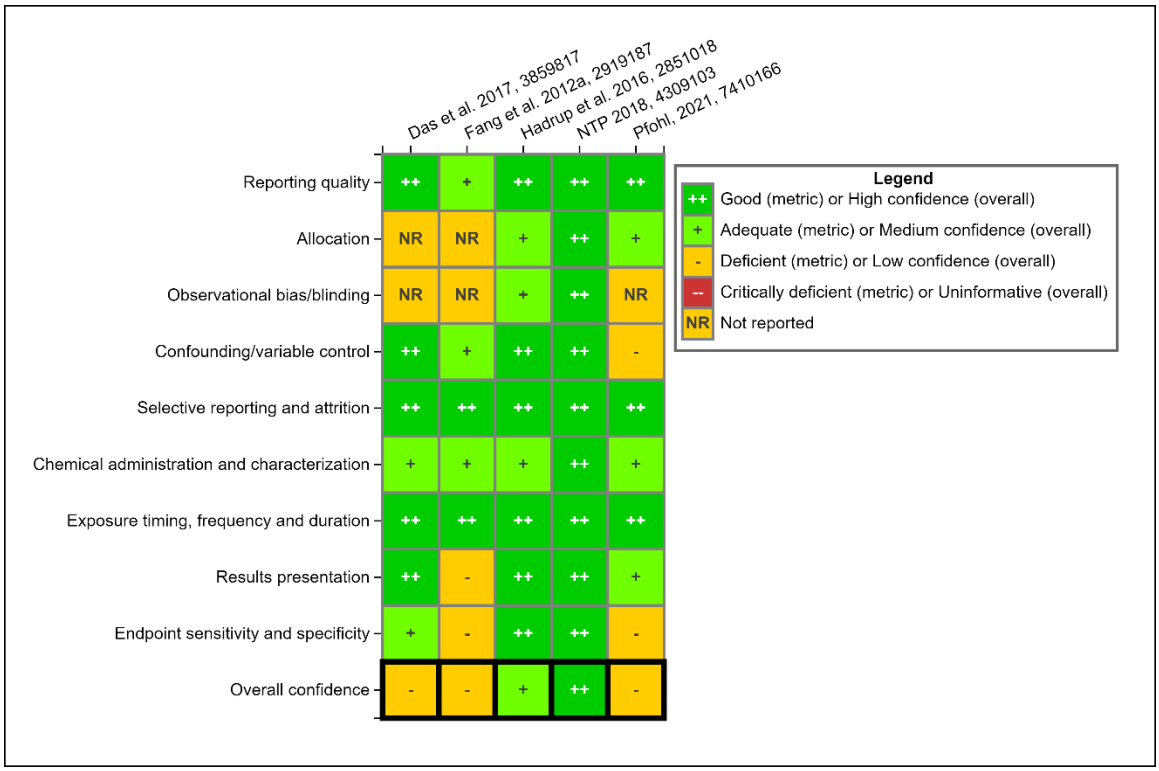


Figure 3-47. Heat map of study quality evaluations of rodent studies that examined PFNA effects on liver histopathology. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Liver-histopathology/.

Treatment-related increases in liver weight can result from various changes in hepatic morphology including hyperplasia of any resident liver cell type, hypertrophy, inflammation, fibrosis, increased hepatocyte size, neoplasia, congestion, or metabolic enzyme induction (Hall et al., 2012; Thoolen et al., 2010; U.S. EPA, 2002a). Liver histopathology has been examined in four of the short-term PFNA exposure studies (NTP, 2018; Das et al., 2017; Hadrup et al., 2016; Fang et al., 2012b) and one subchronic study (Pfohl et al., 2021). Two of these studies were *high* or *medium* confidence for this endpoint, and three were *low* confidence (see Figure 3-47). Consideration of blinding was most influential to these confidence judgments (see HAWC; https://hawc.epa.gov/study/assessment/100500071/). The Das et al. (2017) histological data (cell size/morphometrics) where overall *low* confidence as blinding was not affirmed for these subjective measurements, and Fang et al. (2012b) was *low* confidence primarily due to deficiencies in describing sampling procedures and a lack of quantitative results. Pfohl et al. (2021) did not include an appropriate negative control (mice fed a standard diet) and did not provide details on fasting or adequate descriptions of the scoring metrics and rankings applied for the quantitative lipid histology. In the NTP 28-day study, histopathological examinations were undertaken in males

and females from the two highest dose groups that died early from chemical-induced death (termed natural death as distinguished from moribund sacrifice). Animals were reported to have been observed twice daily for signs of overt toxicity, which mitigated concerns for tissue degradation in non-surviving animals.

Coherent with PFNA-induced increased liver weight, the NTP 28-day study observed consistent dose-dependent increases in hepatocyte hypertrophy, cytoplasmic alterations, necrosis, and vacuolization in both male and female SD rats exposed to PFNA (see Table 3-16). The hepatocyte hypertrophy and cytoplasmic alterations were characterized by an increase in the size of primarily centrilobular hepatocytes associated with the accumulation of cytoplasmic granules or an increase in homogenous eosinophilic cytoplasm (NTP, 2019). These morphological alterations have been shown to be linked to liver inflammation, xenobiotic peroxisome-induced hypertrophy, steatosis, and hepatobiliary cholestasis, although the last is typically localized to the portal tract (NTP, 2014; Tarantino et al., 2008). While the necrosis severity was graded as minimal to mild, time to first incidence was relatively short (12 and 15 days at the high dose in females and males, respectively), and decreased with increasing dose. Additionally, there was a high rate of incidences reported, particularly in males, and that worsened with increasing dose. Furthermore, the preterm mortality in the high dose animals (≥5 mg/kg-day in males with time to first death as early as day 15; ≥12.5 mg/kg-day in females with time to first death as early as day 11) truncated the window of exposure, potentially reducing the progression and severity of the observed hepatocyte degeneration and necrosis.

In male rats, statistically significant increases in mild to marked hepatocyte hypertrophy and cytoplasmic alterations were observed in the NTP 28-day study at ≥0.625 mg/kg-day, with hepatocellular necrosis at ≥2.5 mg/kg-day (see Table 3-16), both at doses lower than those eliciting mortality. Additionally, minimal to moderate vacuolization in centrilobular hepatocytes with cytoplasmic accumulation of microvacuoles occurred in males at 1.25 and 2.5 mg/kg-day, which were morphologically consistent with lipid accumulation (NTP, 2014; Thoolen et al., 2010; Wolf et al., 2008b). The 14-day study in adult male Wistar rats reported a nonsignificant but dose-dependent increase in hepatocyte hypertrophy and a significant decrease in cell borders at PFNA doses up to 5 mg/kg-day (Hadrup et al., 2016). In concordance with the 28-day and 14-day results, a *low* confidence morphometric analysis by Das et al. (2017) observed significantly increased cell sizes and decreased DNA content indicative of hepatocellular hypertrophy following a 7-day exposure to PFNA at 10 mg/kg-day in both adult male wild type and PPARα null mice. Additionally, this study reported statistically significant increases in liver triglycerides in both wild type and PPARα null animals (other hepatocellular endpoints such as necrosis were not evaluated; see "Mechanistic Evidence and Supplemental Data" below for PPARα-related discussions). The *low* confidence subchronic study in male mice showed histopathological accumulation of liver lipids in mice fed a low-fat diet and 0.24 mg/kg-day PFNA compared with a low-fat diet alone, while there was a slight decrease in lipid accumulation in mice fed a high-fat diet and exposed to PFNA

compared with mice fed a high-fat diet alone (Pfohl et al., 2021). However, the lack of a negative control where mice were fed a standard diet make these results difficult to interpret. The 14-day *low* confidence exposure study in adult male SD rats (Fang et al., 2012b) reported increased hepatocyte focal vacuolation at 5 mg/kg-day PFNA.

While the NTP study observed dose-dependent increases in nonneoplastic hepatocellular lesions in both males and females, the magnitude of responses was generally less in females than in males. In female SD rats, significant dose-dependent hepatic hypertrophy and cytoplasmic alterations were also of mild to marked in severity, consistent with males, and the increasing incidences of cytoplasmic alterations were reported at the lowest dose tested (≥1.56 mg/kg-day). However, hepatocellular hypertrophy in females was not observed until ≥6.25 mg/kg-day. Similar to males but at higher doses, hepatic necrosis occurred at 12.5 and 25 mg/kg-day, where survival to term and time to first incidence was reduced, which complicates interpretation. Thus, there is some evidence that with short-term PFNA exposures, male rats appeared to be more sensitive than female rats to hepatocellular alterations, possibly related to the higher liver accumulation and longer serum half-lives of PFNA in males (see Section 3.1).

Table 3-16. Incidences and severity scoring of liver nonneoplastic lesions reported in a *high* confidence 28-day oral toxicity study conducted in adult Sprague-Dawley rats NTP (2018)[a,b,c]

	Dose (mg/kg-d)										
Animal group	**0**	**0.625**	**1.25**	**1.56**	**2.5**	**3.12**	**5[d]**	**6.25**	**10[e]**	**12.5[d]**	**25[e]**
Cytoplasmic alteration											
Male rats (#)	(0/10) 0	***(10/10)** 1.5 t = 29***	***(10/10)** 3.7 t = 29***		***(10/10)** 4.0 t = 29***		***(9/9)** 3.1 t = 24***		***(10/10)** 2.1 t = 15***		
Female rats (#)	(0/10) 0			***(5/10)* 1.0 t = 29***		***(10/10)** 1.0 t = 29***		***(10/10)** 2.4 t = 29***		***(9/10)** 1.6 t = 13***	(0/10) 0
Vacuolization											
Male rats	(0/10) 0	(0/10) 0	***(6/10)** 1.5 t = 29***		***(9/10)** 3.7 t = 29***		(0/9) 0		(0/10) 0		
Female rats	(0/10) 0			(0/10) 0		(0/10) 0		(0/10) 0		(0/10) 0	(0/10) 0
Hypertrophy											
Male rats (#)	(0/10) 0	***(7/10)** 2.3 t = 29***	***(10/10)** 4.0 t = 29***		***(10/10)** 4.0 t = 29***		***(9/9)** 3.6 t = 24***		***(10/10)** 3.0 t = 15***		
Female rats (#)	(1/10) 0			(0/10) 0		(2/10) 1.0 t = 29		***(10/10)** 4.0 t = 29***		***(10/10)** 3.0 t = 13***	***(10/10)** 2.6 t = 11***
Necrosis											
Male rats (#)	(0/10) 0	(0/10) 0	(1/10) 1.0 t = 29		***(5/10)* 1.2 t = 29***		***(6/9)** 2.0 t = 24***		***(9/10)** 1.8 t = 15***		
Female rats (#)	(0/10) 0			(0/10) 0		(0/10) 0		(0/10) 0		***(4/10)* 1.3 t = 16***	***(3/10)* 1.0 t = 12***

[a]# = Dose-dependent changes; bold-italicized font indicates statistically significant changes compared with corresponding controls; shaded cells represent doses not targeted; t = day of first incidence.

[b]Severity averaged to four-point scale as follows: 0 = not detected; 1 = minimal severity; 2 = mild severity; 3 = moderate severity; 4 = marked severity. Number in parenthesis indicate number positive out of total animals examined.

[c]Bar charts for effects on liver histopathology displayed as total number incidences, statistical analysis and trend results can be found in HAWC for male rats at: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna_liver_histopathology/ and for female rats at: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-adult-female-rodent-liver-histopathology/.

[d]Terminal sacrifice, n = 1 for males (5 mg/kg-day), n = 2 for females (12.5 mg/kg-d).

[e]Terminal sacrifice, n = 0. For females (25 mg/kg-day) there was complete mortality by day 15, for males (10 mg/kg-d) there was complete mortality by day 22.

* $p < 0.01$; **$p < 0.001$.

Clinical chemistry

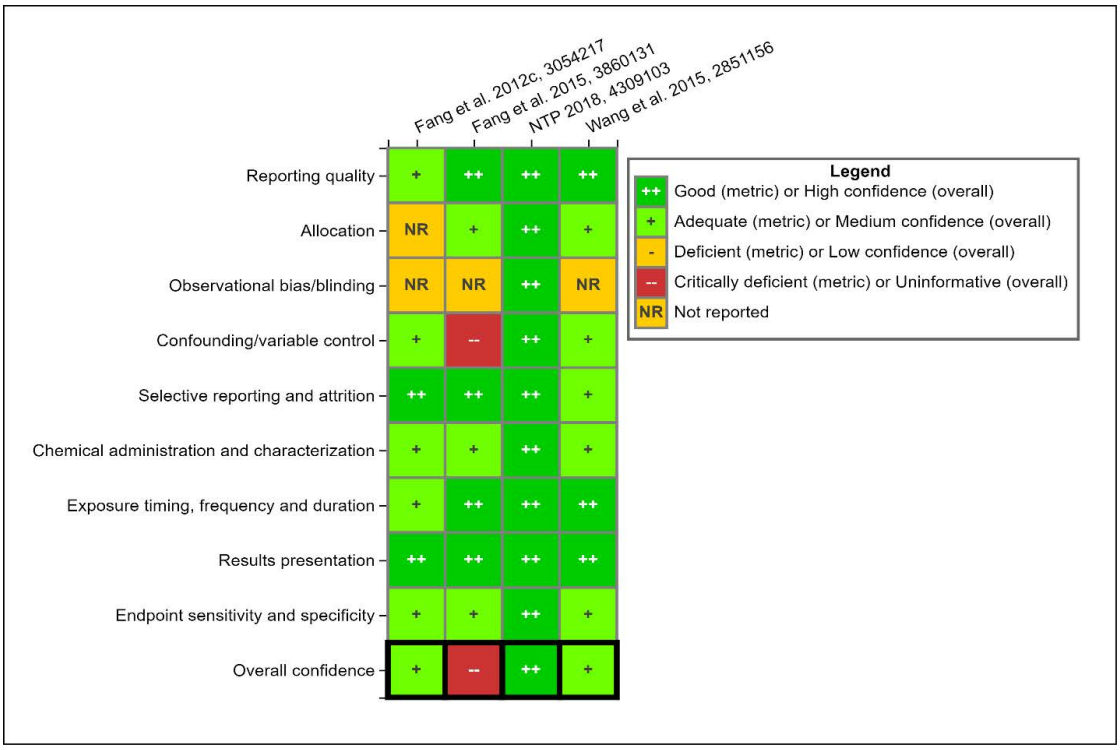


Figure 3-48. Study evaluation heat map of animal studies that measured PFNA effects on liver enzyme levels and hepatobiliary markers. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Liver-enzymes-and-hepatobiliary-markers/.

PFNA effects on serum liver enzymes, blood proteins, and hepatobiliary components were examined in four of the short-term exposure studies (NTP, 2018; Fang et al., 2015; Wang et al., 2015a; Fang et al., 2012c). These studies were considered *high* and *medium* confidence for the clinical chemistry endpoints examined, except for in the 14-day exposure study by Fang et al. (2015) that was found to be *uninformative* and is not considered further due to critical deficiencies in confounding variable control (there was not a negative control with animals that were not diabetically induced and the range of elevated blood glucose across treatment groups was not reported) (see Figure 3-48).

Serum liver enzymes

As indicated earlier, ALT and AST are markers of hepatocellular damage as increased serum levels are thought to be due to hepatocyte injury resulting in release into the blood, and with ALT considered more specific and sensitive (Boone et al., 2005). ALP is localized to the bile canalicular membrane and is more indicative of hepatobiliary damage (Hall et al., 2012; Amacher et al., 1998). Lactate dehydrogenase (LDH) is involved in energy production in most cells and so lacks specificity

to the liver but provides some contributory indication of potential hepatocellular leakage and damage (Klein et al., 2020; EMEA, 2008; Boone et al., 2005).

After 28- or 14-day exposures to PFNA, modest but statistically significant increases in serum ALT of approximately 1.5-fold were observed in male rats at 1.25 or 5 mg/kg-day, respectively (NTP, 2018; Fang et al., 2012c). NTP (2018) also reported mild but dose-dependent elevations (up to 1.4-fold) in serum AST in male SD rats, with a statistically significant 1.9-fold increase in ALP at 1.25 mg/kg-day but that latter finding did not follow a dose gradient (see Figure 3-49). The 14-day exposure by Fang et al. (2012c) also reported significant increases in serum AST (1.3-fold), ALP (1.3-fold), and LDH (1.6-fold) in male SD rats exposed to PFNA at 5 mg/kg-day. Consistent with results in male rats, the NTP 28-day study observed significant dose-dependent increases in serum ALT and ALP in female rats with significant increases (<1.5-fold) at ≥3.12 mg/kg-day (see Figure 3-50). Serum AST was unaffected by PFNA in female rats in the NTP study.

In the only study in mice, Wang et al. (2015a) reported a pronounced 7.6-fold increase in serum ALT and 3.1-fold increases in serum AST in adult males after a 14-day exposure at 5.0 mg/kg-day, suggesting potentially greater sensitivity in mice than rats and aligning with liver weight data. However, there is uncertainty in the results due to co-occurring overt toxicity (26% body weight loss) reported at this dose level.

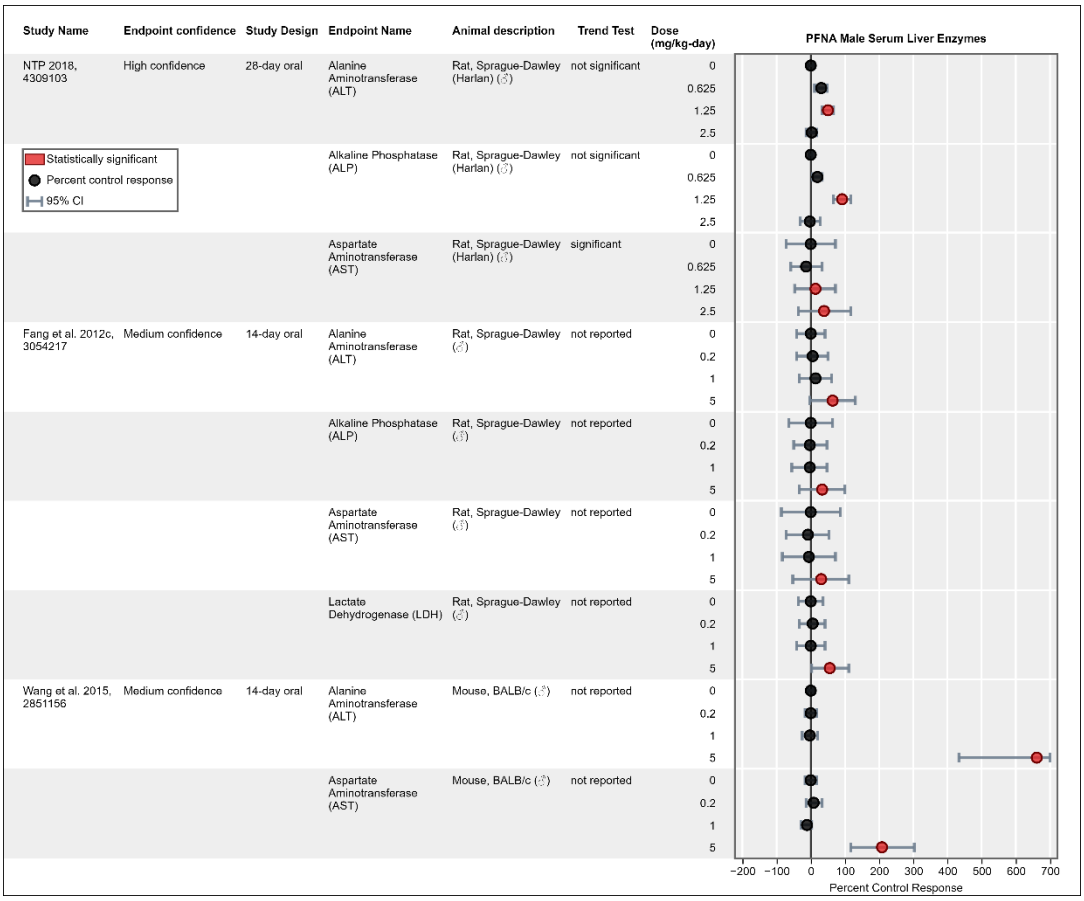


Figure 3-49. PFNA effects on serum liver enzymes in *high* and *medium* confidence studies in adult male rats and mice displayed as percent control responses.[a] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-male-rodent-liver-enzymes/.

[a]Percent change value for Wang et al. (2015a) was 660 ± 226% at 5 mg/kg-d.

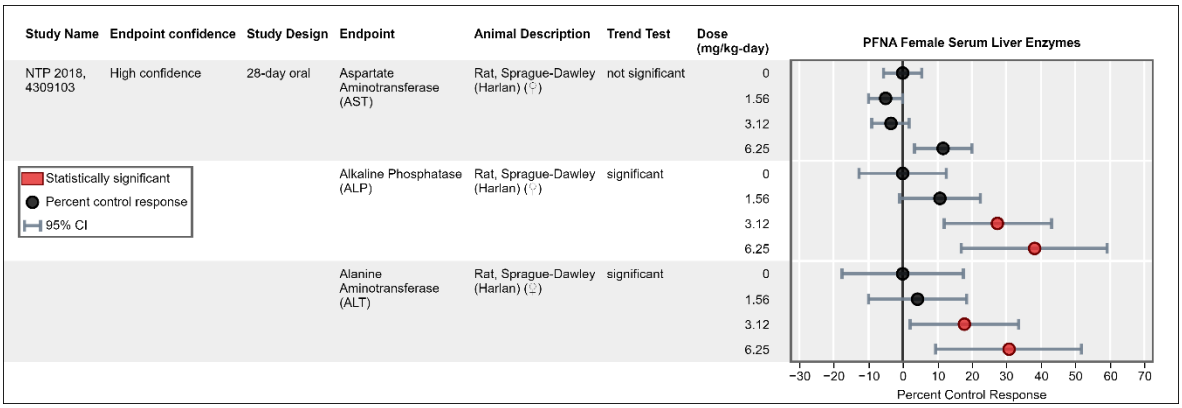


Figure 3-50. PFNA effects on serum liver enzymes in a *high* confidence study in adult female Sprague-Dawley rats displayed as percent control responses. See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-adult-female-liver-enzymes/.

Blood proteins

Blood proteins are routinely measured in diagnostic panels as changes, particularly decreases, can be indicators of protein loss due to kidney disease or impeded production in the liver, such as in liver disease (Boron and Boulpaep, 2017). Two major classes of blood proteins, albumin, and globulin (also measured clinically as total protein) are synthesized by the liver with some globulins also synthesized in lymphoid tissues. Albumin serves as a transporter of bilirubin, lipids, and hormones and other endogenous and exogenous molecules in blood circulation. Globulins include a large grouping of proteins that are also involved in iron, lipid, and hormone transport, and immune system functioning. In male rats exposed for 28 days, modest but significant dose-dependent reductions in serum globulin (1.4–2.1-fold at ≥0.625 mg/kg-day), albumin (1–1.3-fold at ≥1.25 mg/kg-day), and total protein (1.1–1.5-fold at ≥0.625) were reported, which in turn contributed to significant dose-dependent increases in A/G ratios 1.5–1.6-fold at ≥0.625 mg/kg-day (see Figure 3-51) (NTP, 2018). Smaller but still significant dose-dependent increases in albumin and reductions in serum globulin (1.2–1.3-fold) were also reported in females at ≥1.56 mg/kg-day, and the combination of these changes were associated with dose-dependent increases of 1.3- to 1.5-fold in the A/G ratio. The extent to which the perturbed protein levels and increased A/G ratio could reflect disturbances of the hepatic or immune system production and/or metabolism of these proteins is an unknown for this chemical.

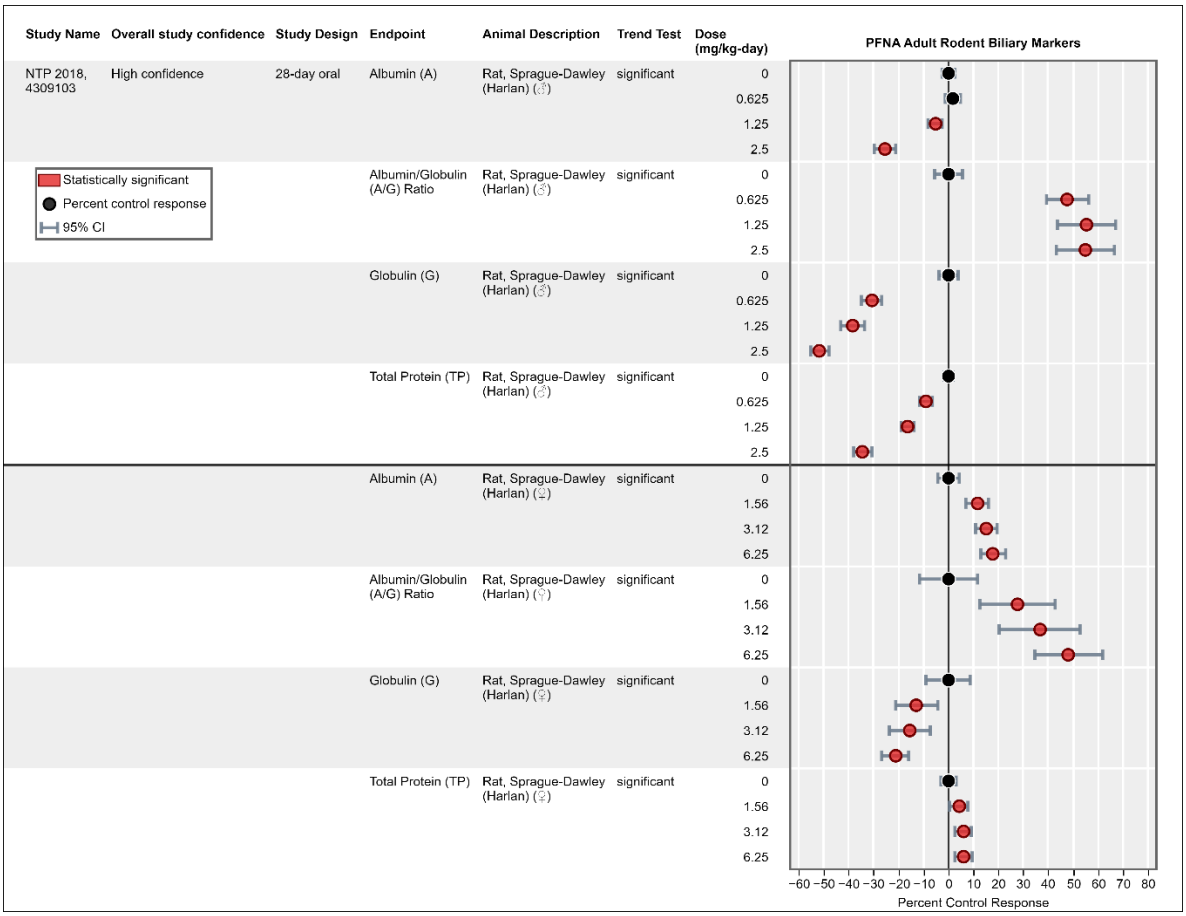


Figure 3-51. PFNA effects on serum proteins in a *high* confidence study in adult male (top) and female (bottom) Sprague-Dawley rats displayed as percent control responses. See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-adult-rodent-serum-protein/.

Hepatobiliary markers

Serum markers of potential hepatocellular dysfunction and hepatobiliary injury (i.e., liver injury affecting the biliary system) include impacts on bile components that are essential for normal bile flow, lipid metabolism, and erythrocyte breakdown (Hall et al., 2012; Ennulat et al., 2010; Boone et al., 2005; U.S. EPA, 2002a; Thompson, 1996). For PFNA, these endpoints were evaluated only in the NTP 28-day study in rats. In this study, similar to findings on the hepatobiliary marker ALP discussed above, significant dose-dependent increases in both serum total bile acids and bilirubin were observed in PFNA-treated adult male and female rats, with a higher magnitude of effect in males (see Figure 3-52). In male rats, pronounced elevations in serum total bile acids were reported at 1.25 mg/kg-day (5.4-fold increase) and 2.5 mg/kg-day (17-fold increase). This magnitude of increase in serum total bile acids could be indicative of potentially obstructed bile flow and/or disrupted bile acid synthesis, metabolism, or elimination (NTP, 2018; Boone et al., 2005; Thompson, 1996). Consistent with elevated total bile acids, total bilirubin (direct + indirect),

direct bilirubin (conjugated + delta bilirubin), and indirect bilirubin (unconjugated from erythrocyte breakdown) were also markedly elevated in males at ≥1.25 mg/kg-day PFNA, all with significant dose-dependence and exceeding a threefold change at 2.5 mg/kg-day. Total, direct, and indirect bilirubin were increased by 4.6-fold, 13-fold, and threefold, respectively, at 2.5 mg/kg-day PFNA (NTP, 2018). Elevated bilirubin may indicate impaired processes of hepatic bile flow, accelerated erythrocyte destruction, or decreased bilirubin metabolism (Hall et al., 2012; Boone et al., 2005; U.S. EPA, 2002a; Thompson, 1996). In adult female rats, NTP (2018) observed significant dose-dependent increases in total bile acids and direct bilirubin consistent with males but of a lesser magnitude; total bile acids increased two- to threefold at ≥3.12 mg/kg-day and bilirubin increased <twofold at 6.25 mg/kg-day. Taken together with the coherent elevations in serum ALP (described above with serum enzymes), these findings provide an indirect indication of bile duct obstruction.

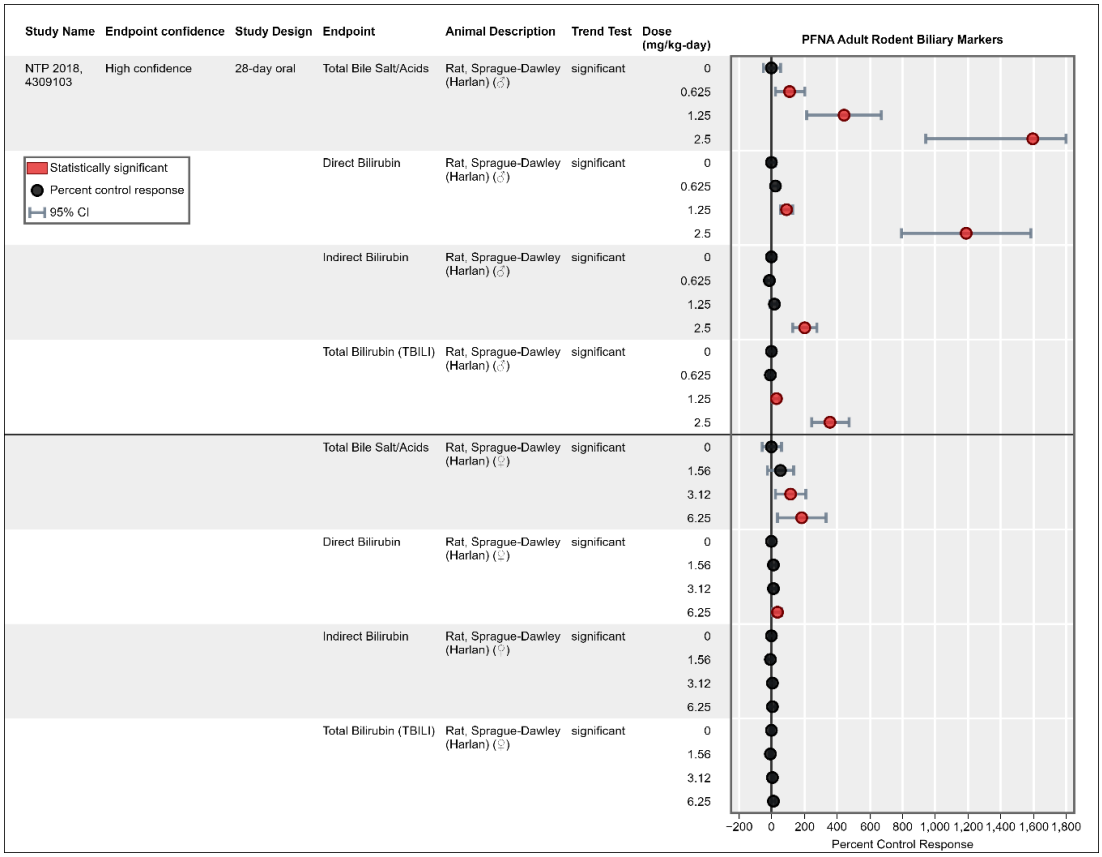


Figure 3-52. PFNA effects on serum hepatobiliary markers in a *high* confidence study in adult male (top) and female (bottom) Sprague-Dawley rats displayed as percent control responses.[a] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-adult-rodent-biliary/.

[a]Increases in total bile acids in males at 2.5 mg/kg-d were 1,594 ± 654%.

Mechanistic Evidence and Supplemental Information

While there has been substantial study of several potential pathways of PFNA-induced hepatic toxicity, particularly as it relates to activation of PPARα, the MOA(s) for PFNA-induced liver damage has yet to be characterized in depth. Mechanistic studies conducted to date indicate that PFNA exerts hepatic toxicity through multiple pathways, including by peroxisomal β-oxidation and activation of PPARα as well as by pathways independent of PPARα, perhaps most notably by activation of the xenobiotic nuclear receptors, constitutive androstane receptor (CAR) and pregnane X receptor (PXR). There has also been some examination of the role of these initiating events in subsequent downstream changes including PFNA-mediated fatty acid perturbations, hepatic lipid accumulation, hepatobiliary disruptions, and oxidative stress (e.g., Wolf et al. (2010), Das et al. (2015), Das et al. (2017), Rosen et al. (2017), and Marques et al. (2022)). These mechanistic findings along with the results from the short-term (<28 day) exposure studies in rodents were considered for potential adversity applying the Hall criteria (Hall et al., 2012) (see discussion below, *Consideration for potentially adaptive versus adverse responses*).

Receptor activation

Mechanistic experiments (e.g., transactivation assays, RT-PCR, microarrays) in several in vivo and in vitro models support PFNA activation of PPARα, CAR, and PXR, with inconsistent results for the other PPAR isoforms, PPARγ and PPARβ/δ. Specifically regarding the other PPAR isoforms, some of the stronger evidence for PPARγ activation was the observed significant overlap between fatty acid gene expression profiles in livers of exposed wild type and PPARα null mice with type 2 diabetic Lepr (*db*/*db*) mice administered a PPARγ agonist (Rosen et al., 2017). ToxCast in vitro high-throughput screening (HTS) screening (e.g., transactivation assays in HepG2 and HepRG cell lines) also reports PFNA activation of PPARγ and various cytochrome p450 regulatory genes (see Appendix C.2 for active hits and a link to null assays), and Zhang et al. (2014) reported PFNA binding to human PPARγ recombinant protein (ligand binding domain or LBD) and HepG2 cells transfected with a reporter plasmid. However, PFNA-treated primary human hepatocytes showed no change in *PPARG* transcripts but upregulated *PPARD* (encoding PPARβ/δ) (Rosen et al., 2013). Li et al. (2019) reported that PFNA could not fully displace a binding probe from the PPARβ/δ-LBD, even at the highest concentration tested (IC_{50} = 128 μM).

The short-term *high* and *medium* oral and developmental toxicity studies with PFNA in wild type and PPARα null mice (Das et al., 2017; Rosen et al., 2017; Wolf et al., 2010) provide supportive evidence that at least some hepatic effects of PFNA (e.g., increased liver weight, cellular hypertrophy, and triglyceride accumulation) are mediated by both PPARα-dependent and -independent (such as CAR) pathways, which is reinforced by gene expression profiling in wild type and PPARα or CAR knockout mice (Rosen et al., 2017; Oshida et al., 2015a; Oshida et al., 2015b). Liver transcript profiles after 7-day PFNA exposures at 1 and 3 mg/kg-day with phenobarbital-treated wild type mice, but not phenobarbital-treated CAR-null mice, suggest that

PFNA activates CAR regardless of PPARα genotype (Rosen et al., 2017). This in vivo microarray testing has shown that most differentially expressed genes (DEGs) in the liver after PFNA treatment were regulated by PPARα but not exclusively (Rosen et al., 2017). In this study, PPARα-independent gene expression ranged from 10% to 17% of the total DEGs in wild type and PPARα null mice.

These results were recapitulated in another study showing that 7-day exposures to 1 and 3 mg/kg-day PFNA-induced CAR activation and increased expression of CAR marker signature gene Akr1b7 in both wild type and PPARα null mice while Cyp2b10, another CAR marker, was only increased statistically in PPARα-null mice (Rooney et al., 2019b). In addition, PFNA-induced hepatomegaly in wild type, CAR-null, and PPARα-null mouse strains, indicating neither PPARα nor CAR alone is not sufficient for this effect ((Zhang et al., 2017). Rosen et al. (2013) also showed PFNA activation of CAR (e.g., increasing *CYP2B6* transcripts) and PPARβ/δ (increasing *PPARD* transcripts) in human but not mouse hepatocytes.

In vivo testing in another mouse strain receiving i.p. injections of PFNA at 20 mg/kg-day over a 3-day period also showed PFNA to be a CAR pathway activator, with additional in vitro reporter assays of human and mouse CAR suggesting PFNA (and other long-chain PFAS) to be indirect phenobarbital-like CAR activators as opposed to direct CAR activators (Abe et al., 2017). ToxCast in vitro HTS assays also show PFNA capable of interaction with the CAR receptor and modulating human *CYP2C9* activity (see Appendix C.2 for active hits and a link to null assays); *CYP2C9* is expressed primarily in liver and is regulated by CAR/PXR (Al-Dosari et al., 2006). In further support, reporter assays in human HepG2 cell lines have shown PFNA activation of human PXR (Zhang et al., 2017).

Regarding PPARα, the NTP 28-day exposure in adult male and female rats reported dose-dependent increases in liver expression of *Acox1/Cyp4a1* genes and associated acetyl CoA enzyme activity, as well as dose-dependent increases in *Cyp2b1* and *Cyp2b2* genes, suggestive of increasing PPARα and CAR activity, respectively. Additionally, gene expression profiles in fetal and postnatal livers of CD-1 mice indicate activation of PPARα-dependent pathways (e.g., *Acox1, Ehhadh, Pdk4*) (Das et al., 2015). In adult rats and mice, i.p. exposures to PFNA resulted in increased peroxisomal β-oxidation indicative of PPARα activation (Kudo et al., 2006; Kudo and Kawashima, 2003; Kudo et al., 2000; Goecke-Flora and Reo, 1996). In vitro screening-level HTS assays under ToxCast in human HepG2 and HEK293 cell lines indicate increased expression of *PPARA* mRNA transcripts, and mRNA transcript and protein production of its heterodimerization partner, retinoid X receptor (RXR) (see Appendix C.2 for active hits and a link to null assays). In vitro transactivation studies in COS-1 cells transfected with human and mouse PPARα have demonstrated PFNA activation of PPARα (Wolf et al., 2014; Wolf et al., 2012; Wolf et al., 2008a). In these and other similar studies, the potency of human and mouse PPARα activation generally increases with PFAS chain length with carboxylates being more potent than sulfonates of the same chain length (Rosenmai et al., 2018; Wolf et al., 2014; Wolf et al., 2012; Wolf et al., 2008a; Takacs and Abbott, 2007; Shipley et al., 2004; Maloney and Waxman, 1999). Analysis of the relative gene expression in primary mouse and human

hepatocytes incubated with short-chain and long-chain PFAS up to C12 also show PFNA (and PFOA) to be among the most potent activators of a select array of predominantly PPARα-dependent genes. Additionally, the lipid metabolism/PPARα pathway is generally well conserved across rodents and humans (Mcmullen et al., 2020; Pawlak et al., 2015; Corton et al., 2014; Hall et al., 2012; Rakhshandehroo et al., 2009; Klaunig et al., 2003). Human and rodent PPARα share structural and ligand binding features (Corton et al., 2014), and in vivo microarrays and in vitro testing have shown that both humans and rats share transcriptional pathways of fatty acid metabolism (Mcmullen et al., 2020; Rakhshandehroo et al., 2009).

Hepatic lipid accumulation/steatosis

Hepatic steatosis is defined as the excess accumulation of triglyceride-rich lipid droplets within hepatocytes, with clinical diagnosis when lipids account for at least 5% of liver weight, also defined as fatty liver disease (Nassir et al., 2015). PFNA has been shown to interact with human liver fatty acid binding protein (hL-FABP) (Yang et al., 2020a; Zhang et al., 2013a), and disruptions in hepatic lipid homeostasis that result in increased uptake and/or reduced clearance of lipids can lead to abnormal lipid accumulation or steatosis, with progression to other types of liver injury such as inflammation and necrosis (Ipsen et al., 2018). PFNA effects on increasing hepatic triglyceride levels has been examined in three *medium* confidence short-term in vivo studies in adult male rats and mice with two of these studies also measuring total cholesterol (Das et al., 2017; Wang et al., 2015a; Fang et al., 2012c). Fang et al. (2012c) reported an 84% increase in liver triglycerides in adult male SD rats exposed to PFNA at 5 mg/kg-day for 14 days, despite exposure conditions that resulted in decreased body weight (approximate 20%; see Section 3.2.1, "Adult rodent body weight") that would be expected to decrease lipid levels. The increased liver triglyceride in this study was significantly reduced with Kupffer cell inactivation by $GdCl_3$, suggesting a possible role for these immune cells in the steatotic pathway although this remains unstudied. No significant changes in liver triglycerides were reported at the lower PFNA doses tested in this study (0.2 and 1 mg/kg-day), nor were there changes in liver cholesterol levels. Wang et al. (2015a) also reported significantly elevated liver triglycerides and cholesterol (up to 134% and 38%, respectively) in male mice exposed to PFNA at 0.2 mg/kg-day and 1 mg/kg-day for 14 days, with no effects at 5 mg/kg-day where body weight loss became statistically significant (Wang et al., 2015a). Likewise, Das et al. (2017) observed a marked 305% increase in liver triglycerides at 10 mg/kg-day following a 7-day treatment that did not significantly affect body weight. This effect was attenuated but remained significantly increased by 81% in PPARα null mice receiving the same dose, suggesting that PPARα-independent pathways contributed to lipid accumulation. The involvement of both PPARα-dependent and -independent pathways is consistent with findings for liver weight and serum markers of liver injury discussed previously. To a more limited extent, these observations also align with the *low* confidence histological findings by Pfohl et al. (2021) and Das et al. (2017). The *low* confidence 90-day dietary exposure study in adult male mice (Pfohl et al., 2021) evaluated lipid accumulation and reported total lipid accumulation was

increased by 50% in mice receiving a low-fat diet plus PFNA (but not in animals receiving a high-fat diet plus PFNA compared with mice fed a high-fat diet alone). Similarly, Das et al. (2017) reported significant increases in lipid accumulation in livers of PFNA-treated animals at 10 mg/kg-day. It is also notable that NTP (2018) reported significant dose-dependent increases in activity of acetyl CoA at doses > 0.625 mg/kg-day, which is involved in lipid metabolism (Alves-Bezerra and Cohen, 2017).

Some of the short-term studies (NTP, 2018; Wang et al., 2015a; Fang et al., 2012c) also showed general reductions in serum triglycerides in PFNA-treated male rodents, although it is not possible to infer relationships between serum levels and liver uptake based on the current evidence (see Section 3.2.9, Figure 3-101). Furthermore, while the existence of relationships between lipid homeostasis and body weight are to be expected in studies that report overt toxicity, the mechanistic interactions underlying PFNA effects on liver lipid accumulations and body weight loss, the latter at higher dose levels, remain unclear as liver lipids were increased by PFNA in both the presence and absence of significant weight loss suggesting complex interactions.

In general alignment with the gavage exposure studies, several i.p. and in vitro exposure studies in rats and mice also show lipid accumulation and other changes consistent with the gavage exposure studies (Louisse et al., 2020; Zhang et al., 2017; Kudo et al., 2006; Kudo and Kawashima, 2003; Kudo et al., 2000; Goecke-Flora and Reo, 1996). However, the high PFNA doses used in these studies make direct comparisons to the in vivo gavage exposures highly uncertain. Additional mechanistic evidence of PFNA-mediated effects on lipid homeostasis included observations of disruptions in the balance of fatty acid accumulation, synthesis, and oxidation in wild type and PPARα null mice (7-day exposure at 10 mg/kg-day) that favored hepatic lipid accumulation (Das et al., 2017). In wild type mouse livers, PFNA-induced upregulation of genes traditionally observed to increase with PPARα activation (e.g., peroxisomal/mitochondrial fatty acid β-oxidation and microsomal fatty acid ω-oxidation genes) as well as genes involved in fatty acid and triglyceride synthesis and transport. These transcriptional patterns with high but nontoxic doses of PFNA were also generally observed in livers of PPARα null mice along with hepatic lipid accumulation, but at more modest levels compared with wild type mice. An interpretive challenge is the increase in fatty livers in control PPARα null animals, which has been reported previously and is not unexpected given the role of PPARα in fatty acid oxidation (Corton et al., 2014).

A few gene expression studies have examined a possible role for sterol regulatory element-binding protein (SREBP) pathways in PFNA-induced perturbations of lipid and cholesterol homeostasis, including those that do not result in overt toxicity. SREBPs are master transcriptional regulators of cellular lipid and cholesterol metabolism (Shimano and Sato, 2017). Fang et al. (2012c) measured an increase in expression of the *Srebf1* gene encoding SREBP-1c lipogenesis protein involved in fatty acid metabolism in adult male rats exposed to PFNA at doses ≥1 mg/kg-day. Similarly, microarray and computational studies in wild type and PPARα null mice by Rooney et al. (2019a) suggest that activation of sterol SREBPs are specifically linked to PFNA-induced lipid

accumulation in both mouse strains. In contrast, the microarray analysis by Das et al. (2017) in PFNA-exposed SV129 and PPARα null mice at high doses (10 mg/kg-day) showed no increase in either *Srebf1* or *Srebf2* subtypes in either strain although some target genes were upregulated in wild type or both strains (e.g., *Fasn*, *Scd1*, *Hmgcs1*), which is generally consistent with findings for another long-chain PFAS PFOA (Yan et al., 2015).

Oxidative stress

In vivo testing that targeted oxidative stress markers in association with liver injury reported statistically significant increased concentrations of malondialdehyde and hydrogen peroxide in livers of male rats exposed to PFNA for 14 days at 5 mg/kg-day (Fang et al., 2012a). PFNA also activated Nrf2, a transcription factor that induces an array of antioxidant genes in response to redox imbalance, in the livers of wild type and PPARα null mice after 7 days of treatment with both 1 and 3 mg/kg-day (Rooney et al., 2019b). In vitro testing by Wielsøe et al. (2015) considered the potential of PFNA, PFOA, PFOS, and several other PFAS to alter total antioxidant capacity and increase DNA damage and reactive oxygen species (ROS). In this study, HepG2 cells were exposed to PFNA for 24 hours at non-cytotoxic concentrations. PFNA was found to cause significant DNA damage and ROS generation with no significant effects on total antioxidant capacity (Wielsøe et al., 2015). PFNA was also a potent inhibitor of mitochondrial respiration in isolated rat liver mitochondria compared with shorter chain PFAS (Wallace et al., 2013). In general, while there is largely consistent evidence of increased hepatic oxidative stress after PFNA exposure, the available studies were not designed to assess whether these increases were a cause or consequence of other hepatic changes.

Summary of mechanistic findings

The PFNA mechanistic evidence in human and rodent cell lines provided strong support for PFNA activation of PPARα and CAR/PXR pathways in vivo in rodent studies and in vitro in multiple human and rodent cell lines. The available in vivo and in vitro mechanistic testing was generally consistent in observations of PFNA-mediated triglyceride accumulations in the liver, but not cholesterol, with evidence supporting involvement of both PPARα-dependent and PPARα-independent activation. There is also evidence suggesting possible involvement of the lipid regulator, SREBP and its target genes by pathways that remain to be more fully delineated, as well as potential oxidative stress pathway inductions. Elevated hepatic triglycerides are also observed in rodents exposed to other perfluorinated compounds, such as PFHxS, PFOA, PFOS, and PFDA (Nakagawa et al., 2012; Bijland et al., 2011; Kudo and Kawashima, 2003; Kawashima et al., 1995). There was evidence for a role for both PPARα and CAR pathways in the liver effects, but with inconsistent evidence for activation of the other known PPAR isoforms, PPARγ and PPARβ/δ. The findings in rodents are interpreted to be relevant to humans. While PPARα signaling pathways may be less responsive in humans, the MOA leading to the observed liver effects remains unclear and also involves activation of CAR/PXR and other possible PPARα-independent pathways.

Additionally, the generally consistent in vivo and mechanistic evidence of elevated liver triglyceride and steatosis in rodents is considered directly relevant to humans as the lipid metabolism pathway is generally well conserved across rodents and humans (Mcmullen et al., 2020; Pawlak et al., 2015; Corton et al., 2014; Hall et al., 2012; Rakhshandehroo et al., 2009; Klaunig et al., 2003).

Consideration for potentially adaptive versus adverse responses

As described in the protocol (see Appendix A), the evidence of PFNA-induced hepatic effects is based on studies in rodents that may have species-specific responses is considered in the context of potentially adaptive versus adverse responses using recommendations outlined in Hall et al. (2012) and others (e.g., U.S. EPA (2002a), EMEA (2008), Thoolen et al. (2010), and Boone et al. (2005)). Hepatotoxicity observed in male and female rats and mice administered PFNA for short durations (28 days or less), mouse offspring in developmental studies, as well as in supplemental mechanistic testing includes increases in liver weight; hepatocellular hypertrophy, cytoplasmic alterations, and necrosis; elevated ALT/AST in mice with mild increases in serum ALT/AST/ALP in rats; pronounced increases in hepatobiliary components in rats (serum total bile acids and bile salts, bilirubin, and liver accumulation of triglycerides); altered blood proteins (albumin, globulin); peroxisomal β-oxidation and activation of PPARα; and activation of CAR/PXR pathways.

Chemically induced increases in liver weight and hepatocellular hypertrophy may be interpreted as potentially adverse when accompanied by degenerative or necrotic alterations, including: hepatocellular necrosis, fibrosis, inflammation, and steatotic vacuolar degeneration; biliary/oval cell proliferation, degeneration, fibrosis, and cholestasis; or necrosis/degeneration of other liver cells (Hall et al., 2012). For PFNA, the NTP 28-day study reported dose-dependent hepatocellular hypertrophy and cytoplasmic alterations characterized by accumulation of cytoplasmic granules or increases in homogenous eosinophilic cytoplasm in male and female rats that is consistent with potential liver inflammation, peroxisome-induced hypertrophy, steatosis, and/or hepatobiliary cholestasis (NTP, 2014; Tarantino et al., 2008; Terasaki et al., 1993). The severity of hepatocellular necrosis was reported to be minimal to mild at ≥2.5 mg/kg-day in adult male rats and ≥12.5 mg/kg-day in female rats (which was inconclusive due to body weight loss), consisting of a few widely scattered, randomly distributed foci of necrotic hepatocytes co-occurring with mononuclear inflammatory cells (NTP, 2018). Given the preterm mortality in higher dose animals (≥5 mg/kg-day in males; ≥12.5 mg/kg-day in females), it is possible that the resulting reduced window of exposure reduced the progression and severity of the observed hepatocyte degeneration and necrosis. Thus, the short-term testing with PFNA indicates that increases in liver weights co-occurred with a constellation of histopathological lesions, including clearly adverse lesions such as necrosis (in rats).

The lack of longer duration exposures was a substantial source of uncertainty. This data gap prevented full consideration of whether the liver enlargements and concurrent effects (e.g., hepatobiliary markers) at lower PFNA doses could elicit adaptive mechanisms or, as indicated by the histopathology data at higher PFNA doses, could progress to more severe liver disease.

Therefore, although the linkage between liver hypertrophy and histological evidence of necrotic changes supports adversity, the short-term data were further evaluated based on additional criteria set forth in Hall et al. (2012) that considers dose-dependent and biologically significant changes in at least two clinical pathology parameters as confirmatory indicators of hepatocellular damage. These parameters include increases in ALT of two- to threefold; biologically significant changes in markers of hepatobiliary damage (ALP, AST, GGT, GLDH, etc.); or a biologically significant change in other clinical pathology markers indicating liver dysfunction (albumin, bilirubin, bile acids, coagulation factors, cholesterol, triglycerides, etc.) (Hall et al., 2012). There were differences in the magnitude of the increases in liver enzymes between rats and mice administered PFNA. In the NTP 28-day study, while histopathological changes were observed at the lowest dose in both sexes, changes in serum liver enzymes ALT/AST/ALP in rats were generally mild with less than twofold increases at doses up to 2.5 mg/kg-day in males and 6.25 mg/kg-day in females. However, in male mice, ALT and AST were increased by 7.5-fold and 3.1-fold, respectively, after 14 days of exposure to 5 mg/kg-day. Thus, the serum liver enzyme findings suggest some differences in these markers across rodent species exposed to PFNA with heightened sensitivity in mice possibly linked to pharmacokinetic differences (Wang et al., 2015a). Finally, the human evidence showed some indication of modest but consistent positive associations between serum PFNA and ALT in adults, as well as generally consistent increases in AST and total bilirubin, which are somewhat aligned with the animal evidence although the epidemiology evidence is uncertain due to the small magnitude of effects. While increases in these markers in rats are not interpreted to provide clear support for adversity and the weight of evidence for these changes in humans was similarly considered insufficient, the observed large increases in mice would meet one of the criteria for adversity described by (Hall et al., 2012).

The 28-day study in rats also observed dose-dependent increases of large magnitude in total bile acids and bilirubin in adult males (NTP, 2019, 2018). Total bile acids were increased 5.4-fold to 17-fold at ≥1.25 mg/kg-day and total bilirubin and direct bilirubin were increased 4.6-fold and 13-fold, respectively, at 2.5 mg/kg-day in males. Increases in biliary components of this magnitude are considered to be indicators of impaired bile flow or intrahepatic cholestasis (NTP, 2019). While there were also dose-dependent trends in female rats in this study, the magnitude of increase was less than in males. Increases in serum bilirubin with concurrent increases in serum total bile acids are considered reliable indicators of potential loss of hepatic function and toxicity, and fulfills the second criterion for adversity (Hall et al., 2012). In addition, there were dose-dependent reductions in blood proteins (albumin, globulins) in adult male and female rats with associated increases in the A/G ratio that could relate to further perturbations of liver metabolic pathways and protein production. Finally, there was generally consistent and coherent in vivo (gavage and i.p. exposures) and in vitro evidence of increasing triglyceride accumulation and disrupted liver lipid homeostasis that aligned with some of the histopathology (cytoplasmic vacuolization). As indicated above, steatosis is a common liver response in animals associated with

exposure to other long-chain PFAS, including PFOA and PFDA (Costello et al., 2022). Sustained steatosis can progress to steatohepatitis and other adverse liver diseases such as fibrosis and cirrhosis (Angrish et al., 2016).

Thus, taken together, the evidence of PFNA-mediated liver toxicity includes a constellation of multiple, coherent effects including histopathological evidence of hepatocellular hypertrophy and necrotic changes, increasing liver weights, hepatic steatosis, and elevations in some serum markers (ALT/AST in mice, hepatobiliary cholestasis markers in rats) that meet the criteria set forth by Hall et al. (2012) for adversity. The lack of rodent bioassays with PFNA of longer duration at lower dose levels are a source of uncertainty as this would allow evaluation of whether effects worsened over time and if lower doses administered over a longer period elicited similar adverse responses.

Evidence Integration

There is *moderate* evidence in humans that higher exposure to PFNA is associated with liver injury in adults across *medium* confidence studies. The available studies showed consistently increased serum ALT, AST, GGT, and total bilirubin in most studies in adults, indicating potentially impaired liver function, although some uncertainty exists regarding the biological significance of the small positive associations observed in the individual studies. The available evidence further suggests that these associations are unlikely due to confounding by other PFAS based on mixture modeling in a subset of studies. The results in children and adolescents were inconsistent. The positive associations with serum enzymes and generally consistent increases in total bilirubin provide some cross-evidence stream coherence with the observed effects in animal studies.

In addition, there is *robust* evidence in rodents of PFNA-induced liver injury based on consistent and coherent treatment-related increases in liver weight, histopathological effects, hepatobiliary disruption, triglyceride accumulation, and some clinical chemistry markers (e.g., increased ALT that was modest in rats but pronounced in mice) across multiple studies, species, rodent strains, sexes, and lifestages. Short-term PFNA exposures of 28 days or less resulted in increased liver weights in adult male and female rats, mice, and gestationally exposed mouse offspring; increased hepatocellular hypertrophy, cytoplasmic alterations, and vacuolization, with some evidence of greater sensitivity in male rodents likely related to pharmacokinetic differences (see Section 3.1). Increasing liver weight and histopathological responses were consistent in direction across species and generally exhibited a monotonically increasing dose-response gradient. In the only study to evaluate hepatocellular necrosis, the NTP 28-day study reported a significant increasing trend of mild necrosis in male and female SD rats exposed to PFNA at ≥2.5 mg/kg-day and ≥12.5 mg/kg-day, respectively, with a generally shortened time to first onset with increasing dose and a high rate of incidences, particularly in males.

Providing additional coherence, pronounced increases in serum total bile acids and bilirubin (i.e., total, direct, and indirect) were observed in PFNA-exposed male rats at doses ≥0.625 mg/kg-day after a 28-day exposure. Effects were dose-responsive and of a large magnitude

(as high as 17-fold increase in total bile acids) that indicates obstructed bile flow and intrahepatic cholestasis. Female rats in the same study also showed significantly elevated total bile acids and direct bilirubin at doses ≥3.12 mg/kg-day, although the increases were not of the magnitude seen in males. Increases in total bile acids and bilirubin were of similar magnitude to those observed for other long-chain PFAS (PFDA), and of greater magnitude than PFOA, which were also evaluated by (NTP, 2019). While a couple PFNA studies reported increases in serum ALT, AST, and ALP in rats, overall effects of short-term PFNA exposures on serum liver enzymes of rats were mild. However, there were large elevations in ALT (7.5-fold increase, considered adverse by Hall et al. (2012)) and AST (4.6-fold increase) in adult male mice after 14 days of exposure to 5 mg/kg-day that aligned with hepatic hypertrophy measured at higher doses in mice of a different strain. Other indicators of potential liver injury observed in male and female rats were the dose-dependent decreases in serum albumin (males only) and globulin with an associated increased A/G ratio at doses ≥0.625 mg/kg-day, signaling possibly decreased synthesis of these proteins in the liver. Increases in ALT and AST, and reductions in serum proteins are also consistent with observations for other PFAS, including PFDA, PFHxA, and PFOA (ATSDR, 2021; NTP, 2019). Together, the observed constellation of PFNA-induced hepatic changes in the liver, including increases in necrosis in rats, concurrent elevations in serum bilirubin and total bile acids, and large elevations in ALT and AST in mice, are adverse and indicative of hepatic injury (Hall et al., 2012); thus, the consistent findings of liver hypertrophy are interpreted as nonadaptive markers of this adverse effect.

While the MOA for PFNA-induced hepatotoxicity likely involves activation of PPARα-signaling pathways, which may be less responsive in humans, the toxicity mechanisms leading to liver dysfunction continue to be unclear and appear to also involve CAR/PXR activation and other PPARα-independent pathways. There is also generally consistent in vivo and mechanistic evidence of PFNA-induced liver triglyceride accumulation and steatosis in rodents that is considered directly relevant to humans as both humans and rats share pathways of fatty acid metabolism (Mcmullen et al., 2020; Corton et al., 2014; Hall et al., 2012; Rakhshandehroo et al., 2009). Taken together, although some uncertainties remain, the findings in rodents are interpreted as relevant to humans.

Additional uncertainties in the available evidence base include the limited exposure durations (≤28-day exposures) that make unknown how extended PFNA exposures might affect the progression of observed liver effects to chronic conditions, such as cholestatic liver disease and NAFLD, and how this might manifest in populations with comorbidities (as reviewed by Foulds et al. (2017), Hardy et al. (2016), and Treviño and Katz (2018)). The dose-dependent liver enlargement in prenatally exposed mice at relatively low maternal doses (≥0.83 mg/kg-day) also suggests susceptibility during early lifestages.

Together, the ***evidence indicates*** that PFNA exposure likely causes hepatotoxicity in humans given sufficient exposure conditions based on a combination of generally consistent and

coherent evidence from human, animal, and mechanistic studies (see Table 3-17).[12] There was consistent epidemiological evidence across most studies of increased serum ALT, AST, GGT, and total bilirubin with median concentrations of 0.6–2.0 ng/mL PFNA, although some uncertainty exists regarding the biological significance of the small changes in these biomarkers of liver injury. Short-term animal studies indicate consistent and coherent effects on liver weight, histopathology, hepatobiliary cholestasis, triglyceride accumulation, and clinical pathology generally at ≥0.625 mg/kg-day in adult male rats and ≥1.56 mg/kg-day in adult female rats and increasing liver enzymes in male mice. Corroborating mechanistic findings provide evidence that the observed changes in hepatic parameters are biologically plausible, adverse, and relevant to humans. Human relevance is supported by mechanistic evidence demonstrating PPARα-dependent and PPARα-independent activation and the involvement of hepatic lipid accumulation and cholestasis.

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

Table 3-17. Evidence profile table for hepatic effects

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.3. Hepatic Effects, Human Studies)					⊕⊕⊙ ***Evidence indicates*** **(likely)**
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Liver enzymes</u> Twelve *medium* confidence studies and 3 *low* confidence studies	• All 9 *medium* confidence studies in adults reported higher ALT levels with higher PFNA exposures (6 statistically significant). Majority of studies for AST and GGT also reported positive associations, most statistically significant. Confounding by other PFAS is unlikely based on available mixture modeling results. • Results in children and adolescents were less consistent across 3 *medium* confidence studies and 1 *low* confidence study.	• *Consistency* across studies in adults • General *coherence* in direction of association across serum markers (ALT, AST, GGT, total bilirubin) within and across studies • *Medium* confidence studies reporting an effect	• Unclear biological significance of small changes	⊕⊕⊙ *Moderate* Primarily consistent evidence of small but significant associations with increased liver enzymes in adults. This judgment does not apply to children or adolescents	*Primary basis:* *High* and *medium* confidence studies in rodents across multiple species, sexes, and study designs showing multiple markers of hepatic injury at ≥0.625 mg/kg-d PFNA; supported by in vivo and mechanistic evidence of cholestasis and liver triglyceride accumulations, and consistent human evidence of increased liver enzymes and bilirubin in adults.

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
<u>Liver disease</u> Two *low* confidence studies	• One study reported an inverse association with non-alcoholic fatty liver disease while the second study found a positive association in women	• No factors noted	• Inconsistency • *Low* confidence studies		*Human relevance:* Effects in rodents and mechanistic data are considered relevant to humans, particularly since the available evidence supports involvement of both PPARα-dependent and -independent pathways (see Mechanistic and Supplemental information section and results for PPARα knockout rodents). *Cross-stream coherence:* Generally *coherent* findings of increased serum markers in humans exposed to median exposure levels of 0.7–2.0 ng/mL PFNA support the findings in rodents. *Susceptible populations and lifestages:* Although some testing in mice indicates early lifestages may be susceptible, the
Evidence from in vivo animal studies (see Section 3.2.3. Hepatic Effects, Animal Studies)					
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Liver weight</u> Nine *high/medium* confidence studies: • 28 d (1× rat) • 14 d (2× rat; 1× mouse) • 7 d (2× mouse) • GD 1–17 (mouse) • GD 1–18 (2× mouse)*	• All *high/medium* confidence studies in wild type rodents reported increased relative liver weights, including in 2 species, both sexes, adults, and early lifestages • Three *high/medium* confidence studies in PPARα null mice (males, nonpregnant females, offspring) reported generally	• *Consistent* and *dose-response* gradient for increased weight across studies, species, sex, and lifestage • *Coherence* with histopathology, hepatobiliary markers, and lipid accumulation in male and female rats and male mice	• No factors noted	⊕⊕⊕ *Robust* Consistent, dose-dependent, and coherent increases in liver weight, hepatic hypertrophy and necrosis, and hepatobiliary	

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
Two *low* confidence studies in mouse offspring and adult rats: • GD 1–18 (mouse)* • Acute (4-hr) inhalation (rat) *Only absolute weights reported in 1 study (dams and offspring, *medium* and *low* confidence, respectively)	consistent increases in relative liver weights but with no effects in PPARα null dams • Increased relative liver weight in male PPARα null mice at ≥3 mg/kg-d, nonpregnant PPARα null females at ≥1.1 mg/kg-d, and gestationally exposed PPARα null offspring at 2 mg/kg-d in *high* and *medium* confidence studies	• *Large magnitude of effect*, up to 195% increase (pregnant wild type mice) in a *high* confidence study • Effects observed in *high* and *medium* confidence studies		disruptions, generally at ≥0.625 mg/kg-d in male rats and ≥1.56 mg/kg-d in female rats, with mice generally exhibiting increased sensitivity. The constellation of findings is considered adverse (see Section 3.2.2. "Mechanistic Evidence and Supplemental Information")	evidence base is limited to effects on liver enlargement and overall inconclusive
<u>Histopathology</u> *high/medium* confidence studies in adult rats: • 28 d • 14 d *Low* confidence studies in adult mice and rats: • 14 d (mouse) • 7 d (rat)	• Hepatocellular hypertrophy at ≥0.625 mg/kg-d (male) and ≥6.25 mg/kg-d (female) rats in a *high* confidence study • Vacuolization in male rats at mid doses (1.25 and 2.5 mg/kg-d); cytoplasmic alterations at ≥0.625 mg/kg-d in males and females in a *high* confidence study • Mild necrosis at ≥2.5 mg/kg-d (male) and ≥12.5 mg/kg-d (female) rats in a *high* confidence study	• *Consistent* hepatic hypertrophy across studies, species, and sexes • *Coherence* with liver weight increases • *Dose-response gradient* for hypertrophy in multiple studies and necrosis in a single study for male and female rats • Effects observed in *high* and *medium* confidence studies • *Biological plausibility* for vacuolation and related histopathology supported by mechanistic evidence of	• No factors noted		

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
		liver lipid accumulation and lipid regulation pathways			
<u>Clinical chemistry (serum liver enzymes, blood proteins, hepatobiliary markers)</u> Three *high/medium* confidence studies in adult rats and male mice: • 28 d (rat) • 14 d (2× mouse)	• Hepatobiliary disruptions indicative of cholestasis (increased total bile acids and bilirubin) in rats exposed at ≥0.625 mg/kg-d (male) and ≥3.12 mg/kg-d (female) in a *high* confidence study • Marked increases in serum ALT and AST in male mice at 5 mg/kg-d; modest increases in serum ALT/AST/ALP in rats at 5 mg/kg-d (male) in *high* and *medium* confidence studies and in ALT/ALP at ≥3.12 mg/kg-d (female) in a *high* confidence study • Decreased globulin and increased A/G ratio at ≥0.625 mg/kg-d (male) and ≥1.56 mg/kg-d (female) rats in a *high* confidence study	• *Consistent* increases in ALT, AST, total bile acids, bilirubin, and A/G ratio in several studies in rats and in male mice • *Large magnitude of effects*, as high as 17-fold for hepatobiliary markers and 7.5-fold increase in serum ALT in male mice • Effects observed in *high* and *medium* confidence studies • *Dose-response* gradients for hepatobiliary effects in a *high* confidence study • *Coherence* with increased liver weight and histopathology • Biological plausibility for cholestasis provided by mechanistic evidence of perturbations in hepatobiliary homeostasis (see mechanistic table below)	• Unclear biological significance of <twofold increases in serum liver enzymes in rats	

Summary of human, animal, and mechanistic evidence			Evidence integration summary judgment
Mechanistic evidence and supplemental information (see subsection above)			
Biological events or pathways	**Summary of key findings and interpretation**	**Evidence stream judgment**	
Molecular events – PPARα and other nuclear receptor activations	Evidence for activation of PPARα and CAR/PXR, in vivo and in vitro (human and mouse). Some inconsistent evidence for activation of PPARγ. Interactions with PPARγ and PPARβ/δ pathways were not well characterized and effects at lower doses represent a data gap.	PPARα-dependent and -independent pathways reported in rodents, in vivo and in vitro, and in human cell lines, provides biologically plausible support for multiple pathways operant in PFNA-induced liver effects	
Cellular and organ events – Hepatic lipid accumulation and steatosis	Several studies reported generally consistent increasing accumulation of liver triglycerides in rats and mice in both the presence and absence of significant weight loss suggesting complex interactions. In vitro testing in human cell lines show increases in cellular triglycerides with PFNA. Significant increases in liver triglycerides reported in PPARα null mice after short-term exposure (but attenuated from wild type mice). Disruptions in the balance of fatty acid accumulation, synthesis, and oxidation, in wild type and PPARα null mice favored liver lipid accumulation, and therefore appears to be partially independent of PPARα activation and indicates multiple mechanisms are involved, with possible involvement of SREBP pathways.		
Cellular and organ events – Hepatobiliary cholestasis	Cholestasis reported in C57BL/6, CAR-null, and PPARα-null mice with disruption of total bile acids/salts, and bilirubin (total, direct, and indirect).		

1

3.2.4. Male Reproductive Effects

This section describes and integrates the evidence informative to assessing the potential for PFNA to cause male reproductive toxicity based on human epidemiological and experimental animal studies, supported by additional mechanistic evidence. The evidence base includes studies that examined effects on male reproductive development, and thus there is some overlap in this section with the evidence synthesis and integration summary of developmental health effects (see Section 3.2.2).

Human Studies

Twelve epidemiology studies (14 publications) examined the association between PFNA exposure and male reproductive effects. Outcomes evaluated in these studies included semen quality parameters, reproductive hormones, and timing of pubertal development.

Semen parameters

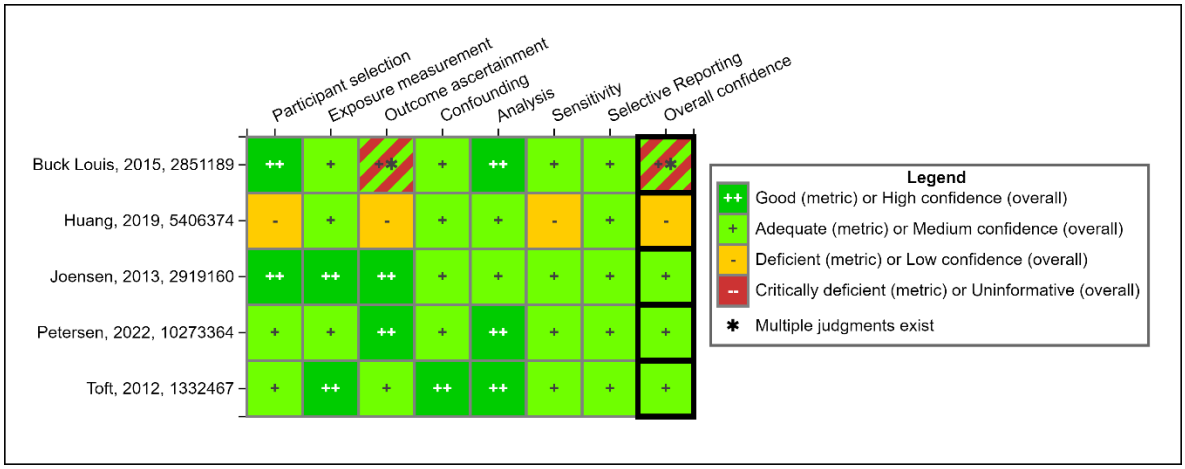


Figure 3-53. Summary of study evaluation for epidemiology studies of semen quality parameters. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500200/.

Sperm concentration, motility, and morphology were considered primary endpoints for the assessment of semen quality parameters. Key issues for the assessment of semen quality involve the timing of sample collection and 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 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 is relatively short (86 days including maturation) in comparison to the longer estimated blood half-life of PFNA (average $T_{½}$ estimated to be 5.6 years in men, see Section 3.1). There are no major concerns for reverse causality with this outcome.

Five epidemiology studies (seven publications) examined the association between PFNA exposure and semen quality parameters. The evaluations for these studies are summarized in Figure 3-53, and additional evaluation details can be obtained from HAWC. All five studies were cross-sectional. Four were *medium* confidence, including an analysis of male partners (18 years and older, mean 26–31 years by country) in a pregnancy cohort (Toft et al., 2012), a study of healthy young men (18–22 years) being considered for military service (Joensen et al., 2013), men selected from a birth cohort (18–21 years) (Petersen et al., 2022), and a study of men (18 years and older, mean 32 years) from couples discontinuing contraception (Buck Louis et al., 2015). One study (Huang et al., 2019a) included men from couples seeking infertility assessment and was *low* confidence. All the studies analyzed PFNA in serum and measured serum parameters using appropriate methods; thus exposure and outcome misclassification are expected to be minimal, except for sperm motility in Buck Louis et al. (2015), which is *uninformative* due to the delay between semen collection and analysis.

The results for the association between PFNA exposure and semen parameters in the four *medium* confidence studies are presented in Table 3-18. The studies analyzed the outcomes differently, so the effect estimates are not directly comparable. There was a decrease in sperm motility in two studies (Petersen et al., 2022; Joensen et al., 2013), reduced percent normal morphology in one study (Toft et al., 2012), and reduced concentration in one study (Petersen et al., 2022) but none were statistically significant and otherwise the results indicated no decrease in semen quality or amount in association with exposures to PFNA. The *low* confidence study reported no association with sperm concentration, motility, or morphology. No evaluation of confounding across PFAS was performed due to the lack of consistency in results, and none of the studies presented multipollutant analyses; this was true for other male reproductive effects as well.

Table 3-18. Associations between PFNA and semen quality parameters in medium confidence epidemiology studies

Reference, confidence	Population	Median exposure (IQR) (ng/mL)	Effect estimate	Sperm concentration	Sperm motility	Sperm morphology (% normal)
Petersen et al. (2022)	Cross-sectional analysis within cohort (2017–2019), Denmark, 1,041 men (18–21 yr)	0.5 (5th–95th 0.3–0.9)	Percent difference for tertiles vs. T1	T2: −8 (−19, 4) T3: −10 (−21, 3)	T2: −6 (−11, 0) T3: −5 (−11, 1)	T2: 0 (−10, 10) T3: −3 (−13, 8)
Joensen et al. (2013)	Cross-sectional study of men evaluated for military service (2008–2009), Denmark; 247 men (18–22 yr)	1.1 (0.9–1.4)	β (95% CI) for 1-unit increase	Cubic root transformed 0.11 (−0.14,0.35)	Square transformed −223 (−584,138)	Square root transformed −0.06 (−0.26,0.13)
Toft et al. (2012)	INUENDO cohort cross-sectional analysis (2002–2004), Greenland, Ukraine, Poland; 588 men	1.2 (P33–P66: 1.0–1.5)	Adjusted difference T2 and T3 vs. T1	(mill/mL) T2: −1 (−19,18) T3: 7 (−13,28)	% motile T2: 0 (−12,11) T3: −1 (−13,11)	% normal T2: −12 (−26,2) T3: −8 (−23,7)
Buck Louis et al. (2015)	LIFE preconception cohort cross-sectional analysis (2005–2009), U.S.; 462 men	1.7 (1.2–2.2)	β (95% CI) for ln-unit increase	(mill/mL) 5.22 (−11.9,22.3)	U	% normal 3.97 (−0.17,8.10)

*$p < 0.05$, U = *uninformative,* T = tertile.

Reproductive hormones

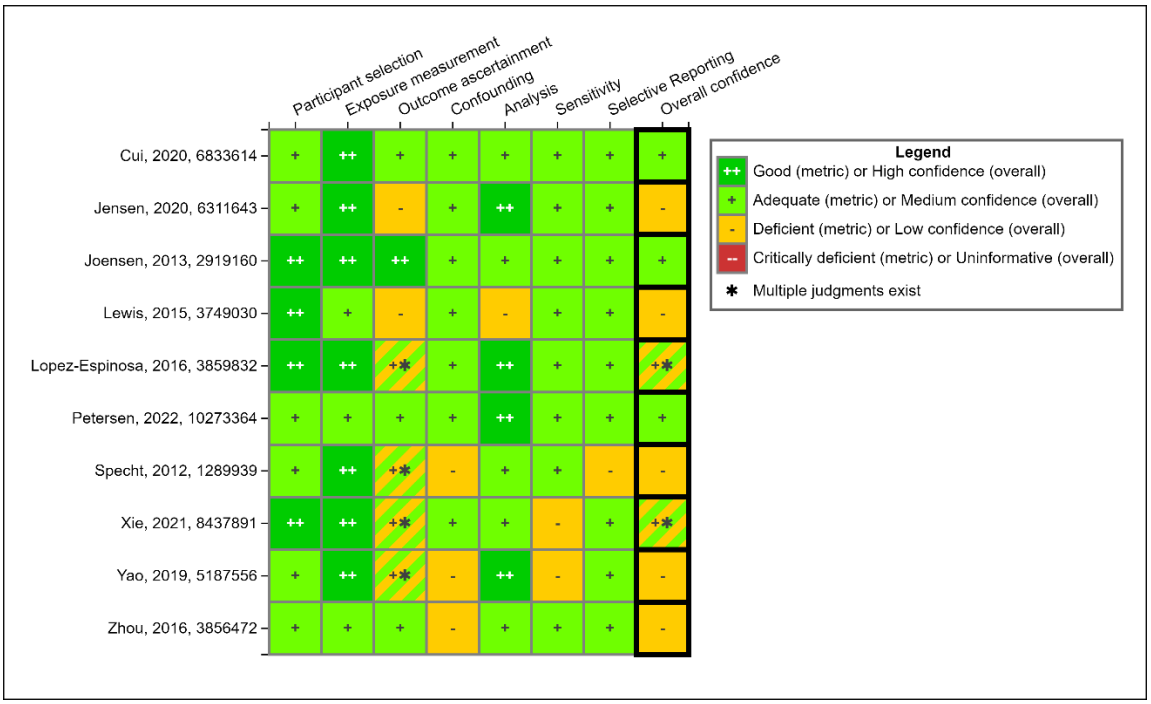


Figure 3-54. Summary of study evaluation for epidemiology studies of male reproductive hormones. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500201/.

Testosterone and estradiol were considered the primary endpoints for serum levels of reproductive hormones in men and boys. Luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex hormone binding globulin (SHBG) were also reviewed where available. The key issue for the evaluation of these studies was the timing of sample collection. Due to diurnal variations in testosterone, LH, and FSH, blood sample collections should be in the morning, and if not, the time of sample collection should be adjusted for in the analysis (Brambilla et al., 2009; Rosner et al., 2007). If there is no consideration of time of collection for these hormones, the study was classified as deficient for outcome ascertainment and *low* confidence or *uninformative* overall (depending on other identified limitations). Sample collection and analytic methods were also considered. Additional outcome-specific considerations are available in the protocol (see Appendix A).

Ten studies (11 publications) examined associations between PFNA and serum levels of reproductive hormones in males (Figure 3-54). Most studies only evaluated testosterone and estradiol with all measuring exposure and outcome concurrently. Relationships between PFNA exposures and serum levels of reproductive hormones were examined in six studies in adult men (Petersen et al., 2022; Xie et al., 2021; Cui et al., 2020; Lewis et al., 2015; Joensen et al., 2013; Specht et al., 2012), three studies in adolescent boys (Zhou et al., 2016; Lewis et al., 2015), one study in children (Lopez-Espinosa et al., 2016), and two studies in infants (Jensen et al., 2020b; Yao et al.,

2019). Study evaluations are summarized in Figure 3-54. Five studies were rated *medium* in overall study confidence (Petersen et al., 2022; Xie et al., 2021; Cui et al., 2020; Lopez-Espinosa et al., 2016; Joensen et al., 2013) and five were *low* confidence (Jensen et al., 2020b; Yao et al., 2019; Zhou et al., 2016; Specht et al., 2012). However, although Lopez-Espinosa et al. (2016) and Xie et al. (2021) were *medium* confidence overall, the results for testosterone were considered *low* confidence due to lack of consideration of collection timing.

The potential for associations between PFNA exposure and serum testosterone was studied in adults, adolescents, children, and infants. In adult men, three studies were *medium* confidence and three were *low* confidence. One *medium* confidence study reported a statistically significant inverse association with exposure measured in both serum and semen for total testosterone (−3.99% change, 95% CI −7.01, −0.87 in serum), and an inverse but not statistically significant association with free testosterone. Another *medium* confidence study (Joensen et al., 2013) in a population of young Swedish men reported a small inverse association between PFNA and ln-testosterone (β: −0.059 per 1 ng/mL change in PFNA, 95% CI: −0.118, 0.001, which is around 2% of the mean testosterone concentration in the population). The third *low* confidence study reported no association. Among the *low* confidence studies, results were inconsistent. Xie et al. (2021) reported no association with PFNA exposure. Lewis et al. (2015) reported a positive association in 40–<60-year-old participants but no association in either younger (20–<40 year-old) or older (60- to 80-year-old) participants (Lewis et al., 2015). The third *low* confidence study (Specht et al., 2012) reported inconsistent results across regions of Greenland, Poland, and Ukraine (quantitative results not shown). In adolescent boys, all three studies were *low* confidence. One study (Zhou et al., 2016) observed an inverse association (β: −0.423 per 1 ng/mL change in PFNA, 95% CI: −0.700, −0.147) while the other two studies reported no association (Xie et al., 2021; Lewis et al., 2015). In children, a *low* confidence study (Lopez-Espinosa et al., 2016) reported an inverse association (β: −2.1, 95% CI: −5.5, 1.3) while two *low* confidence studies in infants reported positive associations ((Yao et al., 2019) β: 0.14, 95% CI: −0.05, 0.33; (Jensen et al., 2020b) β: 0.42, 95% CI: −0.06, 0.90).

Eight studies examined the potential for associations between PFNA exposure and estradiol in male subjects. Of five *medium* confidence studies, four reported reduced estradiol with elevated PFNA exposures, but only one was statistically significant. Joensen et al. (2013) found a change of approximately 1.7% of the mean estradiol concentration in the population in young men β: −0.075 per 1 ng/mL change in PFNA (95% CI: −0.013, −0.019). Also in young men, Petersen et al. (2022) reported a 7% decrease in estradiol (95% CI: −20, 7) per unit increase in PFNA, while in men in couples attending an infertility clinic, a 3% decrease was observed (95% CI: −8.45, 2.84) (Cui et al., 2020). Xie et al. (2021) reported no association with estradiol. In children Lopez-Espinosa et al. (2016) reported a 2% decrease for an IQR change in PFNA concentration (95% CI: −5.5, 1.3). Among *low* confidence studies Zhou et al. (2016) and Yao et al. (2019) reported nonstatistically significant higher estradiol with higher exposure. The remaining study by Specht et al. (2012)

reported inconsistent associations across three countries. For other reproductive hormones, one study reported an inverse association ($p < 0.05$) with SHBG (Cui et al., 2020), while three other studies found no association (Petersen et al., 2022; Joensen et al., 2013; Specht et al., 2012). None of the same four studies found an association with FSH or LH. No association with DHEA, DHEAS, androstenedione, or 17-OHP was observed in Jensen et al. (2020b).

Other male reproductive effects

Two *medium* confidence birth cohorts in Denmark (Ernst et al., 2019) and the United States (Carwile et al., 2021) examined associations of the timing of pubertal development with PFNA exposures. Tanner staging is used in clinical practice to assess pubertal progression and involves evaluation of the development of genitalia (scrotum appearance, testes, and penile size) and pubic hair in boys. In Ernst et al. (2019), there was generally a later age at each Tanner stage for genital and pubic hair development, although these differences were not statistically significant. Conversely, acne, voice break, and first ejaculation occurred earlier with higher exposure. Looking at an outcome that combined these endpoints into a single puberty indicator, there was later age of puberty with higher exposure, but this was nonmonotonic across the tertiles and not statistically significant (age difference in months [95% CI] for tertile 2 versus 1: 4.45 [−0.93, 10.11], tertile 3 versus 1: 1.63 [−3.82, 7.08]). In Carwile et al. (2021), there was greater pubertal development in early adolescence with higher PFNA exposure using a combined pubertal development score as well as a corresponding younger age at peak height velocity (a proxy measure for timing of puberty).

Animal Studies

Eight studies in rats and mice have evaluated the effects of PFNA exposures on the male reproductive system (Singh and Singh, 2019b, c, d; NTP, 2018; Hadrup et al., 2016; Das et al., 2015; Feng et al., 2010; Feng et al., 2009). Endpoints in these studies include evaluation of PFNA effects on sperm number and quality, reproductive organ weight and histopathology, and serum hormone concentrations, all of which are considered reliable indicators of male reproductive health (Creasy and Chapin, 2018; Creasy et al., 2012; Sellers et al., 2007; U.S. EPA, 1996). One study evaluated effects following subchronic 90-day exposures in prepubertal to adult mice (Singh and Singh, 2019b), and two studies examined effects following gestational exposures (Singh and Singh, 2019c; Das et al., 2015). The remaining bioassays were short-term exposures of 28 days or less in prepubertal animals (Singh and Singh, 2019d), postpubertal adolescents (Hadrup et al., 2016), and adults (NTP, 2018; Feng et al., 2010; Feng et al., 2009). Table 3-19 shows the overall evaluation results for the available studies.

Generally, subchronic studies are preferred to fully evaluate chemical effects on male reproductive tissue histopathology and associated sperm parameters because rat and mouse spermatogenesis, from spermatogonia to mature spermatozoa, takes approximately 52 and 35 days to complete, respectively (Creasy and Chapin, 2018). The available 90-day study in mice was

determined to be *low* confidence for these reproductive parameters, as well as for reproductive hormones, due to inadequate reporting of methods, tissue pooling, sample sizes, and results presentation (however, evaluation of reproductive organ weights was considered *medium* confidence) (Singh and Singh, 2019b). Additionally, a *medium* confidence developmental toxicity study in mice by Das et al. (2015) evaluated the effects of PFNA gestational exposures on some endpoints of reproductive system development (timing for attainment of preputial separation; this endpoint is also a developmental milestone; see Section 3.2.2, "Postnatal developmental landmarks"). A second *low* confidence developmental toxicity study by Singh and Singh (2019c) also evaluated PFNA effects on several neonatal (PND 3) testicular endpoints, including testis weight, histology, testosterone, and protein markers, after gestational exposure through the latter half of pregnancy (GD 12–20) (Singh and Singh, 2019c). While the exposure period from GD 12 to 20 was considered satisfactory for capturing the masculinization programming window of mice (Amato and Yao, 2021; Scott et al., 2009), there were several inadequacies in the animal allocation, reporting and attrition, and endpoint sensitivity domains that made the study overall *low* confidence (the maternal endpoints were considered *medium* confidence; see Section 3.2.5).

For the short-term testing by NTP (2018) and Singh and Singh (2019d), spermatogenesis related endpoints were judged to be overall *low* confidence due to potential insensitivity (a predicted bias toward the null) due to the short exposure duration. The NTP 28-day study also reported elevated mortality in adult male rats at higher PFNA treatments of 5 and 10 mg/kg-day (see Section 3.2.1, "Adult rodent mortality"). Thus, this synthesis considers PFNA treatments at 2.5 mg/kg-day to be the highest dose with reportable results for this study for all male reproductive outcomes except the histopathology where tissue analyses were performed at the time of early death (NTP, 2018).

Table 3-19. Overall evaluation results of male reproductive toxicity studies examining the effects of PFNA exposures in rodents[a]

Reference	Species, strain (lifestage)	Exposure design	Exposure route and dose range	Organ weight	Histopathology	Hormones/ sexual maturation	Sperm evaluation
Das et al. (2015)	Mouse, CD-1, pubertal	Developmental GD 1–17	Gestational 0, 1, 3, 5 mg/kg-d	NM	NM	+	NM
Feng et al. (2009)	Rat, Sprague-Dawley (adult)	Short-term 14 d	Gavage 0, 1, 3, 5 mg/kg-d[d]	NM	-	+	-
Feng et al. (2010)	Rat, Sprague-Dawley (adult)	Short-term 14 d	Gavage 0, 1, 3, 5 mg/kg-d	NM	-	+	NM
Hadrup et al. (2016)	Rat, Male Wistar-Hannover (postpubertal)	Short-term 14 d	Gavage, 0, 0.013, 0.25, 5 mg/kg-d	++	NM	+	NM
NTP (2018)[b]	Rat, Sprague-Dawley (Harlan) (adult)	Short-term 28 d	Gavage 0, 0.625, 1.25, 2.5, 5, 10 mg/kg-d	++	++	++	-
Singh and Singh (2019b)[c]	Mouse, Parkes (P) (prepubertal to adult)	Subchronic (90-d) PND 25–114	Gavage 0, 0.2, 0.5 mg/kg-d	+	-	-	- --
Singh and Singh (2019c)	Mouse, Parkes (P) (dams/offspring)	Developmental GD 12-parturition	Gestational 0, 2.0, 5.0 mg/kg-d	-	-	-	NM
Singh and Singh (2019d)	Mouse, Parkes (P) (prepubertal)	Short-term 14 d, PND 25–38	Gavage 0, 2, 5 mg/kg-d	+	-	+	NM

[a]Dark green (++) = *high* confidence; light green (+) = *medium* confidence; yellow (-) = *low* confidence; red (--) = *uninformative*; NM = not measured. Study evaluation details for all outcomes are available in HAWC.

[b]5 and 10 mg/kg-d dose groups not evaluated due to high mortality, except for the histopathology that was evaluated at time of death.

[c]For overall confidence, the sperm count, and viability evaluations were judged to be *low* confidence and the sperm motility results were *uninformative*.

Organ weight

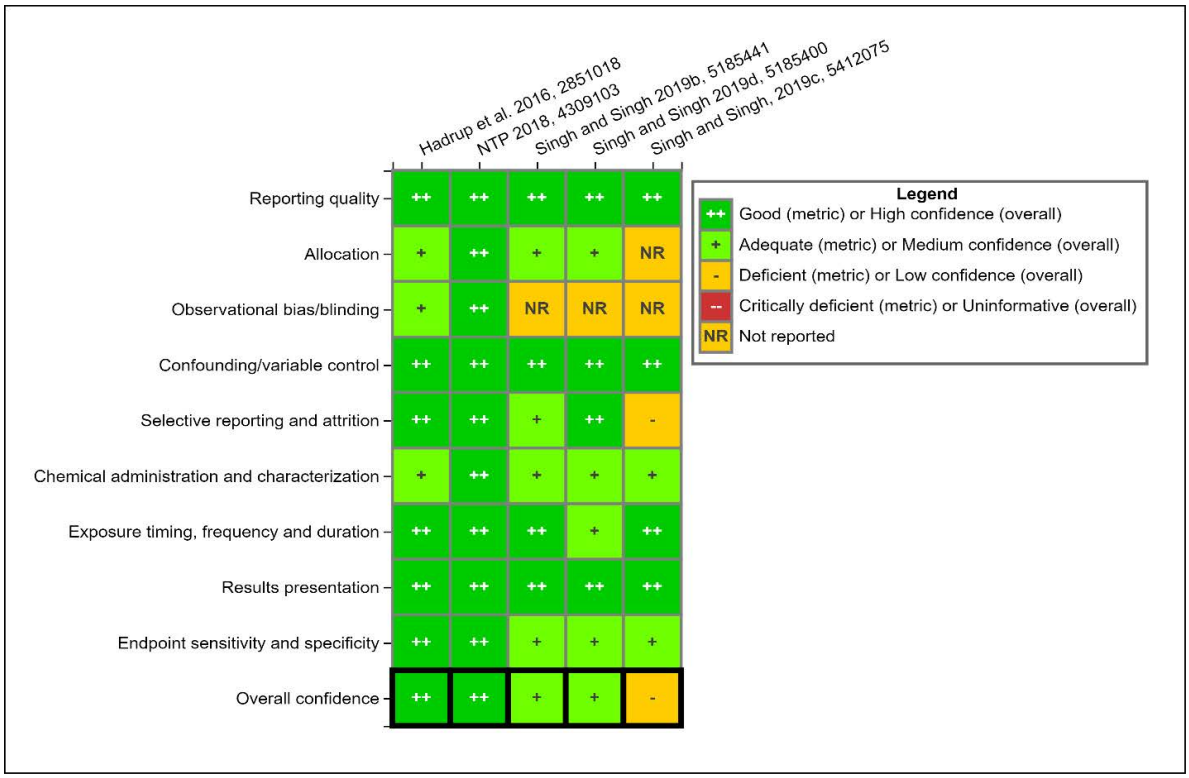


Figure 3-55. Summary of study evaluation of animal studies that examined PFNA effects on male reproductive organ weight. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Male-reproductive-organ-weights/.

PFNA effects on testicular and epididymal organ weights have been examined in adult SD and adolescent Wistar rats treated for 28 and 14 days, respectively (NTP, 2018; Hadrup et al., 2016), prepubertal mice exposed to PFNA for 14 and 90 days (Singh and Singh, 2019b, d), and neonatal mice exposed gestationally (Singh and Singh, 2019c). Most of the organ weight data were found to be *high* and *medium* confidence with minor limitations identified in the mouse studies (see Figure 3-55). The gestational exposure study in mice was determined to be *low* confidence due to lack of clear reporting on the sample design and sizes, and whether litter was accounted for in the sampling and analyses.

For epididymal and testicular weights, absolute values are preferred given the lack of proportionality of these endpoints to body weight. These organ weights are shown to be generally maintained in adult rodents with body weight reductions at upward of 30% (Creasy and Chapin, 2018; Bailey et al., 2004; Creasy, 2003), which also reduces concern for potential confounding due to body weight decreases that are less than severe. Thus, the reporting herein focuses principally on effects to absolute organ weights. NTP (2018) observed dose-dependent reductions in the absolute weight of the epididymis and cauda epididymis of adult SD rats after 28-day exposures.

Epididymal weight was reduced by 7%, 13%, and 35% at 0.625, 1.25, and 2.5 mg/kg-day, respectively, and cauda epididymis weight was decreased by 11% and 33% at 1.25 and 2.5 mg/kg-day PFNA, respectively (see Figure 3-56). In addition to declining epididymal weights, statistically significant trends of decreased absolute right and left testicular weights were reported. The absolute weight of the left testis was statistically significantly reduced by 7% in the 1.25 mg/kg-day dose group, and the absolute weights of the left and right testes were each reduced by statistically significant levels 20% at the 2.5 mg/kg-day dose. NTP (2018) and Hadrup et al. (2016) reported some increases in relative testis weight, but this effect was observed at doses where decreases in body weight were approaching or exceeding 30%. The severe body weight loss (44%) in males at 2.5 mg/kg-day in the NTP 28-day study introduces uncertainty. Although the extent of its impact is currently an unknown, these results are considered with caution when drawing evidence integration judgments.

Studies by Singh and Singh (2019b, 2019d); reported nonsignificant reductions in absolute testes weights of prepubertal male mice exposed to PFNA for 14 and 90 days at doses up to 5 mg/kg-day and 0.5 mg/kg-day, respectively, and with 14-day results of a similar magnitude as observed in adult rats (NTP, 2018). In the 14-day study, absolute testis weight was reduced by 14% and 20% relative to controls in prepubertal mice treated with PFNA at 2 and 5 mg/kg-day, respectively. In the 90-day study the absolute testis weight reduction was 1.3% and 7.9% at PFNA doses of 0.2 and 0.5 mg/kg-day. The *low* confidence gestational exposure study also reported a nonsignificant decrease in absolute testes weight of neonatal mice on PND 3, with a 20% and 24% reduction at 2 and 5 mg/kg-day respectively (Singh and Singh, 2019c).

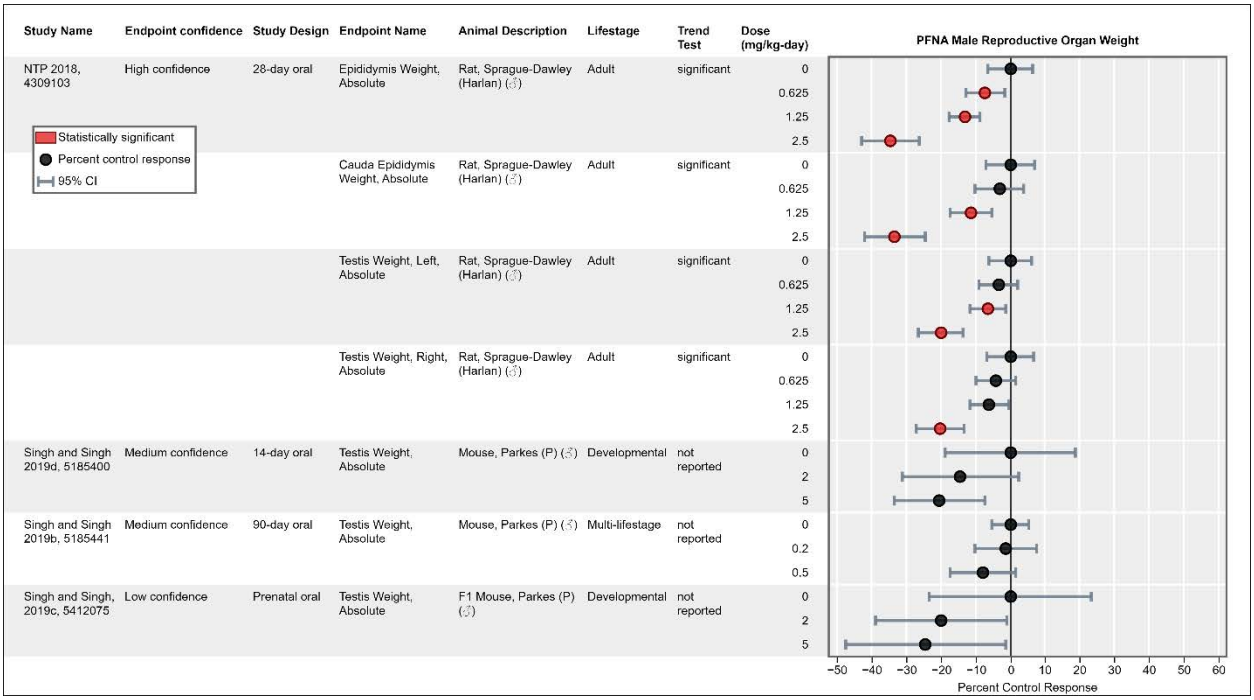


Figure 3-56. PFNA effects on epididymis and testis weights in *high/medium* confidence studies in adult rats exposed short-term (NTP, 2018) and prepubertal mice exposed for 14 and 90 days from PND 25 to 38 (Singh and Singh, 2019d) and PND 25–114 (Singh and Singh, 2019b), plus a *low* confidence gestational exposure study in neonatal mice (Singh and Singh, 2019c). See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-male-reproductive-organ-weight/.

Histopathology

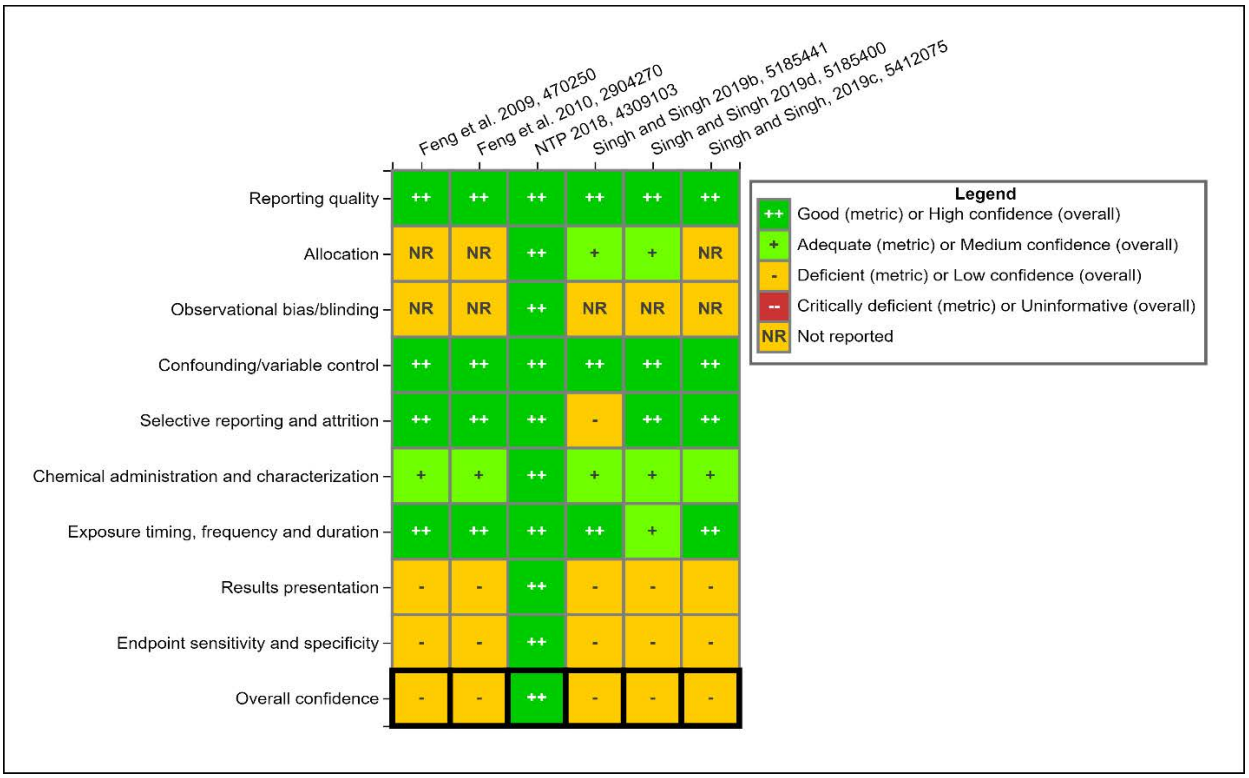


Figure 3-57. Summary of study evaluation of animal studies that examined PFNA effects on male reproductive histopathology. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Male-reproductive-histopathology/.

PFNA-induced histopathology was examined in six studies, including a 90-day exposure in prepubertal to adult mice (Singh and Singh, 2019b), and in short-term PFNA exposure studies in adult rats (NTP, 2018; Feng et al., 2010; Feng et al., 2009) and neonatal and prepubertal mice (Singh and Singh, 2019c, d). The histopathology results in the 28-day toxicity study by NTP (2018) were judged *high* confidence whereas the other studies were considered *low* confidence (see Figure 3-57). For histopathology, investigator blinding is generally not recommended in initial exams as masked evaluations can impede separating treatment-related changes from normal tissue variations that may potentially result in subtle lesions being overlooked (Crissman et al., 2004). NTP (2018) reported a second blinded evaluation of the PFNA histopathology results after an initial unblinded screening that mitigated concerns for potential observational bias. In addition to a lack of any reported blinding in the histological evaluations, the other studies had inadequacies in other domains, including a lack of sufficient reporting on methodologies, sampling design, and results.

Table 3-20 summarizes results of the NTP (2018) examination of reproductive tissue histopathology responses in adult rats treated with PFNA. Statistically significant results were generally observed at doses of 2.5 mg/kg-day or higher concurrent with severe body weight loss

(≥44%; see Section 3.2.1, "Adult rodent body weight"), which introduces uncertainty. Although the extent of its impact is currently an unknown, these results are considered with caution when drawing evidence integration judgments. Histopathological examinations were undertaken in animals from the 5 and 10 mg/kg-day dose groups that died early as a result of chemical-induced death (termed natural death as distinguished from moribund sacrifice), with observations taken twice daily for signs of overt toxicity. The routine checks for morbidity and mortality mitigated concerns for tissue degradation in nonsurviving animals.

According to the diagnostic criteria of Creasy et al. (2012), minimal to mild interstitial (Leydig) cell atrophy was reported in all animals starting at PFNA doses of 2.5 mg/kg-day, worsening to moderate/marked incidences with earlier onset in the 5 and 10 mg/kg-day dose groups. Additionally, significant incidences of mild to moderate germinal epithelium degeneration and spermatid retention in seminiferous tubules was noted at the 2.5 mg/kg-day treatment that also worsened to moderate/marked incidences with earlier onset at the 5 and 10 mg/kg-day dose levels. In the testes, histopathological observations were generally reported prior to study termination for ≥5 mg/kg-day dose groups. In the highest dose group, all animals showed germinal epithelium degeneration, spermatid retention in seminiferous tubules, and interstitial cell atrophy by day 15.

In the epididymis, mild to moderate hypospermia and moderate to marked ductal germ cell exfoliation were reported in animals exposed to PFNA at 2.5 mg/kg-day and 5 mg/kg-day, respectively. Mild to moderate epithelial germ cell exfoliation and apoptosis were also reported in animals treated with 5 mg/kg-day of PFNA. At 10 mg/kg-day doses, significant increases in incidences of mild granuloma sperm lesions were observed in the epididymis, as well as mild to moderate hypospermia and epithelial apoptosis and moderate to marked exfoliation of ductal epithelial germ cells. No lesions were observed in the examined accessory sex glands, including preputial gland, prostate, and seminal vesicle. The *low* confidence histopathological results were generally consistent with adverse effects observed in the *high* confidence NTP reporting with three 14-day studies (Singh and Singh, 2019d; Feng et al., 2010; Feng et al., 2009) and the 90-day study (Singh and Singh, 2019b) reporting degenerative changes in seminiferous tubules. However, the *low* confidence histological data from the short-term exposures in prepubertal animals by Singh and Singh (2019d) and Singh and Singh (2019c) were difficult to interpret due to the possibility that full reproductive maturity was not achieved by terminal sacrifice. A fifth *low* confidence study reported no changes in the seminiferous tubules of neonatal mice (Singh and Singh, 2019c).

Table 3-20. Incidence and severity of male reproductive histopathological observations of *high* confidence in the NTP (2018) 28-day oral toxicity study of PFNA[a]

Organ, effect	Dose (mg/kg-d)					
	0	0.625	1.25	2.5	5[b]	10[b]
Testes						
Interstitial (Leydig) cell atrophy	0 (0/10)	0 (0/10)	1.0 (1/10) t = 29	***1.7 (10/10)*** **t = 29**	***3.1 (9/9)*** **t = 24**	***3.5 (10/10)*** **t = 15**
Germinal epithelium degeneration	0 (0/10)	0 (0/10)	0 (0/10)	***2.2 (6/10)*** **t = 29**	***3.1 (9/9)*** **t = 24**	***3.7 (10/10)*** **t = 15**
Spermatid retention, seminiferous tubules	0 (0/10)	0 (0/10)	0 (0/10)	***2.8 (6/10)*** **t = 29**	***3.0 (9/9)*** **t = 24**	***3.0 (10/10)*** **t = 15**
Epididymis						
Hypospermia	0 (0/10)	0 (0/10)	0 (0/10)	2.5 (2/10) t = 29	***3.7 (9/9)*** **t = 24**	***2.9 (10/10)*** **t = 15**
Ductal germ cell exfoliation	0 (0/10)	0 (0/10)	0 (0/10)	***2.7 (6/10)*** **t = 29**	***3.7 (9/9)*** **t = 24**	***3.6 (10/10)*** **t = 15**
Epithelial germ cell exfoliation	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	2.0 (1/9) t = 25	0 (0/10)
Epithelial apoptosis	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	***2.0 (8/9)*** **t = 24**	***2.1 (10/10)*** **t = 15**
Granuloma sperm	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/9)	***2.0 (4/10)*** **t = 15**
Accessory sex glands						
Prostate	0 (0/10)	NE	NE	0 (0/10)	0 (0/10)	0 (0/10)
Seminal vesicle	0 (0/10)	NE	NE	0 (0/10)	0 (0/10)	0 (0/10)
Preputial gland	0 (0/10)	NE	NE	0 (0/10)	0 (0/10)	0 (0/10)

[a]Bold-italicized font indicates statistically significant changes compared with controls; NE = not examined; severity averaged to a four-point scale as follows: 0 = not detected; 1 = minimal severity; 2 = mild severity; 3 = moderate severity; 4 = marked severity. Values in parentheses indicate frequency out of total number of animals examined. t = timepoint (in days) of first incidence.

[b]Animals that did not survive until study termination reported as NATD (natural death).

Reproductive hormones and sexual maturation

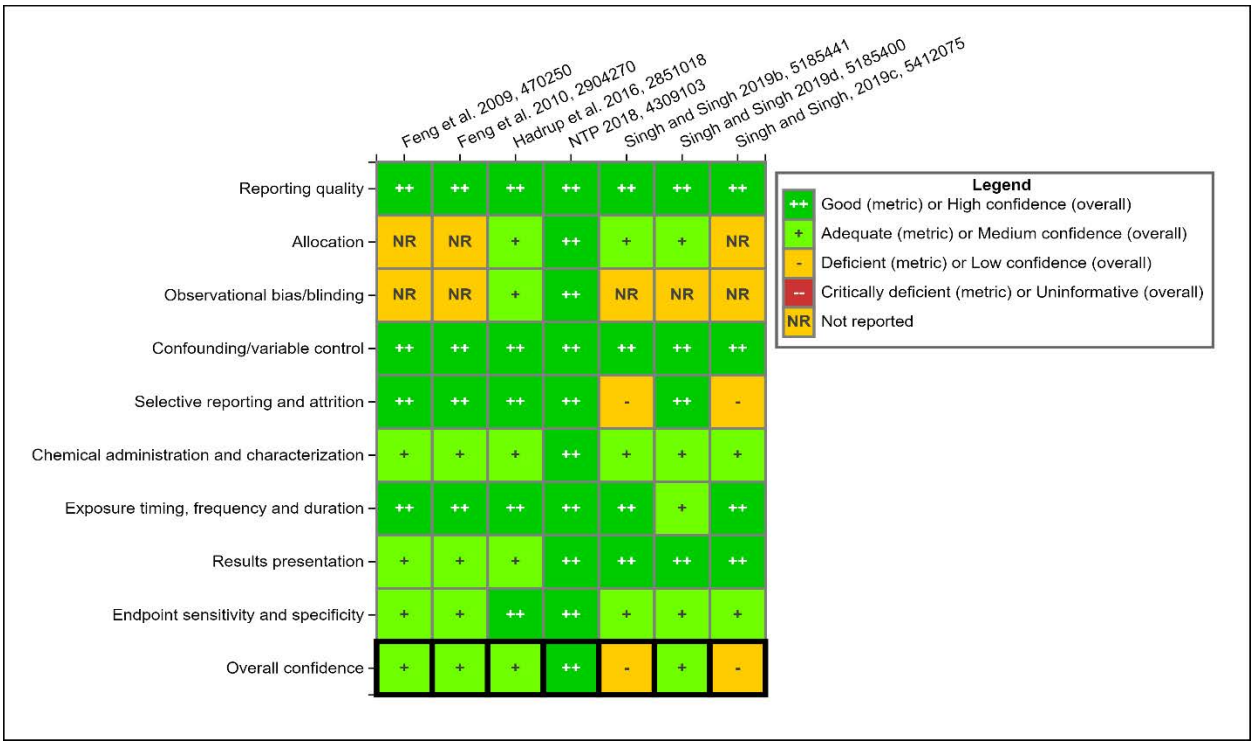


Figure 3-58. Summary of study evaluation of animal studies that examined PFNA effects on testosterone and other hormones. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Male-reproductive-hormones/.

PFNA effects on serum testosterone were examined in the *low* confidence 90-day study in prepubertal to adult mice (Singh and Singh, 2019b), and in five *medium* or *high* confidence short-term studies in adult rats (NTP, 2018; Hadrup et al., 2016; Feng et al., 2010; Feng et al., 2009) and prepubertal mice (Singh and Singh, 2019d), the latter of which also examined testosterone levels in testes (see Figure 3-58). Serum testosterone results in the 90-day study by Singh and Singh (2019b) were judged to be *low* confidence due to inadequate reporting on sample sizes and sampling design. Singh and Singh (2019c) also examined testicular testosterone in gestationally exposed mouse neonates (PND 3), but results were considered *low* confidence due to inadequate reporting on sampling design and whether litter was the statistical unit for the evaluations.

The short-term testing with PFNA was generally consistent across animal models and lifestage in showing statistically significant reductions in serum testosterone exceeding ~70% relative to controls at PFNA doses ≥2 mg/kg-day (see Figure 3-59), with many of the large and significant decreases observed at PFNA doses below those causing notable body weight decreases. Specifically, a 14-day study in prepubertal male mice reported dose-dependent reductions in serum testosterone at PFNA doses of 2 mg/kg-day (72% decrease) and 5 mg/kg-day (85% decrease), concurrent with reductions in testicular testosterone (a direct measure of testicular hormone

production) by 20% and 38%, respectively (Singh and Singh, 2019d). In adult male SD rats, the NTP 28-day study reported dose-dependent decreases in serum testosterone with statistically significant reductions of 81% at 2.5 mg/kg-day PFNA (NTP, 2018). In another study in adult male SD rats, Feng et al. (2009) also reported serum testosterone to be significantly reduced by 86% at 5 mg/kg-day PFNA after a 14-day exposure, with increases in serum testosterone and null effects at 1 mg/kg-day and 3 mg/kg-day, respectively, that are difficult to interpret due to the high variability of results. At lower PFNA doses of 0, 0.0125, and 0.25 mg/kg-day, levels of plasma testosterone were unaffected in adolescent male rats (Hadrup et al., 2016). Finally, the *low* confidence findings in the 90-day and gestational exposure studies were generally consistent with the *high* and *medium* confidence short-term testing although of a lesser magnitude (i.e., 32% at 0.5 mg/kg-day in the 90-day study and 42% at 5 mg/kg-day in gestationally exposed neonates) (Singh and Singh, 2019b, c).

Coherent with the reporting of reduced testosterone in prepubertal and neonatal mice, Das et al. (2015) also reported dose-dependent delays in reproductive system development (i.e., preputial separation) in mouse offspring exposed gestationally to PFNA (discussed in Section 3.2.2, "Postnatal developmental landmarks"). Preputial separation was delayed by 2 and 5 days at gestational doses of 3 and 5 mg/kg-day, respectively (Das et al., 2015). Puberty is controlled by the hypothalamic-pituitary-gonadal (HPG) axis, which stimulates the release of pituitary gonadotropins and then gonadal maturation and reproductive hormone production, notably testosterone (Creasy and Chapin, 2018; Sisk and Foster, 2004). Offspring hormone levels were not evaluated by Das et al. (2015), although the decreased testosterone reported in other rodent studies suggest a potential role in pubertal advancement.

Additionally, examination of potential PFNA effects on reproductive hormones other than testosterone were examined only in 14-day experiments in adult rats (see Figure 3-60) (Hadrup et al., 2016; Feng et al., 2010; Feng et al., 2009). Feng et al. (2010) reported statistically significant, dose-dependent reductions in serum inhibin B at all doses (9%, 11%, and 19% at 1, 3, and 5 mg/kg-day, respectively), as well as increases in müllerian inhibiting substance (MIS) at higher doses (Feng et al., 2010). MIS is produced in Sertoli cells and plays a critical role in fetal sexual differentiation, in addition to involvement in maintaining gonadal competence (mice over-expressing MIS have reduced testosterone and other phenotypic abnormalities (Trbovich et al., 2001)). This observation aligns with the reduced testosterone seen in other short-term testing with PFNA. Inhibin B is also produced by Sertoli cells in testicular seminiferous tubules and mediates FSH secretions by negative feedback to help mediate spermatogenesis (Buzzard et al., 2004). In vitro and in vivo studies suggest PFNA perturbs Sertoli cell functioning and spermatogenesis (discussed in the "Mechanistic Evidence and Supplemental Information," below). However, in vivo 14-day exposures to PFNA had no effect on serum FSH (or LH) in two studies in rats dosed up to 5 mg/kg-day PFNA (Hadrup et al., 2016; Feng et al., 2009). Serum levels of prolactin as well as other steroid hormones in the steroidogenesis pathway (androstenedione and dihydrotestosterone) were also unaffected by PFNA at lower doses up to 0.25 mg/kg-day (Hadrup et al., 2016). Serum estradiol

(E2) was shown to be significantly elevated in male rats exposed to 5 mg/kg-day, which corresponded with the decrease in serum testosterone observed at this dose in the same study (Feng et al., 2009). However, the relevance of increasing E2 in this single study is hard to discern given the limited evidence.

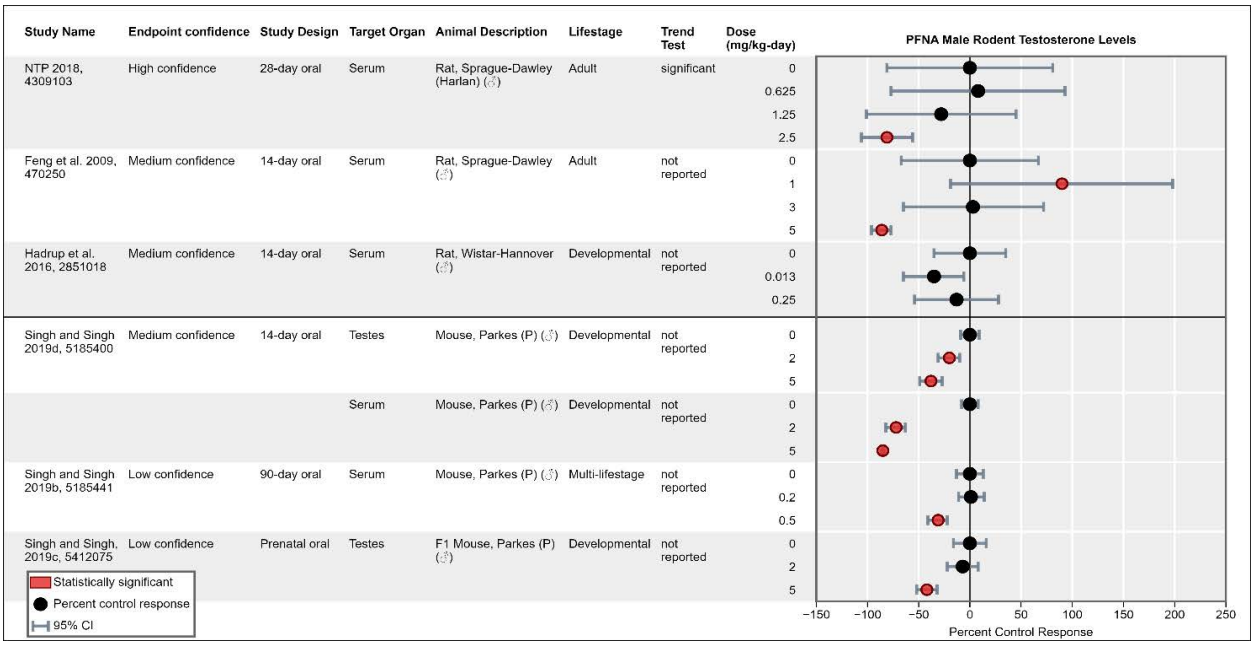


Figure 3-59. PFNA effects on serum and testes levels of testosterone in high and medium confidence short-term toxicity studies in adult male rats (top) and medium and low confidence studies in mice exposed gestationally (GD 12–21), prepubertally (PND 25–38), and to adulthood (PND 25–114) (bottom). See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/PFNA-Male-Rodent-Testosterone-Levels-All-Data-2/.

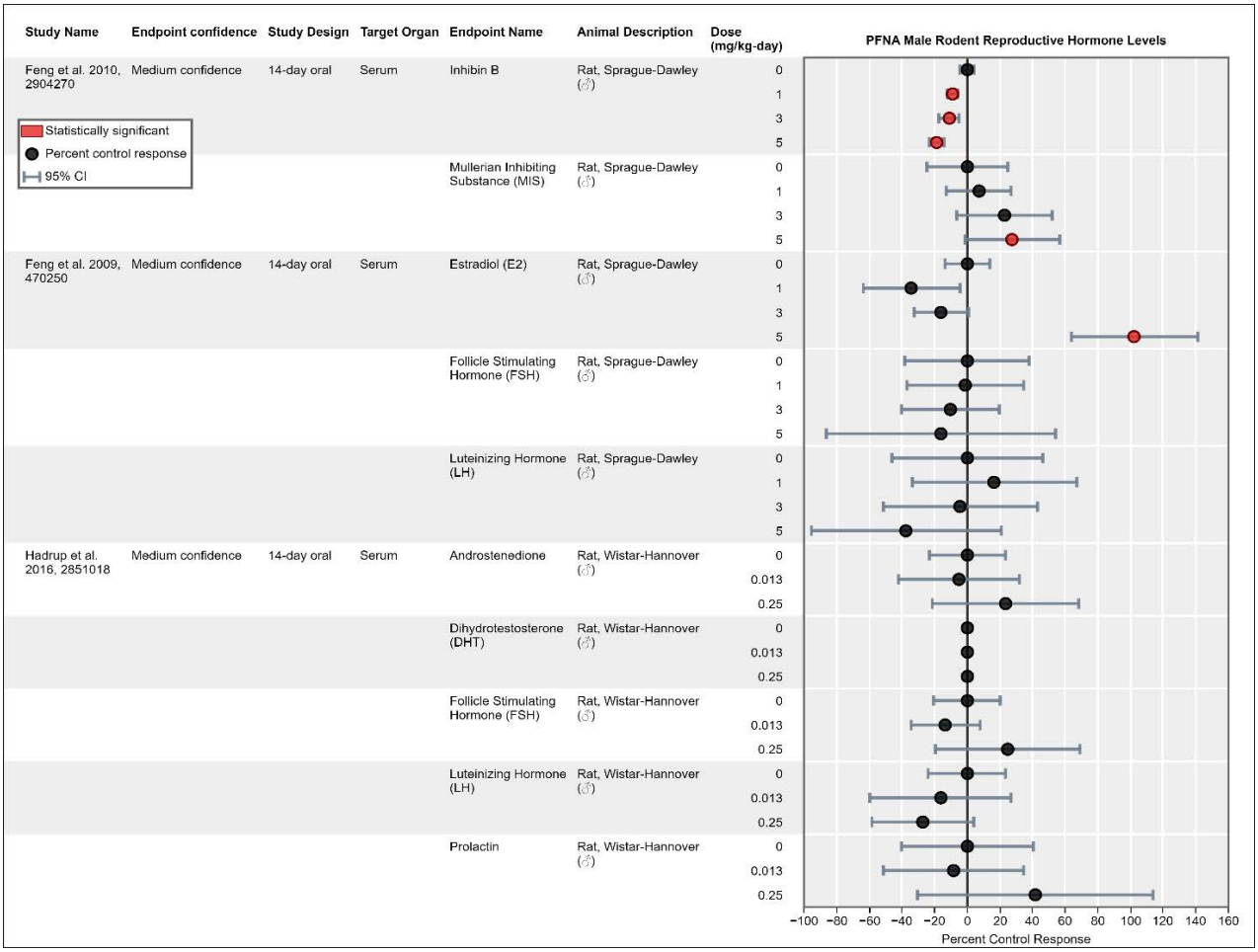


Figure 3-60. PFNA effects on serum levels of non-testosterone reproductive hormones in *high* and *medium* confidence short-term toxicity studies in adult male rats. See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/pfna-male-rodent-other-reproductive-hormones/.

Sperm evaluation

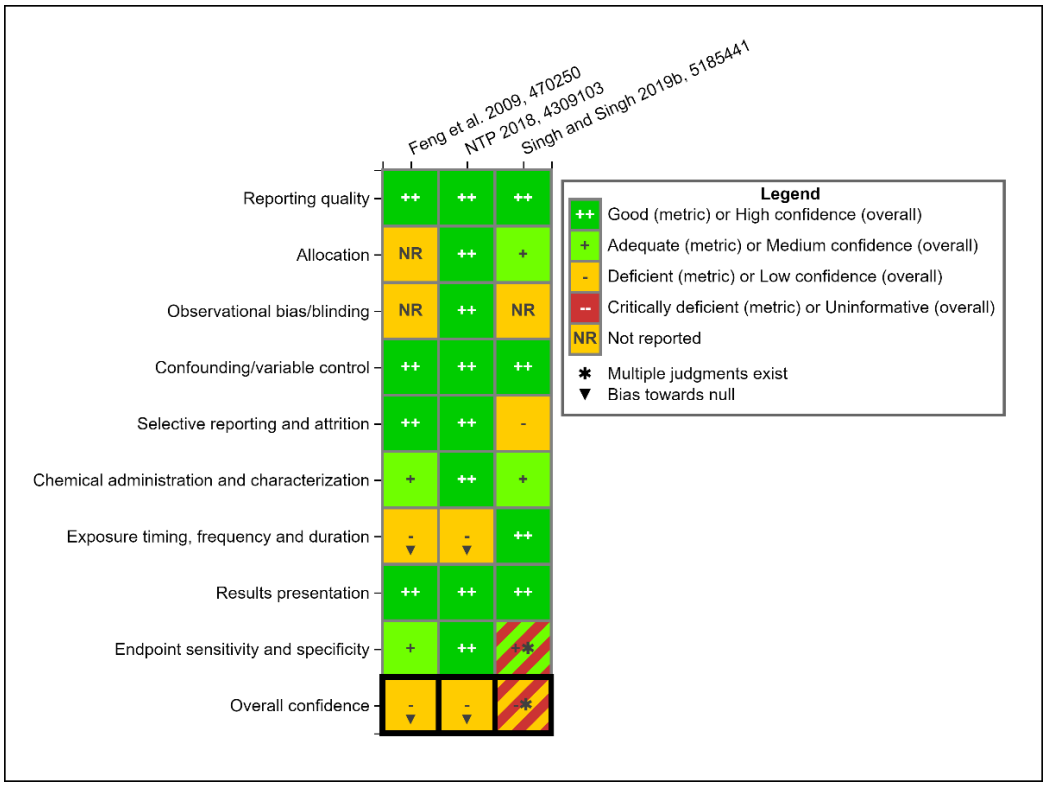


Figure 3-61. Summary of study evaluation of animal studies that examined PFNA effects on reproductive sperm quality.[a] See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Sperm-evaluations/.

[a]In Singh and Singh (2019b), for the endpoint sensitivity domain, the sperm counts, and viability data were considered adequate, and the sperm motility methods were found critically deficient; For the overall confidence, the sperm count and viability evaluations were judged to be *low* confidence and the sperm motility results were *uninformative*. For NTP (2018) and Feng et al. (2009), sperm results were *low* confidence due to potential exposure insensitivity and an expected bias to the null as the exposure duration of 28 days did not encompass the full period of spermatogenesis (~56 days in rats).

PFNA effects on sperm quality were examined in the 90-day study that continuously treated mice with PFNA beginning before puberty, and 28- and 14-day studies in adult rats (Singh and Singh, 2019b; NTP, 2018; Feng et al., 2009). As discussed earlier, the sperm count and viability endpoints in the 90-day study were judged *low* confidence due to inadequacies in reporting on sample sizes and sampling design (see Figure 3-61). Sperm motility results in this study were *uninformative* as there was neither description of evaluation methods nor reporting on experimenter blinding; these results are not discussed further. Additionally, the 28-day and 14-day studies in adult rats were limited by the exposure duration not covering the full period of rat spermatogenesis (~52 days), therefore presenting a potential for bias toward the null. This potential insensitivity was the only study limitation identified for NTP (2018) and Feng et al.

(2009), which would otherwise have been considered *high/medium* confidence studies for these endpoints.

Even with the shorter exposure duration, NTP (2018) reported dose-dependent reductions in absolute caudal epididymal sperm counts. Absolute sperm counts were statistically significantly decreased by 18% at 1.25 mg/kg-day and 31% at 2.5 mg/kg-day PFNA (see Figure 3-62). Relative sperm counts per mg cauda epididymis were not significantly changed. However, absolute values are preferred in this instance given that sperm contribute to epididymal weight, which was reduced in this study, and so expressing the data as a ratio could mask declines in sperm number (U.S. EPA, 1996). Additionally, statistically significant declines in absolute testicular sperm were reported by NTP (2018) in rats exposed to PFNA at 0.625 mg/kg-day but with no dose dependency or significant changes at higher doses. No effects of PFNA on sperm motility were reported in the NTP 28-day study.

Consistent with the NTP study, the *low* confidence 90-day results in prepubertal/adult mice also reported statistically significant reductions in sperm counts and sperm viability in the cauda epididymis at 0.5 mg/kg-day PFNA (Singh and Singh, 2019b). Finally, the *low* confidence (with a predicted null bias), 14-day exposure study in SD rats by Feng et al. (2009) also observed significant dose-dependent increases in the percentage of apoptotic spermatogenic cells, with large increases in apoptosis of over 400% and 500% reported at 3 and 5 mg/kg-day PFNA.

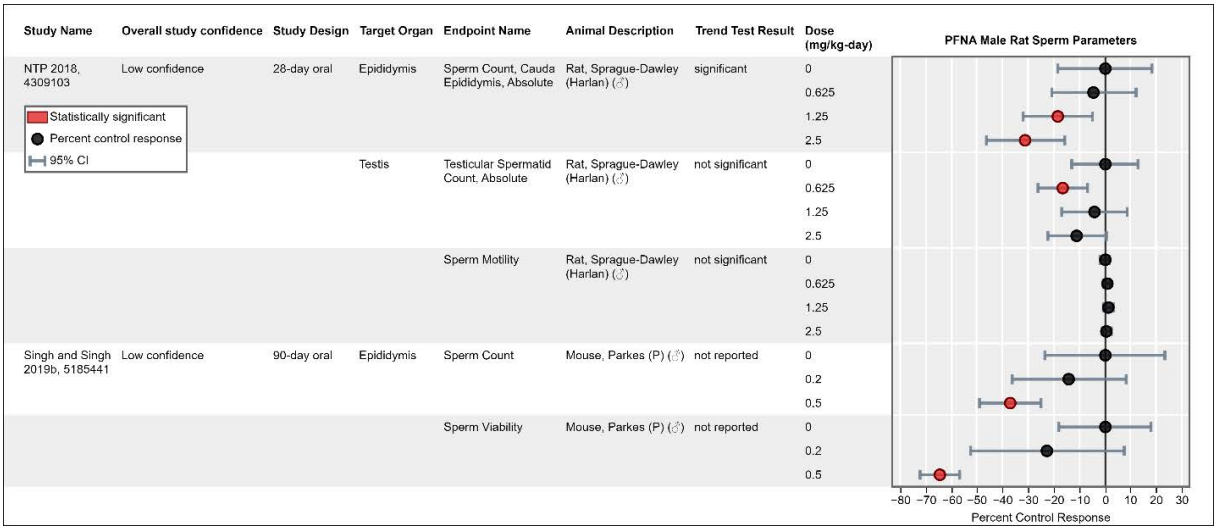


Figure 3-62. PFNA effects on sperm counts and motility in the testis and cauda epididymis in *low* confidence studies in adult rats and prepubertal mice.[a] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-male-rat-sperm-parameters-all/.

[a]NTP (2018) was judged to be *low* confidence due to inadequate exposure durations and uncertainties over potential insensitivity (i.e., biased toward the null). Singh and Singh (2019b) was judged to be *low* confidence due to methodological deficiencies.

Mechanistic Evidence and Supplemental Information

In vivo and in vitro mechanistic data suggest that PFNA may affect spermatogenesis by perturbing Sertoli cell functioning and inducing pathways of spermatogenic cell apoptosis. In addition to reduced serum inhibin B in PFNA-exposed adult male rats (see Figure 3-60), Feng et al. (2010) reported that rat primary Sertoli cell cultures treated with noncytotoxic concentrations of PFNA for 24 hours showed significantly altered regulation of genes encoding Sertoli-specific secreted molecules that support germ cell development and spermatogenesis (i.e., inhibin B, MIS, WT1, ABP, transferrin, FSH-R). Furthermore, oral exposure to PFNA for 14 days in prepubertal mice resulted in decreased testicular glucose, lactate, and lactate dehydrogenase activity (Singh and Singh, 2019a). Glucose is metabolized by Sertoli cells to pyruvate and then lactate by LDH, which is the preferred germ cell energy substrate (Mateus et al., 2018). Thus, while the underlying molecular basis for the germ cell changes has yet to be elucidated, it is possible that functional damage to Sertoli cells, decreased glucose metabolism, and suppression of inhibin B may play a contributing role in the reported spermatogenic impairments. Additional supporting evidence of spermatogenesis inhibition included a 14-day study in prepubertal mice exposed from PND 25 to 38 that reported decreases in the relative percentages of dividing germ cells in prophase meiosis at 5 mg/kg-day and increases in resting cells in G1 at 2 and 5 mg/kg-day (Singh and Singh, 2019a).

Other in vivo mechanistic data suggest interference with male reproductive hormone synthesis in Leydig cells. Quantitative immunohistochemistry reported a decrease in testicular 3β-hydroxysteroid dehydrogenase (Singh and Singh, 2019c), which plays an important role in the production of steroid hormones including progesterone, androstenedione, and testosterone. 3β-HSD was significantly decreased by 29% in PND 3 neonates after dams were exposed to 5 mg/kg-day from GD 12 through parturition. There was also some in vivo evidence of dose-dependent upregulation of spermatogenic cell apoptosis signaling in testis of adult rats treated with PFNA for 14 days at 3 and 5 mg/kg-day (Feng et al., 2009). In addition, testicular oxidative stress was observed in mice exposed to PFNA for 14 and 90 days to 5 and 0.5 mg/kg-day, respectively (Singh and Singh, 2019b, d). Specifically, exposure to PFNA in prepubertal mice increased lipid peroxidation activity and decreased activity of superoxide dismutase (SOD), catalase, and glutathione-*S*-transferases (GSTs) involved in antioxidant activities in the testis. Increases in oxidative stress could potentially contribute to the induction of spermatogenic apoptosis, such as that observed by Feng et al. (2009) in adult rats. Furthermore, in mouse Leydig tumor cells, which partially maintain the properties or original Leydig cells, inhibited progesterone production, which may disrupt Ca^{2+} pathway signaling and steroidogenesis, and decreased mitochondrial membrane potential (Zhao et al., 2017a).

In vitro HTS assays under the ToxCast program have been used to screen PFNA for interactions with the androgen receptor (AR) and estrogen receptor (ER), as well as with the steroidogenesis pathway (aromatase activity; see Appendix C.2 for active hits and a link to null assays). These assays showed PFNA binds both the human and rodent AR and is capable of weak AR

antagonism in a human cell proliferation assay. Reporter-based assays in human cell cultures also showed significant dose-dependent antagonistic effects of PFNA on the AR (Kjeldsen and Bonefeld-Jørgensen, 2013). However, PFNA was estimated to have no activity as either an AR agonist or antagonist in computational modeling that integrated multiple ToxCast assays targeting the AR pathway, including those positive assays summarized here (Kleinstreuer et al., 2017). Additionally, no agonist or antagonist activity was detected in AR transfectant cells in transactivation or translocation assays, with the exception of a non-dose-dependent increase in nuclear translocation in the presence of testosterone compared with testosterone treatment alone (McComb et al., 2019). ToxCast also reported ER activation and inactivation. However, computational modeling integrating these and other ToxCast screening assays in the ER pathway was inconclusive for PFNA-induced ER agonism and antagonism (Browne et al., 2015; Huang et al., 2014). PFNA was also reported to be inactive in another large-scale ER predictive model under the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) that combined multiple computational models of high-throughput data (Mansouri et al., 2016). However, results from other published ER gene reporter assays were inconsistent with Benninghoff et al. (2011) reporting statistically significant upregulation in ER transactivation in the HEK-293T cell line, and Kjeldsen and Bonefeld-Jørgensen (2013) reporting no effects in MCF-7-derived breast cancer cell cultures. Thus, the available in vitro data on potential effects of PFNA exposure on AR and ER pathway function is mixed.

Although the potential effects of PFNA on the steroidogenesis pathway have not been thoroughly evaluated, 14-day oral exposures to 5 mg/kg-day PFNA in adolescent rats led to downregulated expression of several genes in testis and liver of male rats that encode steroidogenic transporters and enzymes, including steroidogenic acute regulatory (StAR) protein, CYP11a, CYP17, and 17β-hydroxysteroid dehydrogenase (Hadrup et al., 2016). Results of in vitro testing varied with weak inhibition of aromatase activity reported in a human placental choriocarcinoma cell line (JEG-3) at high but noncytotoxic doses of PFNA (Gorrochategui et al., 2014). In contrast, other in vitro studies of PFNA effects on aromatase activity were null (Kjeldsen and Bonefeld-Jørgensen, 2013; Kraugerud et al., 2011). Finally, PFNA was inactive in ToxCast bioactivity screening of aromatase inhibition (see Appendix C.2 for active hits and a link to null assays). Overall, while some data suggest the potential for weak inhibitory effects of PFNA on androgen production and activity, the current mechanistic support for this hypothesis is neither consistent nor strong.

Evidence Integration

In the epidemiological evidence base, the evidence of male reproductive toxicity is considered *indeterminate.* There is some suggestion of an inverse association between PFNA exposure and testosterone in the only *medium* confidence study that examined this outcome, but significant uncertainties remain due to a lack of precision in the observation, the small magnitude of the change, and inconsistent findings in the two *low* confidence studies. Although estradiol was similarly slightly decreased in this *medium* confidence study of adults as well as in another *medium* confidence study of children, a third *medium* confidence study of adults and the low confidence

studies showed generally inconsistent (including positive) associations. The epidemiological evidence is also inconsistent for related outcomes including other reproductive hormones, sperm parameters, and timing of pubertal development. The available epidemiological evidence is limited due to the small number of studies per outcome.

Results of animal testing with PFNA provide *moderate* evidence of reproductive toxicity based on generally consistent, dose-dependent, and coherent pattern of perturbations to the male reproductive system of adult and prepubertal rodents following short-term and developmental exposures. PFNA exposure caused decreased reproductive organ weights and serum and testicular testosterone as well as impaired spermatogenesis, and with more uncertain evidence for corresponding structural changes to reproductive tissues. Coherent with reduced testosterone in prepubertal mice, a dose-dependent delay in preputial separation was also observed in male mouse offspring exposed gestationally to PFNA. There is some mechanistic evidence supporting biological plausibility, primarily related to findings related to disrupted spermatogenesis.

Dose-dependent reductions in absolute testis and epididymis weights were observed in multiple *high/medium* confidence studies of adult rats exposed for 28 days and prepubertal and adult mice exposed for 14 or 90 days. While findings in adult rats were observed at PFNA doses generally at ≥1.25 mg/kg-day (NTP, 2018), and small (8%) nonsignificant decreases could be observed in adult mice at PFNA doses as low as 0.5 mg/kg-day after 90 days, decreases of >10% were generally observed at approximately ≥2 mg/kg-day PFNA. Declines in organ weights are coherent with reductions in serum and tissue levels of testosterone in multiple rodent strains and lifestages, and of a notable magnitude (all >70%) with most findings occurring in the absence of overt toxicity. Testicular testosterone, which is a direct measure of testosterone production, was also dose dependently reduced in peripubertal mice exposed to PFNA for 14 days (decreases of 20 and 38% at 2 and 5 mg/kg-day, respectively). In adult rats, the consistent and coherent effects on androgens and androgen-dependent organs may be related to the modest (<40%) but dose-dependent decreases in cauda epididymal sperm counts at ≥1.25 mg/kg-day PFNA (NTP, 2018), as well as the large dose-dependent increase in sperm cell apoptosis (>400%) (Feng et al., 2009), that could be biologically relevant to spermatogenesis and fertility outcomes in humans (Creasy and Chapin, 2018). These evaluations detected effects despite being considered *low* confidence due to the potential insensitivity of the short exposure duration. Decreases in serum testosterone and sperm counts, as well as degenerative changes in seminiferous tubules, were also noted in prepubertal/adult mice exposed to 0.5 mg/kg-day PFNA for 90 days, however results were interpreted with *low* confidence due to sampling design deficiencies (Singh and Singh, 2019b).

The NTP 28-day study included *high* confidence histopathology findings, including germinal epithelium degeneration, spermatid retention in seminiferous tubules, and Leydig cell atrophy in the testes, and ductal germ cell exfoliation in the epididymis that may help to explain the observed organ-level alterations. Germ cell degeneration, depletion, and disorganization are sensitive markers of testicular injury, and sloughed germ cells in the epididymal lumen are indicators of

spermatogenic perturbations (Creasy and Chapin, 2018; OECD, 2008). Additionally, spermatid retention is an early and sensitive indicator of low intratesticular testosterone (OECD, 2008). However, all the histological changes in the NTP 28-day study were observed at doses causing severe body weight loss (≥40%); while the impact of this effect on histopathology cannot be estimated, this introduces uncertainty as to whether these changes would be expected to occur in the absence of overt toxicity. *Low* confidence histopathology results from other studies, although generally consistent with results from the 28-day study, were considered less reliable and are not discussed. Although the mechanistic evidence base is small, studies suggest that functional damage of Sertoli cells may play a role in the observed male reproductive toxicity. There is a sensitive window of Sertoli cell proliferation during fetal and early postnatal periods of testis development (Sharpe et al., 1999; Orth, 1982; Steinberger and Steinberger, 1971), and so the available mechanistic evidence of PFNA-induced Sertoli cell perturbations may be particularly relevant during these early lifestages. It is also notable that Leydig cells, which underwent some PFNA-induced atrophy (NTP, 2018) and are key sites of testosterone synthesis, may also play a contributing role, but this evidence is uncertain given the co-occurring overt toxicity, although some in vitro data shows potential for steroidogenesis disruption. Reduced testosterone levels can contribute to degenerative changes in other male reproductive tissues, epididymal hypospermia, and spermatid retention in the seminiferous tubules, and can also result from Sertoli cell toxicity (Creasy, 2001).

While the animal and mechanistic findings indicate a coherent pattern of PFNA-induced effects on the male reproductive system, the evidence is limited by a general lack of adequate testing encompassing the entire period of spermatogenesis with a bias predicted toward the null. It is possible that the magnitude and severity of effects on spermatogenesis and other male reproductive endpoints may increase with longer exposure or at lower doses (the only study of longer duration and lower doses is the *low* confidence 90-day study in prepubertal/adult mice). Another uncertainty in interpreting some of the findings of NTP (2018) is the large body weight reductions observed in male rats at 2.5 mg/kg-day PFNA. This might suggest an influence of nonspecific toxicity on the reproductive effects observed at this dose. However, absolute testis and epididymis weights, as well as cauda epididymis sperm counts, were significantly reduced at lower dose levels where body weight reductions did not occur (0.625 mg/kg-day) or were modest (17% at 1.25 mg/kg-day), alleviating some concerns for indirect effects.

Taken together, the currently available ***evidence indicates*** that PFNA likely causes male reproductive toxicity in humans given sufficient exposure conditions (see Table 3-21).[13] This conclusion is based primarily on a *high* confidence 28-day oral toxicity study in adult rats that reported a consistent and coherent pattern of adverse reproductive effects, generally at ≥1.25 mg/kg-day PFNA but with some coherent changes at 0.625 mg/kg-day, with additional

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

support from *medium* confidence, short-term studies in adult rats and prepubertal mice observing effects at similar doses (≥2 mg/kg-day). The lack of association in most of the epidemiological studies does not decrease confidence in the animal results given the uncertainties in the epidemiological evidence base.

Table 3-21. Evidence profile table for male reproductive effects

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
Evidence from studies of exposed humans (**see Section 3.2.4. Male Reproductive Effects, Human Studies**)					⊕⊕⊙ ***Evidence indicates (likely)***
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
Semen parameters Four *medium* and 1 *low* confidence studies	• Nonsignificant decreases in sperm motility in 2 *medium* confidence studies, normal morphology in 1 study, and concentration in 1 study	• No factors noted	• Inconsistent results across studies • Imprecision	⊙⊙⊙ *Indeterminate* Some studies indicate inverse associations with testosterone or semen parameters but this is uncertain due to notable inconsistency and imprecision	*Primary basis:* Studies in rats and mice at different lifestages, ranging from short-term to subchronic exposures, generally at doses ≥1.25 mg/kg-d *Human relevance:* Effects in rats and mice are considered potentially relevant to humans based on the conserved role of androgen-dependent pathways in male reproductive functions across species *Cross-stream coherence:*
Male reproductive hormones Five *medium* and 5 *low* confidence studies (3 *medium* and 7 *low* for testosterone)	• Inverse associations with testosterone were observed in 2/3 medium confidence studies for testosterone and 4/5 studies for estradiol (only one study was statistically significant for each hormone). *Low* confidence studies were inconsistent.	• No factors noted	• Imprecision • Unexplained inconsistency within and across age groups • Unclear biological significance of small change in hormone levels		
Other effects Four *medium* confidence studies	• Studies of pubertal development and anogenital distance reported non-coherent results across endpoints	• No factors noted	• Lack of expected coherence across endpoints within studies • Imprecision		

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
Evidence from in vivo animal studies (**see Section 3.2.4. Male Reproductive Effects, Animal Studies**)					Studies in humans were *indeterminate* and neither increase nor decrease confidence in the animal findings. *Susceptible populations and lifestages:* Delays in reproductive system development (i.e., preputial separation) in mouse offspring are observed and prepubertal exposure may affect reproductive hormones and sperm health, although the latter findings are *low* confidence. This suggests the possibility that the developing male reproductive system may be susceptible to PFNA-induced injury.
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Organ weight</u> Two *high* confidence studies • 28 d (adult rats) • 14 d (adolescent rats) Two *medium* confidence studies: • 90 d (prepubertal to adult mice) • 14 d (prepubertal mice) One *low* confidence study in gestationally exposed mice: • GD 12–20 (neonatal mice)	• *Dose-dependent* decreases in absolute epididymal and testicular weights of adult rats exposed short-term generally at ≥1.25 mg/kg-d • Decreases in absolute testicular weights (not statistically significant) of peripubertal to adult mice exposed for 90 and 14 d at up to 0.5 mg/kg-d and 5 mg/kg-d, respectively	• *Consistent, dose-dependent* decreases in absolute testes and epididymal weights in rats and mice • *Coherence* with reduced sperm counts in adult rats, reduced serum and testicular testosterone in rats and mice, and more uncertainly histopathology • *Magnitude of effect*, decreases up to 35% (epididymis) and 20% (testis)	• *Imprecision* of findings in mice	⊕⊕⊙ *Moderate* Consistent, dose-dependent, and coherent effects on decreased organ weights and testosterone, impaired spermatogenesis, and corresponding structural changes to reproductive tissues in adult rats and prepubertal mice.	
<u>Histopathology</u> One *high* confidence study in adult rats • 28 d Five *low* confidence studies • 90 d (prepubertal to adult mice) • 14 d (×3) (1 in prepubertal mice and 2 in adult rats) • GD 12–20 (neonatal mice)	• *Dose-dependent* histopathological responses of the testes and epididymis of adult rats exposed short-term at ≥2.5 mg/kg-d, doses causing severe body weight decreases	• *Dose-dependent* changes in epididymal and testicular tissues with atrophy, degeneration, and sperm loss that increased in incidence, severity, and time to first appearance with dose • *Coherence* with reduced organ weights, sperm counts, and testosterone	• Potential confounding due to overt toxicity for all findings		

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
<u>Reproductive hormones and developmental markers</u> One *high* confidence study in adult rats • 28 d Four *medium* confidence studies • 14 d (2 in adult rats, 1 in juvenile rats, and 1 in prepubertal mice) Two *low* confidence studies • 90 d (prepubertal to adult mice) • GD 12–20 (neonatal mice)	• Reductions in serum testosterone in adult rats exposed short-term at ≥2.5 mg/kg-d • Reductions in serum and testicular testosterone in prepubertal mice exposed subchronically at ≥2 mg/kg-d • Reductions in serum inhibin B and increases in müllerian inhibiting substance (MIS) in adult rats exposed short-term at ≥1 mg/kg-d • Delays in preputial separation in mice at ≥3 mg/kg-d	• *Consistent* reductions in serum testosterone in rats and mice, including subchronic and gestational exposures in mice • *Dose-dependent* reductions in serum testosterone in short-term exposed rats and mice • *Coherent, dose-dependent* reductions in serum and testicular testosterone in short-term exposed mice • *Dose-dependent* delays in androgen-dependent pubertal onset in mice • *Coherence* with decreased organ weights and sperm counts, and histopathology • *Magnitude of effect*, up to 85% reduction in serum testosterone (adult and prepubertal lifestages), • *Magnitude of effect,* 2- to 5-d delay in preputial separation	• Subchronic study in mice is *low* confidence • Potential confounding due to overt toxicity for findings in rats		
<u>Sperm evaluation</u> Two *low* confidence studies in adult rats • 28 d • 14 d One *low* confidence/ *uninformative* study in prepubertal to adult mice	• Reductions in sperm counts of cauda epididymis of adult rats exposed short-term at ≥1.25 mg/kg-d and in prepubertal/adult mice exposed subchronically at 0.5 mg/kg-d	• *Dose-dependent* reductions in epididymal sperm counts in rats and mice, and increases in testicular sperm cell apoptosis in rats • *Coherence* with decreased organ weights and testosterone, and more uncertainly histopathology	• Study in mice is *low* confidence, as is the 14-d rat study (the only limitation in the 28-d rat study introduced a bias toward the null as exposure duration did not cover full spermatogenesis period)		

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
• 90 d		• *Magnitude of effect*, up to 547% increase in sperm cell apoptosis • *Biological plausibility* supported by mechanistic evidence of perturbed Sertoli cell function			
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Summary of key findings and interpretation**			**Evidence stream judgment**	
Molecular events – AR and ER interactions	Toxcast screening assays examining PFNA interactions with the AR and ER were equivocal, but computational modeling of multiple AR and ER suggests that interactions are unlikely.			Evidence of perturbations in Sertoli and Leydig cell functioning and corresponding increases in markers of sperm injury provide biological plausibility for the in vivo animal evidence.	
Cellular level effects – Sertoli and Leydig cell functioning	Perturbed Sertoli cell functioning as indicated by significantly altered mRNA transcripts and proteins secreted by Sertoli cells that are involved in sperm cell development and spermatogenesis. Decreased glucose, lactate, and LDH activity, which are important germ cell energy supplies from Sertoli cells. Decreased 3β-HSD, which catalyzes the synthesis of progesterone, androstenedione, and testosterone in Leydig cells.				
Cellular level effects – Spermatogenic cell apoptosis	One short-term study in adult rats showing dose-dependent upregulation of testicular sperm cell apoptosis pathways. One short-term and one subchronic study showing induced markers of oxidative stress in testes that may contribute to sperm apoptosis.				
Cellular level effects – Steroidogenesis	One short-term study in adult rats showing significant downregulation of some genes encoding steroidogenic transporters and enzymes (StAR, CYP11a, CYP17, 17β-HSD) in testis and liver of male rats exposed at 5 mg/kg-d. In vitro aromatase activity was largely null.				

1

3.2.5. Female Reproductive Effects

This section describes and integrates the evidence informative to the assessment of potential female reproductive health effects following exposure to PFNA. There is some overlap in this section with the evidence synthesis and integration summary of developmental effects as some PFNA studies examined effects on female reproductive system development and maternal health and pregnancy outcomes (see Section 3.2.2).

Human Studies

Epidemiology studies on possible female reproductive effects of PFNA exposure are available for fecundity (characterized using time to pregnancy [TTP]), reproductive hormones, pubertal development, gynecological conditions (endometriosis and polycystic ovary syndrome [PCOS]), ovarian reserve (including primary ovarian insufficiency [POI]), and menstrual cycle characteristics. In total, 26 epidemiology studies are available for these outcomes. The study evaluations are summarized below for each outcome or group of outcomes. In addition, preterm birth and spontaneous abortion could be driven by either female reproductive or developmental toxicity. These outcomes are reviewed in the developmental section of this assessment (see Section 3.2.2) but are also included in the consideration of coherence across outcomes for female reproductive effects.

Fecundity (time to pregnancy)

Eight epidemiology studies reported on the association between PFNA exposure and fecundity or fertility. A summary of the study evaluations is presented in Figure 3-63, and additional details can be obtained from HAWC. One study (Cariou et al., 2015) was considered *uninformative* because of a lack of consideration of any potential confounders and was excluded from further analysis. Of the remaining studies, three were preconception cohorts and were considered *medium* confidence (Crawford et al., 2017; Lum et al., 2017; Vestergaard et al., 2012), and three were pregnancy cohorts and considered *low* confidence (Bach et al., 2018; Bach et al., 2015; Jørgensen et al., 2014). The pregnancy cohorts were rated *low* confidence because of potential selection bias from enrolling women during pregnancy, which would exclude women who were unable to conceive. If there is a true association between PFNA and fecundity, the bias would be against those women expected to be most highly exposed and most sensitive to the effect, which would be expected to result in an underestimate of the association. However, if there is no association, selection would not be related to exposure, and so would be unlikely to cause substantial bias. In addition, one study was a cross-sectional analysis of fertilization rate in women who underwent fully stimulated, assisted reproductive treatment at an IVF clinic; this study was rated *low* confidence primarily due to concerns for residual confounding (Kim et al., 2020c).

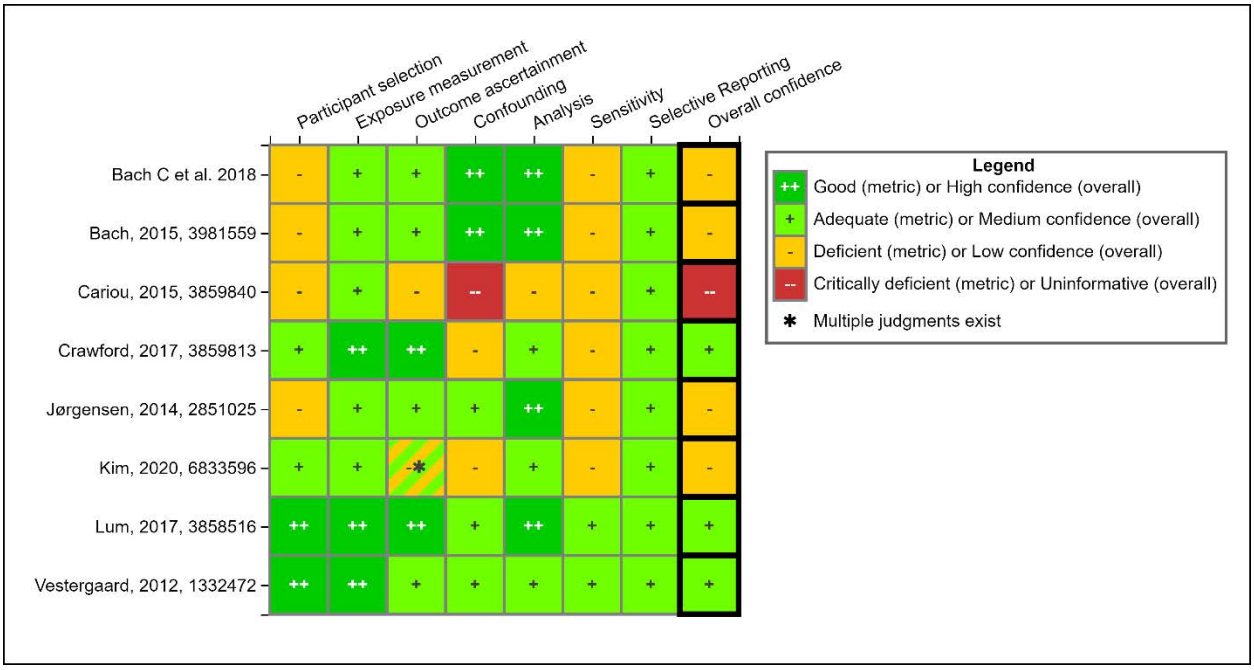


Figure 3-63. Summary of study evaluation for epidemiology studies of fecundity. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500207/.

The results for the association between PFNA exposure and fecundity are presented in Table 3-22. A fecundability ratio less than 1 indicates a decrease in fecundity/increase in time to pregnancy. Of the six studies, decreases in fecundity/increase in time to pregnancy were observed in one *medium* (Lum et al., 2017) and one *low* (Jørgensen et al., 2014) confidence study, with the latter reporting statistical significance, although the association was only observed in some subpopulations in (Jørgensen et al., 2014). Two other studies, one *medium* confidence (Crawford et al., 2017) and one *low* confidence (Bach et al., 2018), reported imprecise results in subpopulations in the same direction (inverse associations). 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 did not observe higher odds with higher exposure. Similarly, the *low* confidence cross-sectional study rate did not report reduced fertilization rates with higher exposure (Kim et al., 2020c).

Overall, the studies with the strongest associations also had high percentages of parous women in their samples. As suggested by Bach et al. (2018), there is a possibility of confounding by factors related to previous pregnancies in the results of parous women. This observation is supported by the lack of association in studies or sub-analyses of nulliparous women (Bach et al., 2018; Bach et al., 2015; Vestergaard et al., 2012), and thus there is considerable uncertainty in the likelihood of a true association between PFNA exposure and fecundity.

Table 3-22. Associations between PFNA and time to pregnancy in epidemiology studies

Reference, confidence	Population	Median exposure (IQR)	Comparison for effect estimate	Fecundability ratio (95% CI)
Bach et al. (2018), *Low*	Danish National Birth Cohort sub-sample (1996–2002), Denmark Nulliparous women (n = 638)	0.5 (0.4–0.6)	Quartiles vs. Q1	Q2: 0.90 (0.72–1.13) Q3: 1.12 (0.88–1.43) Q4: 0.93 (0.74–1.17)
	Parous women (n = 613)			Q2: 0.86 (0.64–1.17) Q3: 0.87 (0.65–1.16) Q4: 0.86 (0.64–1.15)
Vestergaard et al. (2012), *Medium*	Preconception cohort (1992–1995), Denmark; 222 nulliparous women	0.5 (0.4–0.7)[a]	log-unit increase	1.17 (0.88,1.54)
			Above median vs. below	1.25 (0.87,1.79)
Jørgensen et al. (2014), *Low*	INUENDO pregnancy cohort (2002–2004), Greenland, Poland, Ukraine; 938 women	0.6	ln-unit increase	Pooled 0.8 (0.69,0.94)
	Greenland (n = 448, 31% nulliparous)	0.7	Tertiles vs. T1	T2: 0.76 (0.58,1.00) T3: 0.71 (0.54,0.94)
	Poland (n = 203, 92% nulliparous)	0.6		T2: 0.94 (0.62,1.41) T3: 1.31 (0.87,1.98)
	Ukraine (n = 287, 79% nulliparous)	0.6		T2: 0.79 (0.55,1.12) T3: 0.88 (0.62,1.26)
Crawford et al. (2017), *Medium*	Time to Conceive preconception cohort (2008–2009), U.S.; 99 women (40% nulliparous)	0.8 (GM)	dichotomous, cutoff 75th percentile	Cycle-specific model 0.84 (0.46,1.54) D-specific model 0.98 (0.32,2.10)
Bach et al. (2015), *Low*	Aarhus pregnancy cohort (2008–2013), Denmark; 1,372 nulliparous women	0.8 (0.6–1.0)	0.1 ng/mL increase	1.00 (0.98,1.02)
			Quartiles vs. Q1	Q2: 1.05 (0.89,1.25) Q3: 1.03 (0.88,1.22) Q4: 1.00 (0.98,1.02)
Lum et al. (2017), *Medium*	LIFE preconception cohort (2005–2009), U.S.; 483 women (53% nulliparous)	1.2 (0.8–1.7)	Tertiles vs. T1	T2: 0.7 (0.5,1.0) T3: 0.8 (0.6,1.2)

[a]Participants that were pregnant.

Reproductive hormones

Reproductive hormones examined in the evaluated studies include testosterone, estradiol/estrogen, insulin like growth factor 1 (IGF-1), FSH, LH, and progesterone, as well

as SHBG. Key issues for the evaluation of these studies were related to sample collection and processing. Because of diurnal variation for testosterone, LH, and FSH, blood sample collection should be in the morning and if not, time of collection must be accounted for in the analysis (Rosner et al., 2007). 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 PFNA exposure relevant for influencing reproductive hormones is unclear and dependent on several factors, and thus all exposure windows with available data were considered 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. Fifteen studies (16 publications) examined the associations between PFNA exposure and reproductive hormones. One study was deemed *uninformative* due to multiple deficiencies identified in study evaluation (McCoy et al., 2017). Of the remaining studies, most examined only testosterone and estradiol. Most of the available studies were cross-sectional, with a few exceptions that measured exposure prospectively (Timmermann et al., 2022; Harlow et al., 2021; Maisonet et al., 2015). Nine studies examined associations in adults (Harlow et al., 2021; Wang et al., 2021b; Xie et al., 2021; Heffernan et al., 2018; Zhang et al., 2018b; Barrett et al., 2015; Lewis et al., 2015), including two in pregnant women (Timmermann et al., 2022; Yang et al., 2022b), four studies (Xie et al., 2021; Zhou et al., 2016; Lewis et al., 2015; Maisonet et al., 2015) in adolescents, one study (Lopez-Espinosa et al., 2016) in children, and two studies (Jensen et al., 2020b; Yao et al., 2019) in infants. The study evaluations are summarized in Figure 3-64. Two studies examined NHANES data with overlapping dates, but one included both sexes and all ages above 12 years (Xie et al., 2021) while the other included only postmenopausal women (Wang et al., 2021b); the latter study was considered primary for postmenopausal women due to more thorough consideration of potential confounding and more participants due to including multiple NHANES study cycles. In summary, eight studies were *medium* confidence and seven were *low* confidence. However, of the *medium* confidence studies, three did not consider timing of sample collection and was thus *low* confidence for testosterone (Wang et al., 2021b; Xie et al., 2021; Lopez-Espinosa et al., 2016).

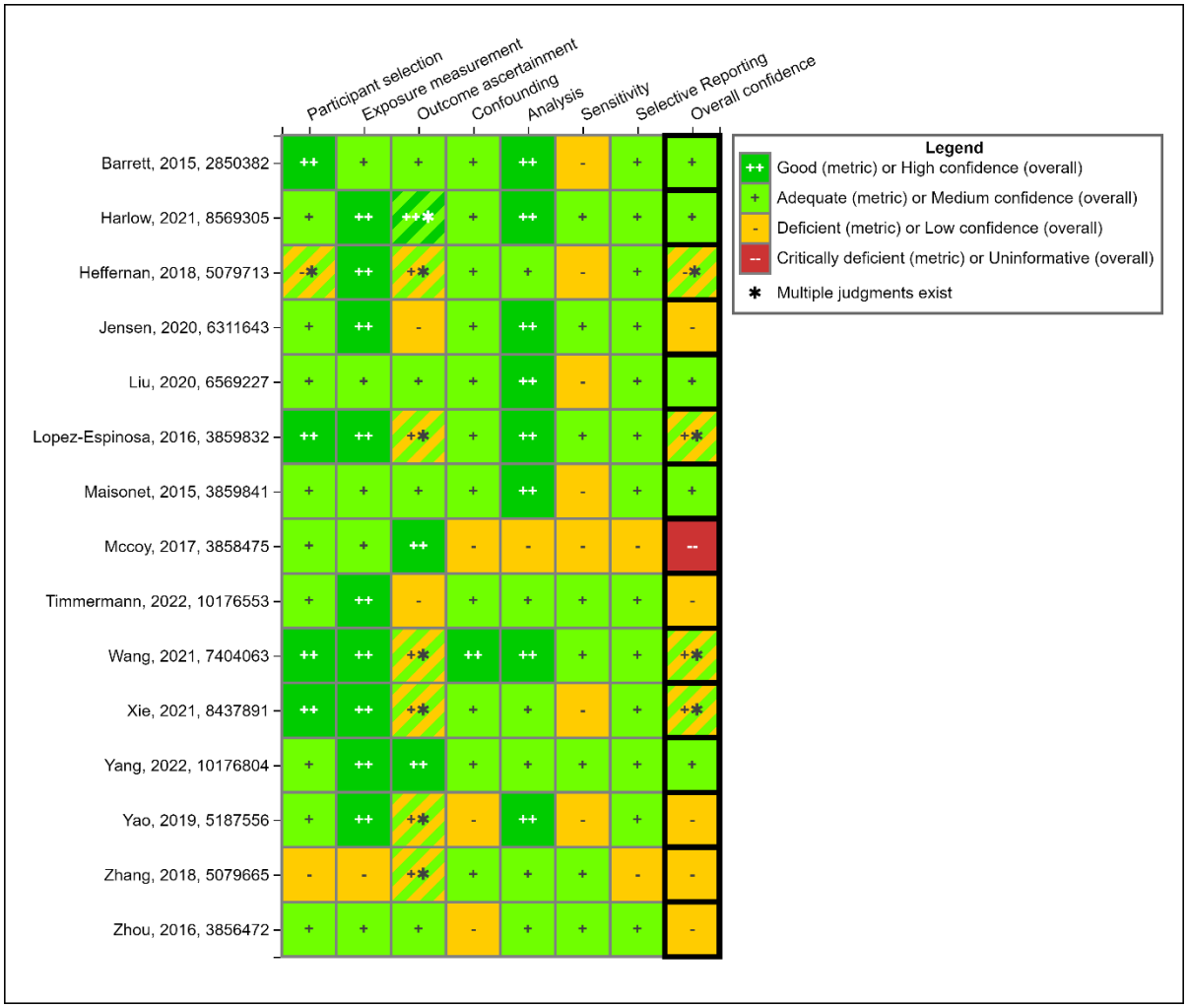


Figure 3-64. Summary of study evaluation for epidemiology studies of female reproductive hormones. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500212/.

*Outcome-specific ratings differ for this domain.

Estradiol

Ten studies examined estradiol levels in association with PFNA. In three studies of adults, there was no clear association with estradiol. One *medium* confidence study reported a statistically significant inverse association (−6.59% change, 95% CI −11.6, −1.30 in tertile 3 versus 1) in premenopausal women (Harlow et al., 2021), while in Xie et al. (2021), among women aged 20–49, an inverse association was also observed, but the association was not monotonic across quartiles (strongest in quartile 3). No association was observed in postmenopausal women in Wang et al. (2021b). A *low* confidence study reported a nonsignificant inverse association in women with POI (Zhang et al., 2018b). In contrast, one low confidence study reported a nonsignificant positive association (Heffernan et al., 2018), and one *medium* confidence study reported no association

(Barrett et al., 2015). In pregnant women, Yang et al. (2022b) reported an inverse association ($p < 0.05$) with estradiol in late pregnancy but positive associations ($p > 0.05$) in early and mid-pregnancy. In adolescents, one study reported an inverse association, but it was not statistically significant and non-monotonic across quartiles (Xie et al., 2021); a *low* confidence study of adolescents reported a nonsignificant positive association (Zhou et al., 2016), while a single *low* confidence study of children (Lopez-Espinosa et al., 2016) reported a nonsignificant inverse association. Lastly, in one *medium* confidence study of infants (Yao et al., 2019), there was a nonsignificant positive association. Overall, there was inconsistency in the direction of association across studies and most of the results were imprecise. There was no apparent pattern of association by study population, confidence, or sensitivity (e.g., exposure levels and contrast).

Testosterone

As described above, most studies were *low* confidence for testosterone. In adult women, six studies were available, five of which were *low* confidence. The *medium* confidence study reported a positive but not statistically significant association with testosterone (3.81% change, 95% CI −2.57, 10.62 in tertile 3 versus 1) (Harlow et al., 2021). In Lewis et al. (2015), the direction of association was not consistent across age groups, with inverse associations observed in 20–<40 years and 60–80 years, but a positive association observed in 40–<60 years (none statistically significant). In different NHANES cycles, no association was observed in premenopausal (Xie et al., 2021) or postmenopausal women (Wang et al., 2021b). In Heffernan et al. (2018), a statistically significant positive association was observed in controls (i.e., women without PCOS), but no association was observed in women with PCOS. No association was observed in women with POI in Zhang et al. (2018b); data on women without POI were not provided for PFNA. In adolescents, four studies were available. Maisonet et al. (2015), the single *medium* confidence study, reported higher testosterone levels in 15-year-old girls with higher PFNA exposure (β: 0.22 (95% CI: −0.09, 0.54), specifically a median PFNA exposure 0.5 ng/mL). The *low* confidence studies were not consistent with this finding, reporting inverse associations, although none were statistically significant (Xie et al., 2021; Zhou et al., 2016; Lewis et al., 2015). One *low* confidence study in children reported an inverse association with testosterone (Lopez-Espinosa et al., 2016) while one *low* confidence study in infants Yao et al. (2019) reported a positive association (neither statistically significant). Overall, the direction of association for testosterone was inconsistent across studies both within and across age groups. No patterns were apparent by exposure levels.

Other hormones

Lopez-Espinosa et al. (2016) reported a significant inverse association between PFNA and IGF-1 (β: −3.5 (95% CI: −6.0, −1.0). Barrett et al. (2015) observed higher luteal phase progesterone with higher PFNA exposure in normally cycling women (β: 0.25 (95% CI: −0.09, 0.60). In infants, Liu et al. (2020b) similarly found a positive association with progesterone (3.28% change, 95% CI −1.18, 7.93). Timmermann et al. (2022) also reported higher prolactin with higher

exposure (2.9% difference, 95% CI −0.9, 6.9). SHBG was not associated with PFNA levels in Maisonet et al. (2015), Harlow et al. (2021), or Heffernan et al. (2018). No association was observed with FSH or LH in POI cases in Zhang et al. (2018b), with FSH in premenopausal women (Harlow et al., 2021) or with dehydroepiandrosterone (DHEA), DHEA sulfate, androstenedione, or 17-hydroxyprogesterone in Jensen et al. (2020b).

Other female reproductive effects

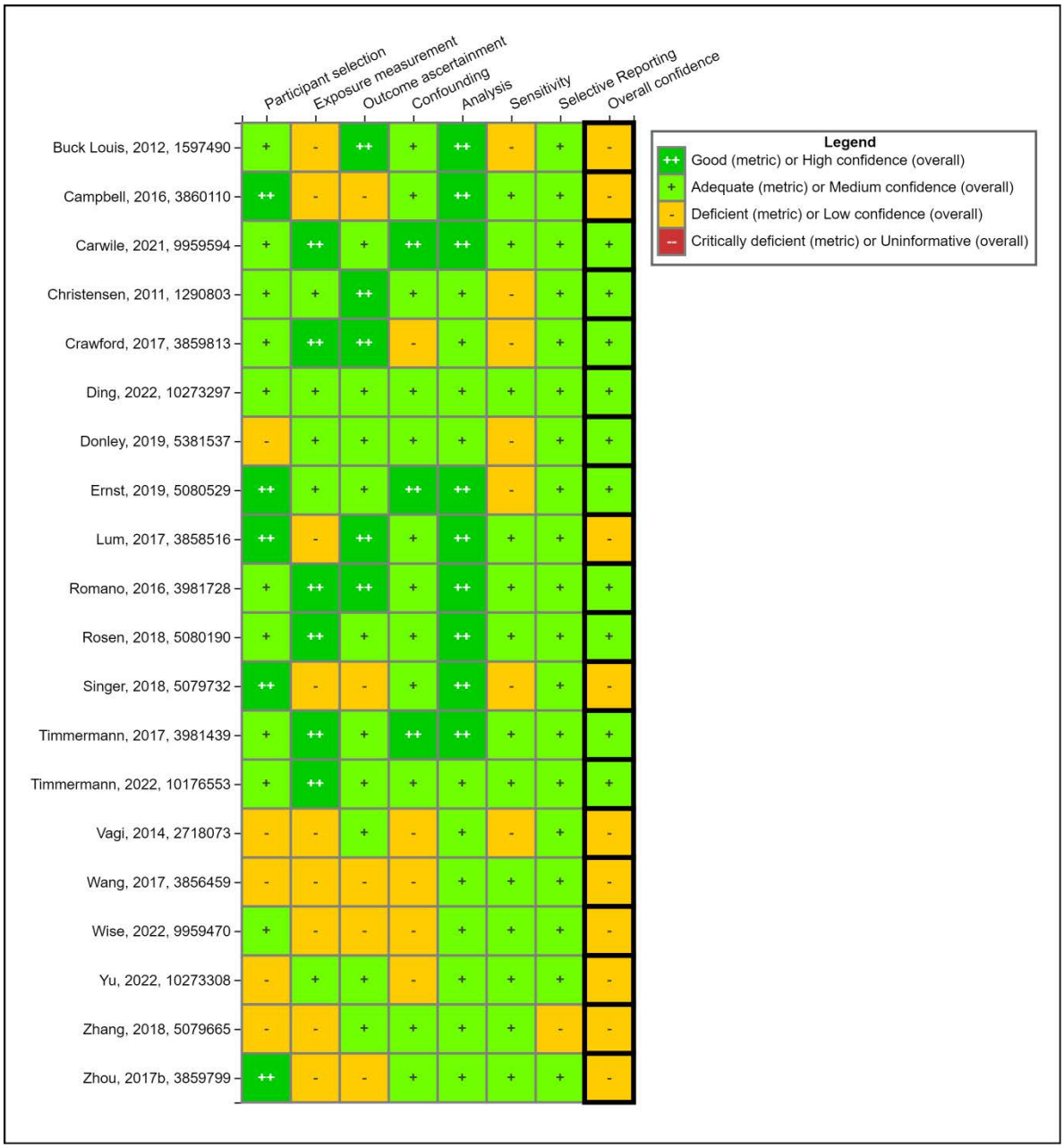


Figure 3-65. Summary of study evaluation for epidemiology studies of other female reproductive effects, including pubertal development, menstrual cycle

characteristics, gynecological conditions, and ovarian reserve. Ratings on this heat map may not reflect all the included outcomes. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500362/.

Pubertal development

Three *medium* confidence studies and two *low* confidence (see Figure 3-65) examined timing of pubertal development with prenatal PFNA exposure. The *medium* confidence studies included birth cohorts in Denmark (Ernst et al., 2019) and in the United States (Carwile et al., 2021) and a case-control study nested in a birth cohort in the United Kingdom (Christensen et al., 2011). The *low* confidence studies were cross-sectional analyses in the United States (Wise et al., 2022; Yu et al., 2022). Ernst et al. (2019) and Carwile et al. (2021) reported results for several pubertal outcome measures, while the other three studies focused on age at menarche (Wise et al., 2022; Yu et al., 2022; Christensen et al., 2011).

In Ernst et al. (2019), with median exposure of 0.5 ng/mL (10th–90th percentile: 0.3–0.7), girls had an earlier age at each Tanner stage for breast development and pubic hair, as well as axillary hair, acne, and menarche, although most of these associations were not statistically significant and some were non-monotonic across the tertiles. Looking at a combined puberty indicator outcome, there was a nonsignificant lower age at puberty (age difference for tertile 2 versus tertile 1: −4.74 (95% CI: −10.45, 0.96); tertile 3 versus tertile 1: −5.06 (95% CI: −10.61, 0.48)). In contrast, in Carwile et al. (2021), girls had less pubertal development in early adolescence with higher exposure and, correspondingly, older age at peak height velocity, a proxy for timing of puberty. No association was observed with age at menarche. In Christensen et al. (2011), with median exposure of 0.6 ng/mL (IQR 0.5–0.8), there was not a clear association, as there were higher odds of earlier age at menarche when PFNA was analyzed as dichotomous (OR 1.15 (95% CI: 0.78, 1.69)) but lower odds when analyzed as continuous (OR 0.91 (95% CI: 0.59–1.40)), neither statistically significant. In the *low* confidence studies, no association was observed with age at menarche (Wise et al., 2022; Yu et al., 2022). Overall, there is considerable uncertainty for this outcome given the inconsistency across studies.

Menstrual cycle characteristics

Four epidemiology studies reported on the association between PFNA exposure and menstrual cycle characteristics. One was a preconception cohort described above for fecundity (Lum et al., 2017). One was a pregnancy cohort in Norway (Singer et al., 2018) and two were cross-sectional studies of participants in a preconception cohort (Zhou et al., 2017a) and Black women in the United States. (Wise et al., 2022). For this outcome, there is potential for reverse causation because menstruation is one of the mechanisms by which PFAS are removed from the body. Thus, all the available studies were considered *low* confidence (see Figure 3-65). There were also concerns of potential outcome misclassification due to self-reporting in all the studies except for Lum et al. (2017), in which the length of menstrual cycle was identified prospectively.

One study (Zhou et al., 2017a) reported higher odds of irregular and long cycle (OR (95% CI) for continuous exposure = 1.50 (1.03, 2.07) and 1.49 (1.05, 2.11), respectively) but lower odds of menorrhagia (i.e., heavy menstrual bleeding) with higher exposure (OR = 0.47 (0.26, 0.86)). Wise et al. (2022) similarly reported lower exposure levels in women with medium and heavy versus low menstrual bleed but found no association with bleed length. Lum et al. (2017) and Singer et al. (2018) reported no association with cycle length. The associations in Zhou et al. (2017a) and Wise et al. (2022) are consistent with the predicted direction of reverse causation (i.e., heavy bleeding leads to higher removal of PFNA, lower PFNA in blood; longer cycle leads to lower removal of PFNA, higher PFNA in blood), so they are difficult to interpret.

Gynecological conditions

Three epidemiology studies reported on the association between PFNA exposure and endometriosis. All the studies were cross-sectional, which decreases confidence for this chronic outcome due to the inability to establish temporality. There is potential for reverse causality, as described above, toward a protective direction since endometriosis can be associated with heavier and more frequent bleeding. Because of this, all studies were classified *low* confidence (see Figure 3-65), although the study by Louis et al. (2012) is considered stronger in some study design aspects because it included two groups of women, one group scheduled for surgery (laparoscopy or laparotomy) and one group identified through a population database who underwent pelvic magnetic resonance imaging (MRI) to identify endometriosis (Louis et al., 2012). The remaining two studies were deficient for outcome ascertainment, specifically due to self-reporting of endometriosis diagnosis (Campbell et al., 2016) and case definition including only endometriosis-related infertility among surgically confirmed cases (Wang et al., 2017). Two studies reported slightly increased odds of endometriosis with higher exposure, although the estimates were imprecise (Louis et al. (2012): operative sample OR: 2.20 (95% CI: 1.02,4.75); population sample OR: 1.52 (95% CI: 0.15, 15.1); and Campbell et al. (2016) OR (95% CI versus Q1: Q2: 3.75 (0.69, 20.66), Q3: 5.27 (1.20,23.06), Q4: 3.24 (0.81, 12.91)). The other *low* confidence study reported lower odds of endometriosis-related infertility with higher exposure (Wang et al. (2017) OR (95%) versus tertile 1: tertile 2: 0.71 (0.39,1.28), tertile 3: 0.52 (0.28, 0.95)).

In addition, one case-control study in the United States examined PCOS and PFNA exposure (Vagi et al., 2014). Study results may also be influenced by reverse causality due to association with menstruation. Because PCOS is associated with irregular menstruation and less frequent bleeding, it is possible that effect estimates will be inflated. This study is *low* confidence for this reason in addition to concerns with participant selection and confounding. Odds of PCOS were higher with higher exposure (OR (95% CI) in tertile 2 versus tertile 1: 1.13 (0.37–4.49); tertile 3 versus tertile 1: 2.25 (0.67, 8.00)), but this result was not statistically significant and, because of the study limitations, is difficult to interpret.

Ovarian reserve

Three studies examined the association between PFNA exposure and ovarian reserve, the fertility potential based on number and quality of eggs. Two *medium* confidence studies examined anti-Müllerian hormone (AMH), including a cohort (Crawford et al., 2017) and a nested case-control study (Donley et al., 2019), and one *low* confidence case-control study examined POI (Zhang et al., 2018b) (see Figure 3-65). AMH is commonly used as an endocrine marker for age-related decline of ovarian reserve in healthy women (Grynnerup et al., 2012), but a single measurement in healthy women may not be informative in predicting fecundity (ACOG, 2019), so these results are interpreted with caution. In contrast, POI is a more specific outcome and is defined as an elevated FSH level greater than 25 IU/L on two occasions more than 4 weeks apart and oligo/amenorrhea for at least 4 months in Zhang et al. (2018b). However, because this definition is closely tied to menstruation, there are concerns for reverse causality as with the previous outcomes, which would be expected to be biased away from the null. In Zhang et al. (2018b), odds were higher of POI in the third tertile of exposure, although not statistically significant (OR (95% CI), versus tertile 1: tertile 2: 0.96 (0.50, 1.85), tertile 3: 1.34 (0.70, 2.54)). In Crawford et al. (2017), there was an inverse association between AMH and PFNA, consistent with decreased ovarian reserve, although this observation was not statistically significant (β: −0.17, $p = 0.3$). No association was observed with AMH in Donley et al. (2019), although exposure levels were lower than in the other two studies (median 0.5 versus 0.8 in Crawford et al. (2017) and 2.1 in Zhang et al. (2018b)). The results of Zhang et al. (2018b) and Crawford et al. (2017) are coherent with each other, but due to the inconsistency with AMH, imprecision of results, study limitations, and the small number of studies, there is still considerable uncertainty.

Menopause

One *medium* confidence cohort study of midlife women examined the association between PFNA exposure and timing of menopause. Ding et al. (2022) defined natural menopause as 12 months of amenorrhea since the last menstrual period without a reason such as hysterectomy. They reported earlier onset of natural menopause with higher exposure (total effect relative survival 0.84, 95% CI 0.71, 0.97).

Breastfeeding duration

Four *medium* confidence birth cohorts examined duration of breastfeeding in relation to exposure to PFNA measured during gestation. Five additional cross-sectional or case-control studies without prospective measurement of exposure that reported analyses predicting PFNA concentrations based on past breastfeeding duration were considered supplemental evidence because of the high probability of reverse causation due to lactation being an elimination route (Kim et al., 2020b; Pirard et al., 2020; Ammitzbøll et al., 2019; Lee et al., 2018; Harris et al., 2017). The results of the included studies are summarized in Table 3-23. Three of the four studies reported results consistent with earlier cessation of breastfeeding with higher exposure

(Timmermann et al., 2022; Timmermann et al., 2017b; Romano et al., 2016), with one of these being statistically significant (Timmermann et al., 2022). In contrast, one study reported a statistically significant inverse association, with less likelihood of cessation of breastfeeding by 3 or 6 months with higher exposure (Rosen et al., 2018). In studies where an adverse association was observed, the associations were clearer for cessation of any breastfeeding (which could include formula supplementation and solid foods) than for exclusive breastfeeding, which had null results in two of the studies; there is no clear explanation for this difference.

Table 3-23. Associations between PFNA and breastfeeding duration in epidemiology studies

Reference, confidence	Population	Median exposure (IQR)	Form and units of effect estimate	Endpoint	Effect estimate
Risk of cessation of breastfeeding (>1 indicates earlier cessation)					
Rosen et al. (2018)	Norwegian Mother and Child Study (1999–2008), Norway, 1,716 women	0.5 (0.3–0.6)	HR (95% CI) for IQR change	Cessation of any breastfeeding by 3 mo	**0.77 (0.63, 0.93)***
				Cessation of any breastfeeding by 6 mo	**0.84 (0.73, 0.97)***
Timmermann et al. (2022)	Odense Child Cohort (2010–2012), Denmark, 932 women	0.6 (0.5–0.9)	HR (95% CI) for doubling	Cessation of any breastfeeding	**1.14 (1.03, 1.27)***
				Cessation of exclusive breastfeeding	0.93 (0.84, 1.02)
Romano et al. (2016)	HOME cohort (2003–2006), U.S., 336 women	0.9 (0.7–1.2)	RR (95% CI) vs. Q1	Cessation of any breastfeeding by 3 mo	Q2: 1.10 (0.85, 1.44) Q3: 1.03 (0.77, 1.36) Q4: 1.12 (0.81, 1.53)
				Cessation of exclusive breastfeeding by 3 mo	Q2: 0.92 (0.81, 1.04) Q3: 0.96 (0.86, 1.07) Q4: 0.96 (0.85, 1.08)
				Cessation of any breastfeeding by 3 mo	Q2: 0.95 (0.77, 1.18) Q3: 0.94 (0.75, 1.17)

Reference, confidence	Population	Median exposure (IQR)	Form and units of effect estimate	Endpoint	Effect estimate
					Q4: 1.13 (0.90, 1.43)
Continuous duration of breastfeeding (<0 indicates earlier cessation)					
Timmermann et al. (2017b)	Two birth cohorts in Faroe Islands (1997–2009), Denmark, 1,092 women	0.6 (0.5–0.8)	Difference in mo (95% CI) for doubling	Duration of any breastfeeding	**−1.3 (−2.0, −0.7)***
				Duration of exclusive breastfeeding	**−0.2 (−0.5, −0.0)***

*$p < 0.05$.

Animal Studies

Six experimental studies in rats and mice have evaluated the effects of PFNA exposures on the female reproductive system (Zhang et al., 2021; Singh and Singh, 2019c; NTP, 2018; Das et al., 2015; Rogers et al., 2014; Wolf et al., 2010). This evidence base includes five developmental toxicity studies evaluating reproductive endpoints in rodent dams exposed to PFNA during gestation (Zhang et al., 2021; Singh and Singh, 2019c; Das et al., 2015; Rogers et al., 2014; Wolf et al., 2010), with two of these studies also evaluating effects on sexual maturation markers in female offspring (Zhang et al., 2021; Das et al., 2015).

The study designs are provided in Table 3-24. As previously discussed, increased mortality was reported in adult female rats in the NTP 28-day study at the two highest doses of PFNA (12.5 and 25 mg/kg-day), making PFNA treatments at 6.25 mg/kg-day the highest dose with reliable results for all outcomes except the histopathology, which was performed at the time of early death (see Section 3.2.1, "Adult rodent mortality").

The 28-day study in rats was judged to be *high* confidence for the female reproductive endpoints evaluated. All five developmental toxicity studies were found to be overall *high* or *medium* confidence for the pregnancy outcomes evaluated in dams with no major concerns noted with exception of Zhang et al. (2021) that was *low* confidence for dams co-exposed to the PPARα inhibitor GW6471 due to inadequate sample sizes and other deficiencies (see Figure 3-66). The offspring marker of sexual maturation (timing of vaginal opening) in CD-1 mice was judged to be overall *medium* confidence with some downgrading due to a lack of blinding of study evaluators when scoring animals (Das et al., 2015). The sexual maturation endpoints in female ICR mouse offspring were found to be *low* confidence due largely to missing reporting on sampling design and whether litter was the statistical unit in results evaluations, among other inadequacies in allocation and observational bias domains (Zhang et al., 2021).

Table 3-24. Overall evaluation results of female reproductive toxicity studies examining the effects of PFNA exposures in rodents[a]

Reference	Species, strain (lifestage/sex)	Exposure design	Exposure route and dose range	Organ weight/ histopathology	Pregnancy outcomes	Hormones/ sexual maturation	Estrous cycle length
Das et al. (2015)[b]	Mouse, CD-1 (dam/offspring)	Developmental GD 1–17	Gavage 0, 1, 3, 5 mg/kg-d	NM	++	+	NM
NTP (2018)[c]	Rat, Sprague-Dawley (Harlan)(adult female)	Short-term 28 d	Gavage 0, 1.56, 3.12, 6.25, 12.5, 25 mg/kg-d	++	NM	++	++
Rogers et al. (2014)	Rat, Sprague-Dawley (dam/offspring)	Developmental GD 1–20	Gavage 0, 5 mg/kg-d	NM	+	NM	NM
Wolf et al. (2010)	Mouse, Wild type 129S1/SvlmJ; PPARα-Knockout PPARa$^{tm1Gonz/J}$ (dam/offspring)	Developmental GD 1–18	Gavage 0, 0.83, 1.1, 1.5, 2 g/kg-d	NM	++	+	NM
Singh and Singh (2019c)	Mouse, Parks (P) (dams at term)	Developmental PND 25–38	Gavage 0, 2.0, 5.0 mg/kg-d	NM	+	NM	NM
Zhang et al. (2021)[d]	Mouse, ICR (dam/offspring)	Developmental GD 1–18	Gavage 0, 0.5, 3 mg/kg-d	+ -	+	-	NM

[a]Dark green (++) = *high* confidence; light green (+) = *medium* confidence; yellow (-) = *low* confidence; red (--) = *uninformative*; NM = not measured. Study evaluation details for all outcomes are available in HAWC.

[b]10 mg/kg-d dose group ceased due to high mortality among treated dams.

[c]12.5 and 25 mg/kg-d dose groups not evaluated due to high mortality, except for the histopathology that was evaluated at time of death.

[d]Maternal organ weight is *medium* confidence with exception to dams co-administered the PPARα inhibitor GW6471 that are *low* confidence. Offspring (PND 45) organ weight and histopathology are *low* confidence.

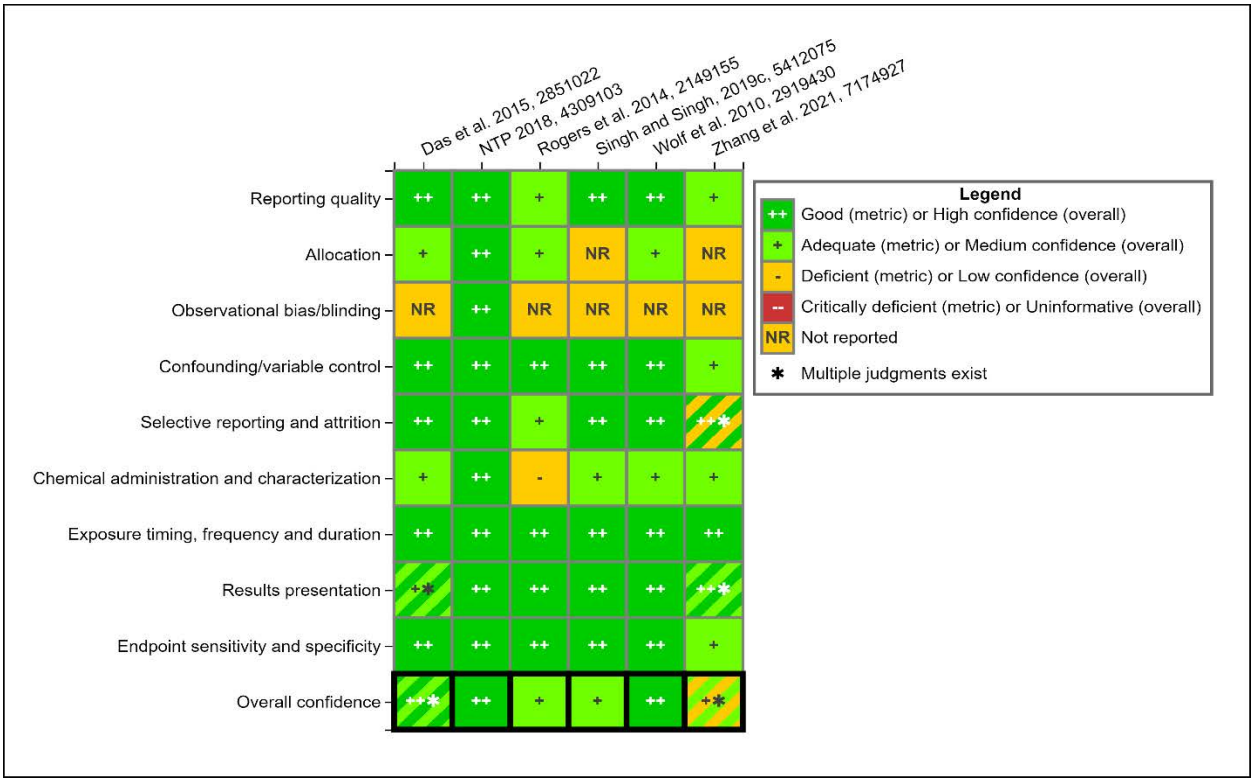


Figure 3-66. Summary of animal study evaluations that examined PFNA effects on the female reproductive system.[a,b] See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Female-reproductive-outcomes-animals/.

[a]Das et al. (2015) was *high* confidence for pregnancy outcomes, and *medium* confidence for offspring markers of sexual maturation.

[b]Zhang et al. (2021) was *medium* confidence for maternal body weight (other pregnancy outcomes were not evaluated), and *low* confidence for offspring markers of sexual maturation.

Pregnancy outcomes

The developmental toxicity studies with PFNA in three wild type mouse strains (CD-1, 129S1/SvlmJ, and Parkes) found no statistically significant changes in number of uterine implants, litter sizes, or litter losses in gestationally exposed dams at any dose levels tested (Singh and Singh, 2019c; Das et al., 2015; Wolf et al., 2010). Das et al. (2015) observed statistically significant increases in maternal body weight gain of CD-1 mouse dams ranging from 24% to 34% and 21% to 41% during late gestation (GD 11–17) at 3 and 5 mg/kg-day, respectively. However, no significant changes in absolute maternal body weight and maternal body weight minus gravid uterine weight were seen at GD 17. Singh and Singh (2019c) reported no significant differences in body weight gain of pregnant Parkes mice exposed to PFNA at up to 5 mg/kg-day from GD 12 to 20. Some nonsignificant elevations in litter loss (i.e., full litter resorption and/or whole litter loss) were observed in wild type mice at the high dose; 35% (6/17) litter loss at 2 mg/kg-day PFNA and 14% (2/14) litter loss in controls (Wolf et al., 2010). This study also reported a statistically significant

declining trend in pregnancy rates of PPARα null mice at ≥0.83 mg/kg-day. Compared with PPARα null controls, which had a pregnancy rate of 75% (18/24), PFNA doses of 0.83, 1.1, 1.5, and 2 mg/kg-day reduced pregnancy rates to 65% (13/20), 58% (14/24), 21% (9/43), and 43% (16/37), respectively. However, PFNA did not affect dam body weight or body weight gain (Wolf et al., 2010). This finding raises the possibility that PFNA may interfere with some pregnancy parameters when PPARα is not functional, but the mechanism by which this might occur is currently unknown. Zhang et al. (2021) reported a statistically significant increase (12%) in pregnancy weight gain of ICR mice exposed to 3 mg/kg-day PFNA, which did not occur in mouse dams co-administered the PPARα inhibitor GW6471 (latter being a *low* confidence result). In contrast to observations in mice, Rogers et al. (2014) reported statistically significant reductions in body weight gain of pregnant SD rats exposed to 5 mg/kg-day PFNA (ranging from 10% to 28% from GD 4 to 19). There were no maternal deaths or other overt toxicity parameters reported in rat dams at this dose.

Reproductive hormones and sexual maturation

Das et al. (2015) reported dose-dependent delays in vaginal opening of approximately 3 and 7 days in CD-1 mice at 3 and 5 mg/kg-day PFNA, respectively (see Section 3.2.2, "Postnatal developmental landmarks"). PFNA effects on the day of first estrus were not evaluated in Das et al. (2015). Consistent with these findings, the *low* confidence findings by Zhang et al. (2021) also reported statistically significant delays (approximately 2 days) in both age of vaginal opening and age of first estrus of gestationally exposed mice at 3 mg/kg-day PFNA. Pubertal onset is activated by the HPG axis leading to increased serum gonadotropins (LH, FSH) that stimulate ovarian maturation, sex steroid production (notably estradiol and progesterone), and sexual maturation (Sisk and Foster, 2004). However, in the mouse, vaginal opening and first estrus may occur days before first ovulation, making them less reliable indicators of the timing of first ovulation/puberty but nonetheless relevant endpoints of reproductive development (Gaytan et al., 2017; Prevot, 2014; Safranski et al., 1993). These latter results are synthesized and integrated with other studies examining developmental outcomes (see Section 3.2.2) given the apparent coherence of findings of developmental delays after PFNA exposure (see integration discussion below).

The only studies to examine PFNA effects on reproductive hormones of females are the *high* confidence NTP 28-day study in adult SD rats (NTP, 2018) that evaluated serum testosterone only, and the *low* confidence developmental effects study in ICR mice by Zhang et al. (2021) that evaluated serum LH, FSH, estradiol, and progesterone in gestationally exposed offspring at PND 30 and PND 45. However, the hormone results in females are difficult to interpret because they were not presented by estrus cycle stage, which influences reproductive hormone levels. The NTP 28-day study observed dose-dependent elevations in serum testosterone in females with increases of 34%, 48%, and 66% at 1.56, 3.12, and 6.25 mg/kg-day PFNA, respectively. In the developmental toxicity study by Zhang et al. (2021), statistically significant reductions in LH were reported in females at

PND 30 and 45 at 3 mg/kg-day PFNA, as well as significant reductions in serum progesterone at PND 45, but with no statistically significant effects on serum estradiol or FSH at these timepoints.

Taken together, the available evidence provides some support that prenatal exposures to PFNA delayed vaginal opening in mice, and short-term exposures elevated serum testosterone in adult female rats although uncertain due to the lack of presentation by estrus cycle stage.

Organ weight and histopathology

The 28-day exposure study with PFNA reported no statistically significant changes in absolute or relative uterine weights in treated female rats (NTP, 2018). In the same study, no histopathological abnormalities were observed at time of necropsy for the ovaries, uterus, clitoral gland, or mammary gland. Histopathology analysis was conducted on the ovaries at all doses up to 25 mg/kg-day PFNA; all other reproductive tissues were examined in control and dose groups of 6.25 mg/kg-day and higher only. The *low* confidence findings by Zhang et al. (2021) reported statistically significant reductions in ovarian weight, corpora lutea number, and follicle counts (antral, primary, and secondary) in PND 45 mice gestationally exposed to PFNA at 3 mg/kg-day.

Estrous cycle length

The 28-day exposure study with PFNA reported no statistically significant changes in the estrous cycle length (NTP, 2018).

Mechanistic and Supplemental Information

Mechanistic studies relevant to female reproductive effects were sparse and limited to screening-level in vitro assays and in vivo reproductive testing in zebrafish. Overall, HTS and in vitro data for effects on the ER were inconclusive or inconsistent (Evans et al., 2022; Li et al., 2020b; Mansouri et al., 2016; Rosenmai et al., 2016; Browne et al., 2015; Judson et al., 2014; Kjeldsen and Bonefeld-Jørgensen, 2013; Benninghoff et al., 2011). While there was some evidence for weak inhibitory effects on the AR, overall, the results were equivocal, and there was no clear effect on aromatase activity (Kleinstreuer et al., 2017; Gorrochategui et al., 2014; Kjeldsen and Bonefeld-Jørgensen, 2013; Kraugerud et al., 2011). ToxCast testing results for PFNA are detailed in Appendix C.2. A 6-month reproductive study in adult zebrafish reported dose-dependent increases in serum E2 in females (Zhang et al., 2016). The same study also reported statistically significant increases in testosterone, reductions in fecundity, and downregulation of some gonadal steroidogenesis genes but none of these endpoints showed dose dependency (Zhang et al., 2016). Taken together, the in vivo non-mammalian and mechanistic evidence base for female reproductive effects is neither consistent nor strong, although in vitro studies suggest that PFNA is unlikely to interact with the ER or affect aromatase activity.

Evidence Integration

The human epidemiological evidence of female reproductive toxicity is considered *indeterminate.* Reported associations between PFNA exposure and female reproductive effects in some human epidemiology studies included reduced fecundity, early onset of puberty, risk of gynecological conditions, decreased ovarian reserve, and reduced anogenital distance. However, there was considerable uncertainty in interpreting these findings due to inconsistency across studies, imprecision of results, potential confounding by parity for fecundity, as well as the high likelihood of reverse causation for menstrual cycle characteristics (irregular cycle length) and gynecological conditions (endometriosis and polycystic ovarian syndrome).

The experimental animal evidence base provides *slight* evidence of female reproductive toxicity with a large amount of uncertainty. A *medium* confidence developmental toxicity study in CD-1 mice reported dose-dependent delays in vaginal opening of 3–7 days in gestationally exposed offspring at ≥3 mg/kg-day PFNA. As discussed previously, this result is potentially consistent with similar reproductive developmental delays reported for other PFAS (e.g., PFBS (U.S. EPA, 2021b), PFBA (U.S. EPA, 2022a), PFOA (U.S. EPA, 2023f)). Because vaginal opening is not indicative of first ovulation in mice, the delayed vaginal opening in mice reported by Das et al. (2015) is not a direct correlate to puberty in humans. However, it is a marker of sexual and/or reproductive development consistent with EPA's Reproductive Guidelines (U.S. EPA, 1996) (see Section 3.2.2). Whether these observed delays in vaginal opening include related perturbations (e.g., timing of first estrus, ovulation, mammary gland development) and impaired reproductive performance is unknown in this model. In support of this observation, consistent but *low* confidence findings in another strain of mouse included statistically significant delays in the timing of vaginal opening and first estrus, with potentially related changes in reproductive organs at PND 45, in offspring gestationally exposed at 3 mg/kg-day.[14] In addition, although developmental PFNA exposure did not substantially affect pregnancy outcomes in four strains of wild type mice and effects on pregnancy weight gain were inconsistent, PFNA-induced reductions in pregnancy rates in PPARα null mice were large and mostly dose dependent. Overall, although the extent to which these sporadic observations in mice may be coherent is unclear, and mechanistic information is inconclusive and studies of related outcomes (e.g., ovulation, mammary gland development) are lacking, these findings suggest some level of potential concern.

In the single study in rats, PFNA generally did not affect pregnancy outcomes, uterine weight, estrous cyclicity, or reproductive organ histopathology. However, dose-dependent increases in serum testosterone were observed in adult female rats at ≥1.56 mg/kg-day (coherent with effects observed in non-mammalian species); the results for other female reproductive hormones were difficult to interpret and inconclusive. The biological significance or consequences of the observed PFNA-related increase in testosterone in nonpregnant females is currently unclear,

[14]Dose-response estimates for developmental endpoints are incorporated into the developmental toxicity sections.

although abnormal increases during pregnancy have the potential to adversely affect fetal development.

Taken together, the available human epidemiological and animal ***evidence is inadequate*** to assess whether PFNA has the potential to cause female reproductive toxicity in humans (see Table 3-25).

Table 3-25. Evidence profile table for female reproductive effects[a]

Summary of human and animal evidence					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.5. Female Reproductive Effects, Human Studies)					⊙⊙⊙ ***Evidence is inadequate*** *Primary basis:* Considerable uncertainty in the epidemiological evidence base due to a lack of consistency and coherence. The limited animal evidence also had substantial uncertainties and consisted of evidence of delays in reproductive development (vaginal opening) in mice, increased testosterone in adult rats, and reduced pregnancy rates in PPARα null mice. *Human relevance:* Evidence in animals is presumed relevant to humans given that mechanisms regulating female reproduction are similar between rodents and humans. An
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Fecundity</u> Three *medium* confidence, 4 *low* confidence studies	• Decreased fecundity/longer time to pregnancy with higher PFNA exposure in some studies, though results were not consistent across subpopulations	• No factors noted	• Unexplained inconsistency within and across studies • Potential confounding by factors related to previous pregnancies in the results of parous women • Imprecision of estimates	⊙⊙⊙ *Indeterminate*	
<u>Reproductive hormones</u> Three *medium* confidence, 6 *low* confidence studies	• For estradiol and testosterone, the direction of association was inconsistent and observed associations were generally not statistically significant	• No factors noted	• Unexplained inconsistency overall and within age groups • Most studies of testosterone were *low* confidence due to potential outcome misclassification		
<u>Other reproductive effects</u> Four *medium* confidence, 3 *low* confidence studies	• Studies of gynecological conditions, ovarian reserve, menopause, and breastfeeding duration reported associations with PFNA exposure • Studies of pubertal development and	• *Medium* confidence studies reporting an effect with menopause and breastfeeding duration	• Unexplained inconsistency across studies for some outcomes • Studies of menstrual cycle and gynecological conditions were *low* confidence due to		

Summary of human and animal evidence					Evidence integration summary judgment
	menstrual cycle characteristics were inconsistent.		potential for reverse causation		exception is vaginal opening in mice that is not a direct correlation to puberty in humans, although it is a marker of reproductive development. *Cross-stream coherence:* Studies in humans were *indeterminate* and neither increase nor decrease confidence in the animal findings. *Susceptible populations and lifestages:* Delays in sexual maturation markers suggest development may be a potentially susceptible lifestage.
Evidence from in vivo animal studies (see Section 3.2.5. Female Reproductive Effects, Animal Studies)					
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Pregnancy outcomes</u> Five *high/medium* confidence studies • CD-1 mice (GD 1–17) • 129S1/SvlmJ wild type, PPARα null mice (GD 1–18) • SD rats (GD 1–20) • Parkes mice (GD 12–21) • ICR mice (GD 1–18)	• No effects on pregnancy outcomes in wild type mice at nontoxic maternal doses • PPARα null mice showed decreased pregnancy rate with unknown MOA • Decreased pregnancy weight gain in maternal rats in a single dose gestational exposure at 5 mg/kg-d • Increased pregnancy weight gain in two wild type mouse strains (CD-1, ICR) at ≥3 mg/kg-d	• Large magnitude of decreases in pregnancy rate in PPAR-α KO mice.	• Inconsistent effects on pregnancy weight gain, pregnancy rate, and related outcomes in rats and mice of different strains	⊕⊙⊙ *Slight* While the evidence base is small with generally null findings, dose-dependent delays in an endpoint of sexual maturation (i.e., vaginal opening) in mice, and increases in serum testosterone in adult rats that is difficult to interpret given the inconsistent hormonal findings. The evidence base could be strengthened through the addition of longer-term and developmental exposure studies on how these effects may impact ovulation, mammary gland	
<u>Reproductive hormones and sexual maturation markers</u> One *high* confidence studies in adult rats • 28 d	• Delays in markers of sexual maturation in mice exposed gestationally at ≥3 mg/kg-d (delays in vaginal opening in both studies and in day of first estrus in the low confidence study) • Increased serum testosterone in adult	• *Dose-dependent* delay in vaginal opening of mice and increase in serum testosterone of adult rats • *Magnitude of effect*, 3- to 7-d delays and up to 66% increase in serum testosterone	• Lack of expected coherence for related female reproductive outcomes in mice and adult rats • Unclear biological significance of increased testosterone in nonpregnant female rats		

Summary of human and animal evidence					Evidence integration summary judgment
One *medium* confidence gestational exposure study • CD-1 mice (GD 1–17) One *low* confidence gestational exposure study • ICR mice (GD 1–18)	female rats exposed for 28 days at the lowest dose tested (≥1.56 mg/kg-d) • Inconsistent perturbations in other serum hormones (LH, FSH, estradiol, progesterone) of gestationally exposed mice or adult rats	• Consistency of delays in markers of sexual maturation with other PFAS		development, and impaired reproductive performance or mating behavior.	
<u>Histopathology and organ weight</u> One *high* confidence study in adult rats: • 28 d One *low* confidence gestational exposure study • ICR mice (GD 1–18)	• No effects on uterine weight or female reproductive organ histopathology in adult rats • Statistically significant reductions in ovarian weights (absolute), corpora lutea number, and follicle counts (antral, primary, and secondary) in PND 45 mice at 3 mg/kg-d	• *Coherence* in reductions of ovarian weights and histopathology in gestationally exposed adults (unclear coherence with delays in maturation markers)	• *Low* confidence study		

[a]Mechanistic and supplemental information was limited in scope and depth to screening-level assays and a single in vivo non-mammalian (zebrafish) exposure study with inconsistent results.

3.2.6. Immune Effects

This section describes and integrates the evidence informative to assessing the potential for immunotoxic effects following exposure to PFNA in epidemiologic and animal toxicological studies. There is some overlap in this section with the evidence synthesis and integration summary of developmental effects (see Section 3.2.2) as some of those PFNA human studies also examined effects on immunosuppression and hypersensitivity outcomes in children and fetuses; these outcomes are discussed in this section.

Methodological Considerations

Immune-related health effects evaluated from human and animal studies are categorized according to immunotoxicity guidelines from the World Health Organization (WHO) (IPCS, 2012) and considered herein for evidence of immunotoxicity, including: (1) immunosuppression; (2) immunostimulation; (3) sensitization and allergic response; and (4) autoimmunity. The available data for potential immune effects for PFNA are considered within these four categories because of common and related mechanisms in each category. Within each category, health effects data were considered from the most to least relevant for determining hazard conclusions about immunotoxicity (IPCS, 2012). For the human data, clinical studies on disease or immune function assays are considered most relevant followed by general/observational immune assays (lymphocyte phenotyping or cytokines), while hematological endpoints, such as blood leukocyte counts, are considered less informative. Similarly, the currently available animal data are presented from most to least relevant for reaching conclusions on potential immunotoxicity. Immune function assays are described first followed by general/observational immune assays that evaluate immune-related markers, such as blood leukocytes, immune organ weights and histopathology, and cytokine levels.

The currently available human and animal studies with PFNA provide relevant information for consideration of immunosuppression as well as sensitization and allergic response. However, there are no human or animal studies with PFNA that have evaluated outcomes related to immunostimulation and autoimmunity, and so these categories are not discussed further.

Human Studies

Epidemiology studies examining immune effects of PFNA 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. Several different outcomes were included and summarized separately in the sensitization and allergic response category. The health effects evidence from human studies is summarized below for each category. Study evaluations for all epidemiological studies of immunosuppression effects (antibody response and infectious disease) and sensitization and allergic response are summarized in Figures 3-67 and

3-68, respectively, and discussed in each outcome section. Studies reported in multiple publications are presented in Figure 3-67 as a single study; expanded rationales are available in HAWC. Potential for confounding across PFAS was considered within individual study evaluations and synthesized across studies.

Immunosuppression: Antibody response outcomes[15]

The production of antigen-specific antibodies in response to an immune challenge (e.g., vaccination in humans or injection with 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 (IPCS, 2012; ICH Expert Working Group, 2005; U.S. EPA, 1998). 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 disease.

Ten studies (11 publications) examined PFNA exposures and antibody responses following vaccination and are summarized in Figure 3-67, along with one study of antibody response following natural infection. Among these studies, there were analyses of antibody responses following vaccinations to diphtheria (six studies), tetanus (seven studies), measles (three studies), rubella (two studies), mumps (one study), Haemophilus influenzae Type B (two studies), and flu (one study). In children, there were four prospective birth cohorts, including three in the Faroe Islands and one in Norway (Granum et al., 2013), and one cohort of children beginning in their first year of life in Guinea-Bissau (Timmermann et al., 2020). The three Faroe Islands studies included non-overlapping populations enrolled at separate times, 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). These cohorts are thus considered independent of each other. Some 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); these are labeled in the results table. Given that the etiologic window for immune effects of PFAS exposure is not known, these studies in the Faroe Islands have the benefit of assessing multiple windows of exposure (e.g., maternal, multiple points

[15]New epidemiological studies on these outcomes continue to be published. The most recent literature update, completed in April 2023, found 13 new studies examining association between PFNA and immune effects since April 2022, including 5 studies with new data on immunosuppression (antibody response, 3 studies; and infectious disease, 2 studies). As described in Appendix B.2, none of these newer studies were interpreted to have a material impact on the synthesis judgments or dose-response decisions. These studies are documented in Appendix B.2 but not incorporated into the text and figures of the Toxicological Review. For antibody response specifically, all three studies reported an inverse but not statistically significant association with PFNA exposure but do not address the uncertainties described in the evidence synthesis.

in childhood) as well as following outcomes over time. Also in children, there were two *medium* confidence cross-sectional studies in the United States (Stein et al., 2016b) and Greenland (Timmermann et al., 2021) and one *low* confidence (due to expected residual confounding) cross-sectional study in Germany (Abraham et al., 2020). In adults, there were two *low* confidence studies; a short-term cohort (with exposure measured at vaccination and follow-up 30 days later) in the United States (Stein et al., 2016a) and a cross-sectional study in Denmark (Kielsen et al., 2016). These studies were *low* confidence because of concerns about potential selection bias and confounding.

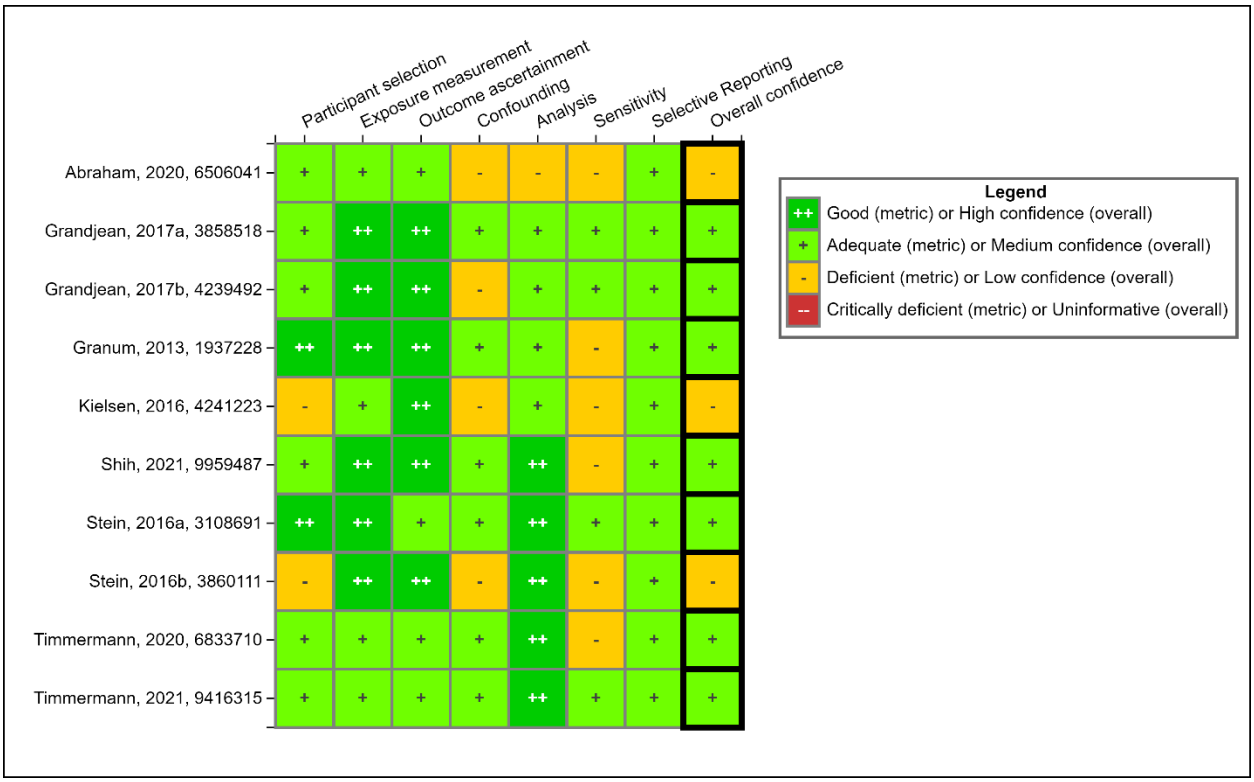


Figure 3-67. Summary of evaluation of epidemiology studies of PFNA and immunosuppression effects (antibody response). See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/pfna-and-immunosuppression-epidemiology-study-eval/.

Note: Multiple publications of the same study: Grandjean et al. (2017a) includes Grandjean et al. (2012). Budtz-Jørgensen and Grandjean (2018a) includes additional analysis of Grandjean et al. (2017a) and Grandjean et al. (2012).

The results for this set of studies are available in Tables 3-26 (children) and 3-27 (adults). 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 with serum PFNA concentrations also measured at different ages. Most studies in children reported a consistent direction of association between higher concentrations of PFNA and lower antibody levels after

vaccination in at least some measurements. These associations were statistically significant for diphtheria vaccination in children at age 5 with childhood exposure measurement (Grandjean et al., 2012) and for rubella vaccination (Granum et al., 2013). There are some results in the opposite direction for sub-analyses of the Faroe Island cohorts (Grandjean et al., 2017b; Grandjean et al., 2017a; Grandjean et al., 2012), including a statistically significant positive association in children aged 5 with exposure measured in infancy (Grandjean et al., 2017b) and in some analyses in Timmermann et al. (2021). No biological rationale has been identified as to whether one period is more predictive of an overall immune response, and thus these differences are difficult to interpret. In adults, one *low* confidence study also reported an inverse association for diphtheria and tetanus vaccination (statistically significant for diphtheria) (Kielsen et al., 2016), while the *medium* confidence cohort reported mixed results depending on timing of exposure measurement and type of vaccine (Shih et al., 2021). The single *low* confidence study of a flu vaccine (FluMist) reported no immunosuppression (Stein et al., 2016a).

Despite the imprecision of many of the individual exposure-outcome analysis pairs, the majority of paired antibody-to-PFNA exposure evaluations provided in Tables 3-26 and 3-27 support a decrease in antibodies with higher PFNA concentration. While some decreases were less than a 1% decrease in antibody concentration per doubling of PFNA concentration, the majority were greater than 5% and several were greater than 10%. There is some uncertainty about the level of adversity of these antibody changes; only one study examined the OR for not being protected against diphtheria (antibody concentrations <0.1 IU/mL), which has clearer clinical significance than continuous changes in antibody levels, and they reported an OR of 1.96 (95% CI 1.07, 3.60), providing support that the changes are functionally relevant. The variability in the results could be related to differences in timing of the boosters, as well as differences in timing of when the antibody measurements were obtained in relation to the last booster. There is some remaining uncertainty resulting from variability in the response by timing/age at exposure, outcome measures, and vaccination exposure type (initial and boosters), which includes different directions in association for diphtheria vaccination in the two subcohorts in Grandjean et al. (2017b); however, overall, the evidence supports an inverse association.

It is plausible that the observed associations with PFNA exposure could be explained by confounding across PFAS, as individuals are not exposed to just one compound in this class of chemicals. Exposure levels of some other PFAS in the Faroe Islands populations were considerably higher (PFOS 17 ng/mL, PFOA 4 ng/mL versus PFNA 1 ng/mL at age 5 years (Grandjean et al., 2012)), and there were moderate correlations between PFNA and PFOS and PFOA (r = 0.48 and 0.54, respectively). There was also a high correlation with PFDA (r = 0.78), although exposure levels to PFDA were comparatively lower (0.3 ng/mL). The authors assessed the possibility of confounding by some of these PFAS in a follow-up paper (Budtz-Jørgensen and Grandjean, 2018a) in which estimates were adjusted for PFOS and PFOA. Details of the analytic modeling from Budtz-Jørgensen and Grandjean (2018a) provided in Budtz-Jørgensen and Grandjean (2018b) show that

the single-PFAS model results were not statistically significant for PFNA for antitetanus antibodies, or for antidiphtheria antibodies in children at age 5 and at age 7 (see Appendix D.1). The effects of PFNA from the single-PFAS models did not show a consistent change when adding control of PFOS and PFOA across the two periods and two antibody endpoints (tetanus and diphtheria), with effect estimates sometimes increasing and at other times decreasing, and even accounting for switching signs. The other available studies did not perform multipollutant modeling that could inform this issue, but some reported correlations between PFAS (Shih et al., 2021; Timmermann et al., 2021; Stein et al., 2016b; Stein et al., 2016a). Correlations were generally moderate to high (mostly 0.4–0.8) between PFNA and PFDA, PFOS, PFOA, and PFHxS. In the other studies of antibody response, specific correlations for each pair of PFAS were not provided, so it is difficult to determine the potential for highly correlated PFAS to confound the effect estimates. Given these mixed findings and the fact that the results for PFNA display a weaker pattern of results than other correlated PFAS such as PFDA, PFHxS, PFOA, and PFOS, with less consistency and smaller magnitude of effect, there is considerable uncertainty in the available evidence as to the extent to which confounding across PFAS may explain the results.

Table 3-26. Summary of PFNA and selected data on antibody response to vaccines in children

Reference, N, confidence	Exposure timing and concentration in serum[a]	Outcome measure timing	Effect estimate as specified	Effect estimate as specified
			Diphtheria vaccine % change (95% CI) per twofold increase in PFNA	Tetanus vaccine % change (95% CI) per twofold increase in PFNA
Grandjean et al. (2012), N = 380–537, *Medium* Grandjean et al. (2017a), *Medium*	Maternal; mean (IQR): 0.6 (0.5–0.8) ng/mL	Children (age 5), prebooster	−14.8 (−31.2, 5.5)	11.2 (−8.6, 35.1)
		Children (age 5), postbooster	−12.9 (−26.7, 3.5)	−3.7 (−23.1, 20.7)
		Children (age 7)	−5.1 (−24.4, 19.2)	22.1 (−4.2, 55.5)
	Children (age 5); mean (IQR): 1.0 (0.8–1.2) ng/mL	Children (age 5), prebooster	−17.7 (−33.0, 1.1)	−5.9 (−21.8, 13.4)
		Children (age 5), postbooster	**−16.1 (−28.8, −1.0)**	−18.2 (−34.0, 1.4)
		Children (age 7)	−17.1 (−32.8, 2.2)	−17.4 (−34.1, 3.6)
	Children (age 7); mean (IQR): 1.1 (0.9–1.5) ng/mL	Children (age 13)	−11.3 (−27.4, 8.5)	31.0 (−2.7, 76.4)

Reference, N, confidence	Exposure timing and concentration in serum[a]	Outcome measure timing	Effect estimate as specified	Effect estimate as specified
	Children (age 13); mean (IQR): 0.7 (0.6–0.9) ng/mL	Children (age 13)	−4.5 (−24.2, 20.2)	15.2 (−16.9, 59.7)
Grandjean et al. (2017b)[b], N = 349, *Medium*	At birth, not reported	Children (age 5), prebooster	4.79 (−18.21 to 34.27)	−7.11 (−26.59, 17.53)
	Infant (18 mo); median (IQR): 1.0 (0.6–1.5) ng/mL	Children (age 5), prebooster	2007–2009 cohort **24.43 (5.72, 46.45)** 1997–2000 cohort −35.28 (−64.95, 19.48)	2007–2009 cohort −6.98 (−21.10, 9.67) 1997–2000 cohort −33.79 (−64.36, 23.01)
	Children (age 5); median (IQR): 1.1 (0.8–−0.6) ng/mL	Children (age 5), prebooster	−8.85 (−23.95, 9.25)	−10.31 (−24.39, 6.40)
Granum et al. (2013), N = 49 *Medium*	Maternal 0–3 d post-delivery; median (IQR): 0.3 (0.2–0.4) ng/mL	Children (age 3)	n/a	−0.01 (−0.41, 0.39)
Timmermann et al. (2021), N = 314, M*edium*	Children (age 7–12); median (IQR): 1.4 (1.1–2.0)	Children (age 7–12)	Adjusted for time since vaccine booster, breastfeeding duration 22 (−6, 58) Additionally adjusted for area of residence −11 (−30, 13)	Adjusted for time since vaccine booster, breastfeeding duration 17 (−5,44) Additionally adjusted for area of residence −19 (−39,9)
	Maternal; median (IQR): 0.9 (0.6–1.3)		−11 (−58, 88)	64 (−18, 228)
			Measles vaccine β (95% CI)	**Rubella vaccine β (95% CI)**
Granum et al. (2013), N = 50 *Medium*	Maternal 0–3 d post-delivery; median (IQR): 0.3 (0.2–0.4) ng/mL	Children (age 3)	−0.55 (−1.51 to 0.41)	**−1.38 (−2.35 to −0.40)**

Reference, N, confidence	Exposure timing and concentration in serum[a]	Outcome measure timing	Effect estimate as specified	Effect estimate as specified
Stein et al. (2016b) N = 1,101–1,190, *Medium*	Children (age 12–19); mean: 0.8 ng/mL	Children (age 12–19)	1.1 (−11.8 to 15.9) (seropositive)	0.6 (−6.7 to 8.5) (seropositive)
Timmermann et al. (2020), N = 237, *Medium*	Children (<1 yr); median (IQR): 0.2 (0.1, 0.3)	Children (<1 yr)	−10 (−22,4)	n/a
		Children (2 yr)	After 1 vaccine (control group) −3 (−18,16) After 2 vaccines (intervention group) 0 (−16, 21)	n/a
			Hib vaccine β (95% CI)	**Mumps vaccine β (95% CI)**
Granum et al. (2013), N = 50, *Medium*	Maternal 0–3 d post-delivery; median: 0.3 ng/mL	Children (age 3)	4.9 (−10.7 to 20.5)	n/a
Stein et al. (2016b) N = 1,101–1,190, *Medium*	Children (age 12–19); mean: 2.5 ng/mL	Children (age 12–19)	n/a	−2.7 (−8.4 to 3.4) (seropositive)

Bold font indicates $p < 0.05$.

[a]Exposure timing is organized into groups based on maternal exposure and childhood exposure (including from birth through age 13).

[b]Results for Faroe Islands Cohort 5 (2007–2009) unless otherwise stated.

Table 3-27. Summary of PFNA and selected data on antibody response to vaccines in adults

Reference, N, confidence	Exposure timing and concentration	Outcome measure timing	Diphtheria vaccine β (95% CI)a	Tetanus vaccine β (95% CI)a	Other vaccine
Shih et al. (2021), Faroe Islands, N = 281, M*edium*	Cord blood; median (IQR): 0.1 (0.1)	Adults (age 28)	% change (95% CI) −1.27 (−15.31, 15.09) Women −11.3 (−28.66, 10.28) Men 9.44 (−11.61, 35.49)	% change (95% CI) −5.88 (−16.69, 6.33) Women −7.01 (−21.83, 10.63) Men −4.78 (−19.72, 12.93)	n/a
	Children (age 7); 0.7 (0.4)		35.02 (−7.9, 97.93) Women 9.76 (−37.77, 93.57) Men 59.27 (−4.09, 164.5)	14.64 (−14.68, 54.05) Women 9.94 (−29.05, 70.37) Men 18.57 (−19.97, 75.65)	
	Children (age 14); 0.7 (0.4)		11.90 (−22.39, 61.36) Women 31.82 (−26.42, 136.16) Men 0.98 (−36.39, 60.32)	−11.02 (−34.9, 21.6) Women −6.38 (−42.99, 53.72) Men −13.85 (−42.01, 27.99)	
	Adults (age 22); 0.9 (0.6)		30.56 (3.39, 64.86) Women 34.31 (−0.4, 81.12) Men 25.02 (−13.64, 80.98)	−5.19 (−21.29, 14.21) Women −6.11 (−26.12, 19.34) Men −3.77 (−28.41, 29.35)	
Kielsen et al. (2016), N = 12, *Low*	Adult (10-d post-vaccination); median (IQR): 0.7 (0.5–0.8) ng/mL	Adult – change from 4 d to 10 d post-vaccination	**−17.90 (−27.99, −6.39)**	−5.96 (−15.41, 4.55)	n/a

Reference, N, confidence	Exposure timing and concentration	Outcome measure timing	Diphtheria vaccine β (95% CI)a	Tetanus vaccine β (95% CI)a	Other vaccine
Stein et al. (2016a), N = 75, *Low*	Adult (18–49 yr old), d of vaccination; mean: 0.8 ng/mL	Adult (18–49 yr old), 30 d post-vaccination	n/a	n/a	FluMist (A H1N1) Seroconversion RR per tertile PFNA by hemagglutinin inhibition: 2nd 6.2 (0.8 to 44.8) 3rd 2.6 (0.3 to 22.7) by immuno-histochemistry: 2nd 1.6 (0.6 to 4.2) 3rd 1.5 (0.6 to 3.9)

Bold font indicates $p < 0.05$.

[a]Linear regression (β or% change in antibody per twofold increase of PFNA). Numbers in parentheses are 95% confidence intervals.

Immunosuppression: Infectious disease

Direct measures of infectious disease incidence or severity, such as respiratory tract infections, pneumonia, gastroenteritis, or otitis media, are relevant for evaluating potential immunotoxicity in humans. Increases in incidence or severity of infectious disease can be a direct consequence of impaired immune function. Given the clear adversity of most infectious diseases, they are generally considered good measures for how immunosuppression can affect individuals and communities. Diagnosis by a healthcare provider is the best way to assess infectious diseases, but these are usually only available for severe diseases and are less likely to be available for infections such as the common cold or gastroenteritis. Self- or caregiver-reported incidence or severity may be less reliable but may be the only way to assess these common ailments. Symptoms of infection are not considered reliable measures of disease because of their lack of specificity.

Twelve studies, including nine prospective birth cohorts, one cohort with exposure measurement in childhood, and two cross-sectional studies examined infectious disease outcomes, with study evaluations summarized in Figure 3-68. Ten of these studies examine effects in children and two in adults (Grandjean et al., 2020) or adults and adolescents (Bulka et al., 2021). Studies in Japan (Goudarzi et al., 2017), Spain (Manzano-Salgado et al., 2019), Denmark (Grandjean et al., 2020), and the United States (Bulka et al., 2021) were *medium* confidence, and the remaining studies were *low* confidence (Kvalem et al., 2020; Timmermann et al., 2020; Impinen et al., 2019; Zeng et al., 2019b; Impinen et al., 2018; Dalsager et al., 2016; Granum et al., 2013). Two of these studies were sub-samples of the Norwegian Mother and Child (MoBa) cohort. The cohort sub-samples for these publications were different, so their study evaluations and results are reported independently, but it is possible that there was some overlap in the participants. The *low* confidence studies were rated as deficient in outcome ascertainment because of the reliance on

parental self-reporting of incidence of common infections or symptoms with no validation of the measures, which is likely subject to recall bias and reduced specificity. However, because the parents are unlikely to know their child's exposure level, this is unlikely to be differential. In contrast, the *medium* confidence studies assessed physician-diagnosed conditions and were limited to more severe illnesses (otitis media, pneumonia, varicella, and respiratory syncytial viral infection), which likely have better recall (Manzano-Salgado et al., 2019; Goudarzi et al., 2017), or used pathogen-specific antibody levels (Bulka et al., 2021) to assess infections. One other study (Zeng et al., 2019b) examined pathogen-specific antibody levels to hand, foot, and mouth disease (HFMD) infection but was considered *low* confidence because of concern for confounding by timing of infection. Grandjean et al. (2020) used biobank samples and national registry data in Denmark to examine severity of COVID-19 illness severity. There was some concern for selection bias in this study because of 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 necessarily a direct measure of immune suppression as other factors may contribute to illness severity.

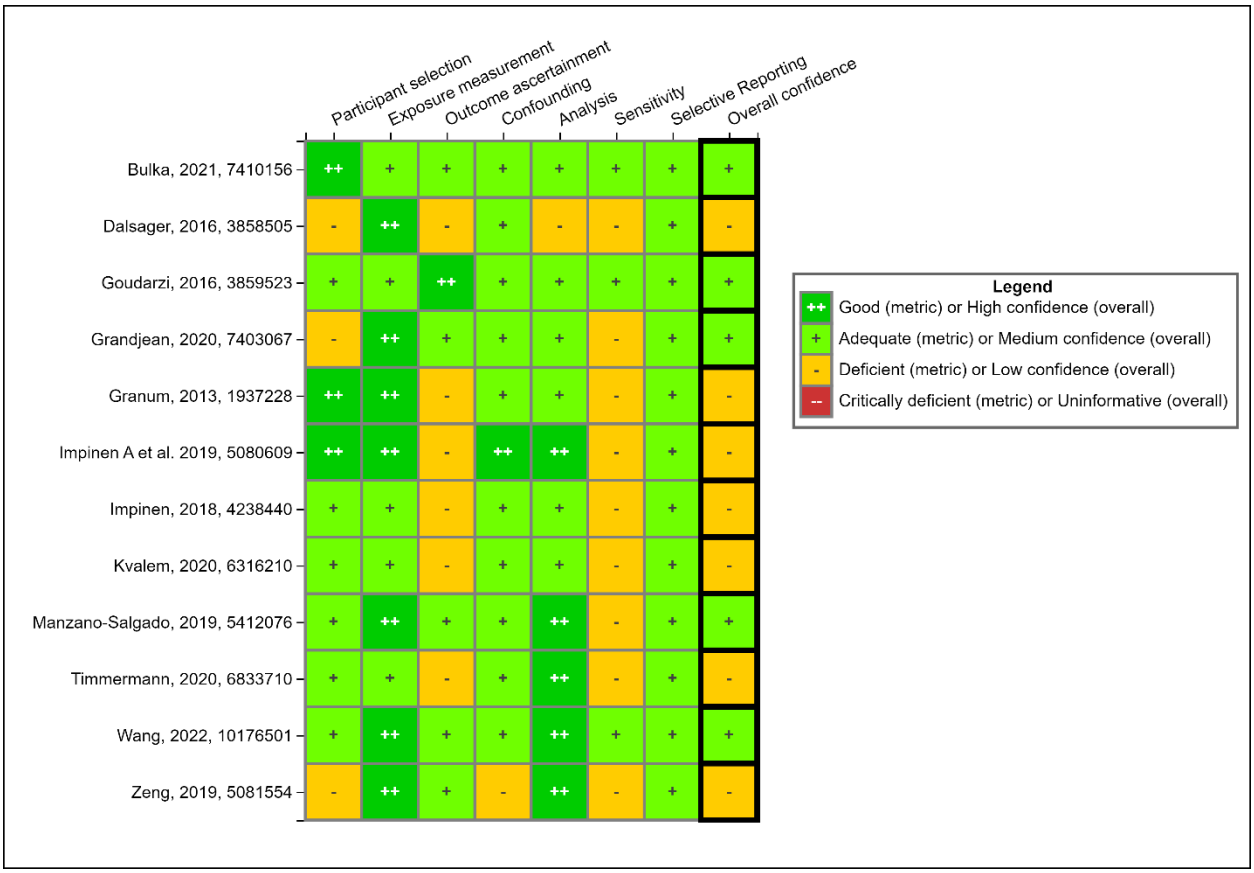


Figure 3-68. Summary of evaluation of epidemiology studies of PFNA and immunosuppression effects (infectious disease). See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-infectious-disease-immunosuppression-8740/.

In children, 5 of the 10 studies reported higher odds of infectious disease with higher PFNA concentrations, but there is considerable inconsistency across specific outcomes within studies and across studies within specific outcomes (see Table 3-28). Among the three *medium* confidence studies in children, only one reported a positive association, with higher odds of lower respiratory tract infection and common cold (Wang et al., 2022). In addition, Impinen et al. (2019) reported higher risk of gastroenteritis, throat infection with streptococcus, and ear infection (from age 6–7). Granum et al. (2013) reported more episodes of common cold with higher exposure, Timmermann et al. (2020) reported higher odds of cough and fever, and Impinen et al. (2018) reported more frequent lower respiratory infection The study of antibody response to HFMD reported lower levels of protective antibody concentrations with higher PFNA exposure ($p < 0.05$ in boys and overall, at birth) (Zeng et al., 2019b). Bulka et al. (2021) reported higher pathogen burden of persistent infections (statistically significant in adolescents) and positive associations with Herpes simplex viruses and *Toxocara spp.* but not cytomegalovirus, Epstein-Barr virus, Hepatitis C or E virus, or *Toxoplaasma gondii*. No clear association was identified with COVID-19 illness severity in Grandjean et al. (2020). Given the inconsistency of the findings, the available evidence on infectious diseases does not inform the immunosuppression observed in the antibody response studies.

Table 3-28. Summary of PFNA and selected data on infectious disease in humans

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFNA results
Total infectious disease[a]	Goudarzi et al. (2017), *medium*	Maternal; median (IQR): 1.2 (0.9–1.6) ng/mL	From birth to age 4	OR (95% CI) Total Q2 1.04 (0.770–1.42) Q3 1.14 (0.842–1.56) Q4 0.918 (0.672–1.25) Trend *p* = 0.748
				Boys: Q2 1.03 (0.673–1.58) Q3 0.899 (0.585–1.38) Q4 0.902 (0.587–1.38) Trend *p* = 0.520
				Girls: Q2 1.09 (0.704–1.70) Q3 1.55 (0.984–2.46) Q4 0.975 (0.617–1.54) Trend *p* = 0.711
Lower respiratory tract infection[b]	Impinen et al. (2018), *low*	Cord blood; median (IQR): 0.2 (0.2–0.2) ng/mL	From birth to age 10	β (95% CI) **0.09 (0.03–0.14)**
	Impinen et al. (2019), *low*	Maternal mid-pregnancy; median	From birth to age 3	RR (95% CI) 1.01 (0.91, 1.13)

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFNA results
		(IQR): 0.5 (0.3–0.6) ng/mL	Age 6–7	0.67 (0.43, 1.05)
	Kvalem et al. (2020), *low*	Child age 10; median (IQR): 0.6 (0.3)	Age 10–16	RR (95% CI) 1.12 (0.94, 1.32)
			Age 16 (last 12 mo)	0.94 (0.52, 1.70)
	Manzano-Salgado et al. (2019), *medium*	Maternal (1st trimester), median (IQR): 0.7 (0.5–0.9) ng/mL	Age 1.5–7	RR (95% CI) 0.95 (0.85, 1.05)
	Wang et al. (2022), *medium*	Maternal; median (IQR): 0.8 (0.6–1.2)	Through age 1	OR (95% CI)
Gastroenteritis (No. episodes/ frequency)	Granum et al. (2013), *low*	Maternal 0–3 d post-delivery; median (IQR): 0.3 (0.2–0.4) ng/mL	From birth to age 3	β (95% CI) 3rd yr −0.46 (−2.27–1.35) All 3 yr −0.10 (−1.36–1.17)
	Impinen et al. (2019), low	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 3	RR **1.06 (1.00, 1.12)**
			Age 6–7	1.08 (0.96, 1.20)
Common cold (No. episodes/ frequency)	Impinen et al. (2018), *low*	Cord blood; median (IQR): 0.2 (0.1–0.2) ng/mL	From birth to age 2	β (95% CI) 0.00 (−0.03–0.03)
	Granum et al. (2013), *low*	Maternal 0–3 d post-delivery; median (IQR): 0.3 (0.2–0.4) ng/mL	From birth to age 3	β (95% CI)[c] **3rd yr 1.24 (0.08–2.40)** **All 3 yr 0.74 (0.05–1.43)**
	Impinen et al. (2019), *low*	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 3	RR (95% CI): 0.99 (0.97, 1.02)
	Kvalem et al. (2020), *low*	Child age 10; median (IQR): 0.6 (0.3)	Age 10–16	OR (95% CI): Reference 1–2 colds 3–5 colds: 1.15 (0.39, 3.40) >5: 1.01 (0.35, 2.89)
			Age 16 (last 12 mo)	OR (95% CI) Reference 0 colds 1–2 colds: 0.86 (0.66, 1.12)

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFNA results
				≥3: 0.63 (0.44, 0.91)
	Wang et al. (2022), *medium*	Maternal; median (IQR): 0.8 (0.6–1.2)	Through Age 1	OR (95% CI) 3.04 (0.77, 11.94) IRR (95% CI) 1.35 (0.81, 2.25)
Cough	Timmermann et al. (2020), *low*	Serum at 4–7 mo; median (IQR): 0.2 (0.1–0.3)	Through 9 mo	OR (95% CI) At 4–7 mo 1.06 (0.78, 1.45) At 9 mo 1.34 (0.87, 2.07)
	Dalsager et al. (2016), *low*	Maternal; median (range): 0.7 (0.2–3.6) ng/mL	Age 1–3	OR for proportion with symptoms (under/above median) Low exposure: Ref Medium: 0.74 (0.43, 1.27) High: 0.59 (0.33, 1.03) IRR for number of d with symptoms Low exposure: Ref Medium: 0.85 (0.66, 1.10) High: 0.82 (0.63, 1.07)
Ear infection	Granum et al. (2013), *low*	Maternal 0–3 d post-delivery; median (IQR): 0.3 (0.2–0.4) ng/mL	From birth to age 3	No significant association with otitis media (data not shown)
	Impinen et al. (2019), *low*	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 3	RR (95% CI): 0.94 (0.88, 1.01)
			Age 6–7	1.12 (0.90, 1.38)
Throat infection with streptococcus	Impinen et al. (2019), *low*	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 3	RR (95% CI): **1.29 (1.11, 1.50)** (no association with other throat infection)
Pseudocrop	Impinen et al. (2019), *low*	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 3	RR (95% CI): 0.98 (0.87, 1.09)
Fever	Timmermann et al. (2020), *low*	Serum at 4–7 mo; median (IQR): 0.2 (0.1–0.3)	Through 9 mo	OR (95% CI) At 4–7 mo 1.29 (0.89, 1.86) At 9 mo 1.20 (0.70, 2.07)

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFNA results
	Dalsager et al. (2016), *low*	Maternal; median (range): 0.7 (0.2–3.6) ng/mL	Age 1–3	OR for proportion of d with symptoms (under/above median) Low exposure: Ref Medium: 1.00 (0.59, 1.71) High: 1.49 (0.86, 2.59) IRR for number of d with symptoms Low exposure: Ref Medium: 0.94 (0.71, 1.24) High: 1.12 (0.84, 1.49)
Diarrhea	Timmermann et al. (2020), *low*	Serum at 4–7 mo; median (IQR): 0.2 (0.1–0.3)	Through 9 mo	OR (95% CI) At 4–7 mo 0.97 (0.63, 1.49) At 9 mo 1.22 (0.73, 2.03)
	Dalsager et al. (2016), *low*	Maternal; median (range): 0.7 (0.2–3.6) ng/mL	Age 1–3	OR for proportion of d with symptoms (under/above median) Low exposure: Ref Medium: 0.75 (0.44, 1.30) High: 0.94 (0.54, 1.65) IRR for number of d with symptoms Low exposure: Ref Medium: 0.46 (0.26, 0.81)* High: 0.74 (0.42, 1.30)
Hand Foot and Mouth Disease Virus Antibodies	Zeng et al. (2019b), *low*	Cord; median (IQR): 0.2 (0.1, 0.3)	Birth and age 3 mo	OR (95% CI) for HFMD antibody concentration below clinically protective level Cord blood: 1.10 (0.79, 1.54) 3 mo: 1.50 (1.04, 2.17)*
Pathogen burden of persistent infections based on antibodies	Bulka et al. (2021), *medium*	Mean: 0.8	Ages 12–49 yr	Relative difference (95% CI) per doubling 12–19 yr: 1.11 (1.03, 1.19)* 20–49 yr: 1.03 (1.00, 1.05) For individual pathogens, Herpes simplex virus and Toxocara spp. had positive association
COVID-19 illness severity	Grandjean et al. (2020), *medium*	Biobank prior to illness; median (IQR): 0.4 (0.2–0.6)	Adulthood	OR (95% CI) for 1 unit increase

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFNA results
				Increased severity based on hospitalization, admission to intensive care and/or death 1.04 (0.54, 2.02) Excluding participants with blood sampled more than 1 wk before or after diagnosis 0.73 (0.25, 2.11)

Bold font indicates $p < 0.05$.
[a]Includes otitis media, pneumonia, respiratory syncytial virus, varicella.
[b]Lower respiratory tract infections include bronchitis, bronchiolitis, and pneumonia.

Sensitization or allergic response

Another major category of immune response is the evaluation of sensitization-related or allergic responses that are a result of exaggerated 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 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. 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. These responses are mediated by circulating factors such as T cells, IgE, and inflammatory cytokines, and 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 or antigen-specific IgE and skin prick tests. Observational tests, such as measures of total IgE levels, are indicators of sensitivity and allergic responses but are not a direct measurement of the response. This section is organized by the different types of measurements, starting with functional measures as the most informative.

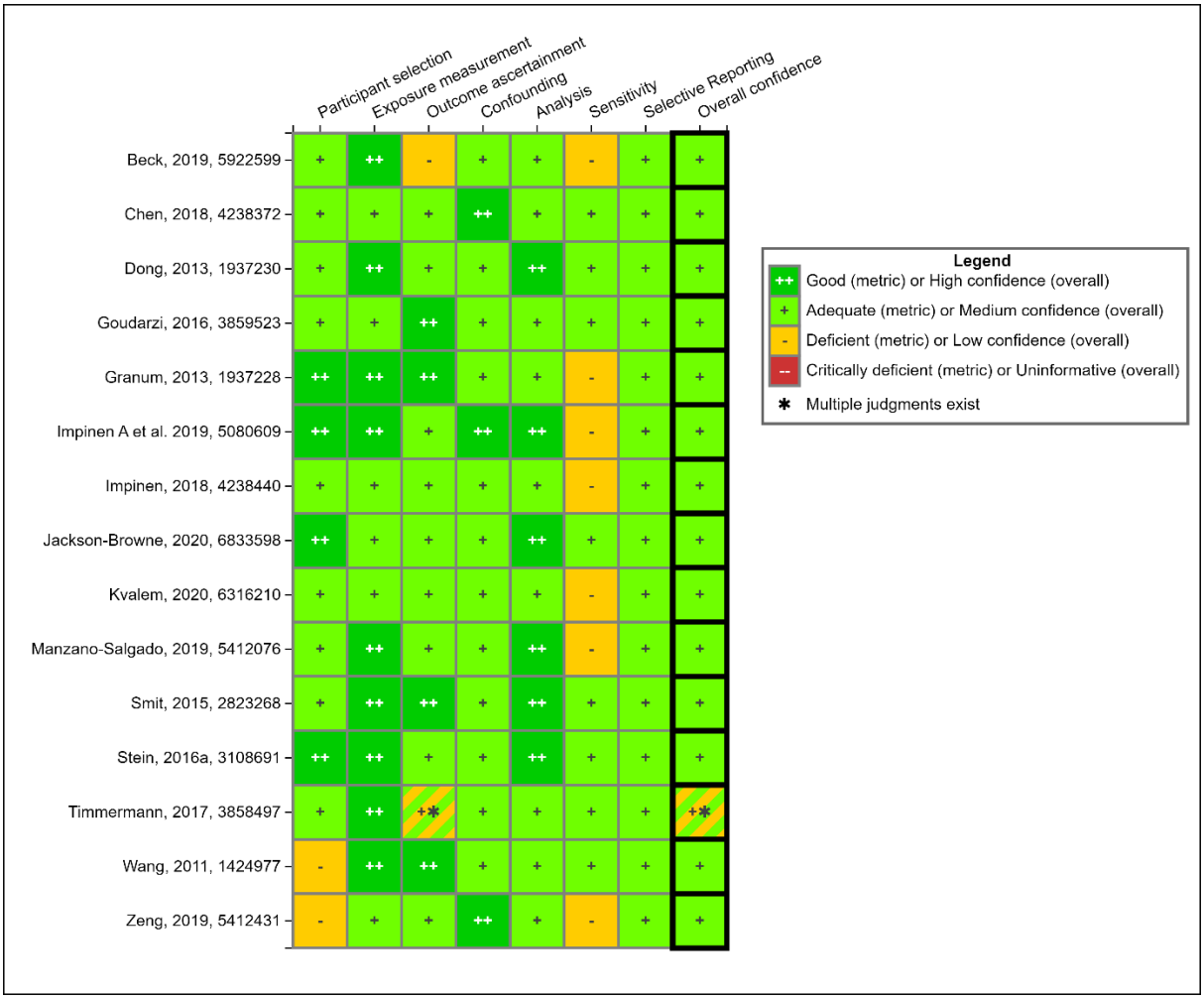


Figure 3-69. Summary of evaluation of epidemiology studies of PFNA and sensitization or allergic response effects. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/pfna-and-hypersensitivity-epidemiology-study-evalu/.

Note: Multiple publications of the same study; Goudarzi et al. (2016) also includes Okada et al. (2014) and Stein et al. (2016b) also includes Buser and Scinicariello (2016) and Humblet et al. (2014) and Dong et al. (2013) also includes Zhu et al. (2016) and Zhou et al. (2017b).

Fifteen studies examined hypersensitivity outcomes in children. The study evaluations are summarized in Figure 3-69. All the studies were *medium* confidence with the exception of asthma in Timmermann et al. (2017a) due to lack of specificity in the outcome measure (parents were asked if their children were "suspected to suffer from asthma"). Among the included studies, as described above for infectious disease, two studies were sub-samples of the Norwegian Mother and Child (MoBa) cohort but were analyzed independently (Impinen et al., 2019; Granum et al., 2013). In addition, three publications of NHANES data are grouped together as one study because there is significant overlap in the populations (Buser and Scinicariello, 2016; Stein et al., 2016b; Humblet et

al., 2014). Ten studies were prospective birth cohorts, with exposure measured during gestation or in cord blood. These were performed in China (Zeng et al., 2019b; Chen et al., 2018), Japan (Goudarzi et al., 2016; Okada et al., 2014), Norway (Impinen et al., 2019; Impinen et al., 2018; Granum et al., 2013), Greenland and Ukraine (Smit et al., 2015), Taiwan (Wang et al., 2011), Spain (Manzano-Salgado et al., 2019), Denmark (Beck et al., 2019), and the Faroe Islands (Timmermann et al., 2017a). In addition, there was a case-control study of asthma in Taiwan reported in multiple publications (Zhou et al., 2017b; Zhu et al., 2016; Dong et al., 2013) and a cohort of children with exposure measured at age 10 (Kvalem et al., 2020). NHANES data, which are cross-sectional, were used for the publications described above (NHANES cycles 1999–2010) as well as an additional study using the 2013–2014 cycle (Jackson-Browne et al., 2020).

Asthma

Twelve studies evaluated different measures related to asthma diagnosis and symptoms in relation to PFNA exposure (see Table 3-29). One study (Dong et al., 2013) examined asthma incidence (i.e., diagnosis within the past year), which is the most specific measure. The remaining studies examined asthma prevalence, either "current" asthma (generally experiencing symptoms in the past year with asthma diagnosis, including new and pre-existing asthma) or "ever" asthma (asthma diagnosis at any time during their life). These measures are less specific and may represent respiratory illnesses more generally. Four studies examined "current" asthma and 11 studies examined "ever" asthma. The single study of asthma incidence reported higher odds of asthma in children 10–15 years of age with higher PFNA exposure, with an exposure-response gradient observed across quartiles in the overall population (Dong et al., 2013). Looking at current asthma, one study (Stein et al., 2016b) out of four reported higher odds, though this was not statistically significant. Two studies also reported a positive association with "ever" asthma, but this was only in a small sub-group (4%, 22 children) of the study population that did not receive MMR vaccination (Timmermann et al., 2017a) and may be due to chance, and in Beck et al. (2019) the association was observed with self-reported asthma and not doctor-diagnosed asthma. In one *medium* confidence study (Manzano-Salgado et al., 2019), an inverse association with maternal PFNA exposure was observed in young children. The remaining studies showed no association with ever asthma. Interactions with sex were observed in Zeng et al. (2019a) and Beck et al. (2019), but in the opposite direction; no interaction with sex was observed in Jackson-Browne et al. (2020).

A strong association (OR > 2 in highest quartile of exposure) with an exposure-response gradient between PFNA exposure and asthma incidence was observed in the study with the most specific outcome measurement (Dong et al., 2013), which is less likely to suffer from outcome misclassification. This study in Taiwan also had exposure levels among the highest of the available studies, and several studies with null results had exposure levels with narrow contrast across participants, which may have reduced sensitivity. While there is considerable uncertainty that is due to inconsistency in the results across studies, the inconsistency may be explained by the better sensitivity and specificity in (Dong et al., 2013).

Allergies/Allergic sensitization

Six studies evaluated allergies and allergic sensitization outcomes (see Table 3-29). Two studies examined food allergies, and both reported higher odds of allergies with higher PFNA exposure (Impinen et al., 2019; Buser and Scinicariello, 2016), although the associations were not statistically significant and the association across quartiles in Buser and Scinicariello (2016) was non-monotonic. Impinen et al. (2019) also examined inhaled allergies and found no increase in odds of allergies. Five studies examined allergic sensitization, and there was no evidence of higher sensitization with higher exposure. Inverse associations were observed in some studies, including a statistically significant finding in Buser and Scinicariello (2016).

Eczema

Ten studies evaluated eczema (see Table 3-29). While the studies used different terminology including eczema, atopic eczema, and atopic dermatitis, most 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. Three studies examined physician-diagnosed atopic eczema, also collected using a questionnaire Impinen et al. (2018), Impinen et al. (2019), Granum et al. (2013), and Kvalem et al. (2020) used a different questionnaire for self-reported eczema. These dermal response conditions can represent hypersensitivity to an antigen exposure from any route. Two *medium* confidence studies reported higher odds of eczema with higher PFNA exposure, but neither was statistically significant, and the other studies reported no association (Chen et al., 2018; Impinen et al., 2018). There is not a clear explanation (e.g., exposure contrast or study confidence) for the differing results.

Observational outcomes

Two epidemiological studies evaluated observational measures of allergic response. Dong et al. (2013) and Stein et al. (2016b) reported statistically significant positive associations between PFNA exposure and total IgE. Dong et al. (2013) also found increases in eosinophilic cationic protein concentration and absolute eosinophilic count with increasing PFNA concentrations in asthmatic children. These findings are coherent with the increase in asthma incidence reported in Dong et al. (2013) and Stein et al. (2016b).

Table 3-29. Summary of PFNA and selected data on hypersensitivity in humans

Reference, confidence	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFNA OR (95% CI)[a] or as specified
Asthma incidence			

Reference, confidence	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFNA OR (95% CI)[a] or as specified
Dong et al. (2013), Medium	Children, current; median (IQR): 0.8 (0.6–1.1) (without asthma)	Children (age 10–15)	Asthma diagnosed in past yr Q2: 1.19 (0.68, 2.09) Q3: 1.54 (0.86, 2.76) **Q4: 2.56 (1.41, 4.65)** **Trend *p* = 0.001**
Current asthma			
Impinen et al. (2019), *Medium*	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 7	0.99 (0.64, 1.52)
Impinen et al. (2018), *Medium*	Cord blood; median (IQR): 0.2 (0.1–0.2) ng/mL	From birth to age 10	1.05 (0.85, 1.29)
Kvalem et al. (2020), *Medium*	Child (age 10); median (IQR): 0.6 (0.3) ng/mL	Child (age 16)	Last 12 mo RR: 0.99 (0.79, 1.23)
NHANES Stein et al. (2016b), *Medium*	Children, current; mean: 0.8 ng/mL	Children (age 12–19)	IQR increase: 1.26 (0.79 2.01)
Ever asthma			
MoBa Granum et al. (2013), *Medium*	Maternal 0–3 d post-delivery; median (IQR): 0.3 (0.2–0.4) ng/mL	From birth to age 3	No significant association (data not shown)
MoBa Impinen et al. (2019), *Medium*	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 7	1.00 (0.73, 1.38)
Smit et al. (2015), *Medium*	Maternal, mean gestational wk 24 or 25; mean (5th–95th): Ukraine: 0.6 (0.3–1.4), Greenland: 0.7 (0.3–2.0)	Children (age 5–9)	0.90 (0.70, 1.14)
Impinen et al. (2018), *Medium*	Cord blood; median (IQR): 0.2 (0.1–0.2) ng/mL	From birth to age 10	0.96 (0.73 1.26)
Kvalem et al. (2020), *Medium*	Child (age 10); median (IQR): 0.6 (0.3) ng/mL	Child (age 10)	Ever asthma RR: 0.94 (0.80, 1.11)
		Child (age 10–16)	Asthma between 10 and 16 yr RR: 0.99 (0.79, 1.23)
Zeng et al. (2019a), *Medium*	Cord blood median (IQR): 0.6 (0.5–0.8)	Child (age 5)	Ever asthma 1.05 (0.15, 7.59) Girls: 0.21 (0.03, 1.47)

Reference, confidence	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFNA OR (95% CI)[a] or as specified
			Boys: 1.10 (0.07, 16.71)
Beck et al. (2019), *Medium*	Maternal, gest wk 8–16; median (IQR): 0.7 (0.5–0.9) ng/mL	Child (age 5)	Ever doctor-diagnosed asthma 0.68 (0.41, 1.14) Boys: 0.58 (0.33, 1.03) Girls: 1.52 (0.47, 4.97) Ever self-reported asthma (≥episodes of wheezing lasting more than a d in past 12 mo) 1.84 (1.03, 3.28) Boys: 2.11 (0.97, 4.58) Girls: 1.50 (0.64, 3.49)
Manzano-Salgado et al. (2019), *Medium*	Maternal (1st trimester), median (IQR): 0.7 (0.5–0.9) ng/mL	Child (age 1.5–7)	Ever asthma **RR: 0.74 (0.57, 0.96)**
Jackson-Browne et al. (2020), *Medium*	Child (age 3–11); median (IQR): 0.7 (0.5–1.1)	Child (age 3–11)	Ever asthma 1.2 (0.8, 1.7)
Timmermann et al. (2017a), *Low*	Maternal, gestational wk 34–36; median (IQR): 0.6 (0.5–0.8)	Child (age 5)	Ever asthma 1.03 (0.67, 1.59)
		Child (age 13)	1.21 (0.77, 1.88)
	Children (age 5); median (IQR): 1.0 (0.8–1.2)	Child (age 5)	**No MMR: 12.52 (1.29, 121.67)**[c] Yes MMR: 0.72 (0.44, 1.18) **Interaction *p* = 0.02**
		Child (age 13)	**No MMR: 6.85 (1.05, 44.69)** Yes MMR: 0.71 (0.44, 1.16) **Interaction *p* = 0.02**
	Children (age 13); median (IQR): 0.7 (0.6–0.9)	Child (age 13)	0.81 (0.52–1.28)
NHANES, Humblet et al. (2014), *Low*	Children (age 12–19); median (IQR): 0.8 (0.5–1.2)	Children (age 12–19)	Continuous: 0.99 (0.88–1.12) T2: 0.95 (0.80, 1.12) T3: 0.99 (0.84, 1.17)
Allergies (food)			
Impinen et al. (2019), *Medium*	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 7	Ever: 1.25 (0.90, 1.74) Current: 1.22 (0.87, 1.71)
NHANES	Children (age 12–19); mean: 0.9 ng/mL	Children (age 12–19)	Q2: 0.83 (0.25 2.75) Q3: 2.09 (0.65, 6.66) Q4: 1.73 (0.54, 5.52)

Reference, confidence	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFNA OR (95% CI)[a] or as specified
Buser and Scinicariello (2016), *Medium*			Trend p = 0.28
Allergies (inhaled)			
Impinen et al. (2019), *Medium*	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 7	Ever: 0.89 (0.65, 1.22) Current: 0.89 (0.53, 1.47)
Allergies (sensitization)			
Impinen et al. (2019); Impinen et al. (2018), *Medium*	Cord blood; median (IQR): 0.2 (0.1–0.2) ng/mL	From birth to age 10	Positive skin prick test or sIgE > 0.35 kU/L 0.95 (0.79, 1.15)
Kvalem et al. (2020), *Medium*	Child (age 10); median (IQR): 0.2 (0.1) ng/mL	Child (age 10)	Positive skin prick test RR: 1.06 (0.91, 1.22) Girls: 1.35 (0.91, 2.02) Boys: 0.94 (0.92, 0.95)
		Child (age 16)	Positive skin prick test RR: 1.03 (0.94, 1.14) Girls: 1.11 (0.86, 1.43) Boys: 0.97 (0.86, 1.10)
Timmermann et al. (2017a), *Medium*	Maternal, gestational wk 34–36; median (IQR): 0.6 (0.5–0.8)	Children (age 13)	Positive skin prick test 0.97 (0.69, 1.38)
	Children (age 5); median (IQR): 1.0 (0.8–1.2)	Children (age 13)	Positive skin prick test 0.79 (0.57, 1.10)
		Children (age 13)	Positive skin prick test 0.82 (0.57, 1.16)
NHANES Buser and Scinicariello (2016), *Medium*	Children (age 12–19); mean: 0.9 ng/mL	Children (age 12–19)	Sensitization (any sIgE >0.35 kU/L) Adj OR (95% CI) Q2: 0.37 (0.12, 1.16) Q3: 0.49 (0.23 1.04) **Q4: 0.51 (0.28, 0.92)** Trend p = 0.15
NHANES Stein et al. (2016b), *Medium*	Children, current; mean: 0.8 ng/mL	Children (age 12–19)	Sensitization (any sIgE >0.35 kU/L) IQR increase: 1.04 (0.80–1.35)
Goudarzi et al. (2017), *medium*	Maternal, gestational wk 28–32; median (IQR): 1.2 (0.9–1.6)	Children (age 4)	Q2: 1.36 (0.89, 2.08) Q3: 0.69 (0.44, 1.08) Q4: 0.87 (0.56, 1.35)

	Reference, confidence	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFNA OR (95% CI)[a] or as specified
Eczema				
MoBa	Granum et al. (2013), *Medium*	Maternal 0–3 d post-delivery; median (IQR): 0.3 (0.2–0.4)	From birth to age 3	Eczema and itchiness or doctor-diagnosed atopic eczema: No significant association (data not shown)
MoBa	Impinen et al. (2019), *Medium*	Maternal mid-pregnancy; median (IQR): 0.5 (0.3–0.6) ng/mL	From birth to age 7	Ever: 0.90 (0.70, 1.17) Current: 0.78 (0.55, 1.10)
	Kvalem et al. (2020), *Medium*	Child (age 10); median (IQR): 0.2 (0.1) ng/mL	Child (age 10)	Ever doctor diagnosed: RR: 0.96 (0.83, 1.10)
			Child (age 10–16)	Ever between 10 and 16 yr RR: 0.83 (0.65, 1.08) Girls: 0.51 (0.35, 0.73) Boys: 0.98 (0.75, 1.28)
			Child (age 16)	Current (last 12 mo) RR: 0.86 (0.94, 1.16)
	Manzano-Salgado et al. (2019), *Medium*	Maternal (1st trimester), median (IQR): 0.7 (0.5–0.9) ng/mL	Child (age 1.5–7)	Ever eczema RR: 0.95 (0.85, 1.06)
Hokkaido	Goudarzi et al. (2017), *Medium*	Maternal, gestational wk 28–32; median (IQR): 1.2 (0.9–1.6)	Children (age 4)	Q2: 1.03 (0.718, 1.48) Q3: 0.90 (0.620, 1.30) Q4: 0.78 (0.528, 1.15) Trend *p* = 0.18
Hokkaido	Okada et al. (2014), *Medium*		Children (age 1 or 2)	Q2: 0.97 (0.70, 1.35) Q3: 0.94 (0.69, 1.29) Q4: 0.77 (0.55, 1.08) Trend *p = 0.15* Girls only **Q2: 0.55 (0.32, 0.91)** Q3: 0.74 (0.47, 1.16) Q4: 0.63 (0.38, 1.02) Trend *p* = 0.122
	Smit et al. (2015), *Medium*	Maternal, mean gestational wk 24 or 25; mean (5th–95th): Ukraine: 0.6 (0.3–1.4), Greenland: 0.7 (0.3–2.0)	Children (age 5–9)	Ever: 0.94 (0.78, 1.14) Current: 1.03 (0.82, 1.30)
	Chen et al. (2018), *Medium*		Children (age 2)	1.53 (0.94, 2.47) per log-unit increase

Reference, confidence	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFNA OR (95% CI)[a] or as specified
	Cord blood; median (IQR): 0.6 (0.5–0.9) ng/mL		Q2: 1.15 (0.67, 1.97) Q3: 1.20 (0.71, 2.05) Q4: 1.47 (0.87, 2.50) Trend p = 0.15
			Girls only **2.22 (1.07, 4.58)** per log-unit increase Q2: 1.10 (0.46, 2.62) Q3: 1.30 (0.57, 2.97) Q4: 2.14 (0.97, 4.74) **Trend p = 0.05**
Impinen et al. (2018), *Medium*	Cord blood; median (IQR): 0.2 (0.1–0.2) ng/mL	From birth to age 10	0–2 yr of age 1.03 (0.85, 1.24) Ever in 10 yr 1.12 (0.75, 1.68)
Timmermann et al. (2017a), *Medium*	Maternal, gestational wk 34–36; median (IQR): 0.6 (0.5–0.8)	Children (age 13)	Atopic eczema 0.81 (0.55, 1.20)
	Children (age 5); median (IQR): 1.0 (0.8–1.2)	Children (age 13)	0.79 (0.51, 1.23)
		Children (age 13)	No MMR: 371.98 (0.16, 8.62×10^5)[b] Yes MMR: 0.77 (0.49, 1.22) Interaction p = 0.12
Wang et al. (2011), *Medium*	Cord blood; mean (SE): 1.98 (0.6)	From birth to age 2	Q2: 1.46 (0.35, 6.07) Q3: 1.53 (0.59, 3.93) Q4: 0.72 (0.23, 2.21)
Total IgE			
Wang et al. (2011), *Medium*	Cord blood; mean (SE): 1.98 (0.6)	Cord blood	β: 0.02 (0.15)
		Children (age 2)	β: 0.04 (0.47)
Timmermann et al. (2017a), *Medium*	Maternal, gestational wk 34–36; median (IQR): 0.6 (0.5–0.8)	Cord blood	% difference per doubling PFNA 0.94 (−17.67–23.75)
		Children (age 7)	% difference per doubling PFNA 21.11 (−11.70–66.12)
	Children (age 5); median (IQR): 1.0 (0.8–1.2)	Children (age 7)	% difference per doubling PFNA −5.02 (−27.83–25.00)
NHANES, (Stein et al., 2016b), *Medium*	Children, current; mean: 0.8 ng/mL	Children (age 12–19)	% change per doubling PFNA **19.4 (8.3–31.7)**

Reference, confidence	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFNA OR (95% CI)[a] or as specified
Dong et al. (2013), *Medium*	Children, current; median (IQR): 0.8 (0.6–1.1) (without asthma)	Children (age 10–15)	Mean IgE (95% CI) quartiles PFNA in asthmatics Q1: 410.9 (230.6–591.2) Q2: 704.5 (524.1–884.9) **Q3: 828.8 (651.6–1006.0)** **Q4: 790.9 (610.1–971.6)** **Trend *p* = 0.001**

Bold font indicates $p < 0.05$.

[a]All estimates are presented as OR (95% CI) for the odds of the outcome per twofold increase in PFNA concentration unless otherwise stated.

[b]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.

Summary of human immune studies

The epidemiologic evidence of an association between PFNA exposure and immune effects is driven primarily by studies of antibody response following vaccination. Despite imprecision in the results, the antibody results present a consistent pattern of findings that higher prenatal and childhood concentrations of PFNA were associated with suppression of at least one measure of the antigen-specific antibody response to common vaccines. This consistency is observed across multiple well-conducted studies, including two birth cohorts in the Faroe Islands, a birth cohort in Norway, and a cross-sectional study in the United States, supported by a *low* confidence study of adults in Denmark. However, while there was consistency across vaccination types, there were only two statistically significant results showing immunosuppression, one for rubella (Granum et al., 2013) and one for diphtheria (Grandjean et al., 2012); in contrast a statistically significant positive association (indicating higher immune response) was reported in Grandjean et al. (2017b). The other available studies of immunosuppression, specifically examining infectious disease, were inconsistent and did not inform the interpretation of immunosuppression. Despite the general consistency in the antibody response studies, there is considerable remaining uncertainty in the antibody response evidence resulting from potential confounding across PFAS as well as variability in the response by age of exposure and outcome measures and vaccination exposure type (initial and boosters).

The evidence for hypersensitivity was less consistent and had more uncertainties. One study reported a clear positive association with asthma incidence and an exposure-response gradient. There are possible explanations for null results in other studies of "current" and "ever" asthma, including greater potential for outcome misclassification and reduced sensitivity based on exposure levels. Other outcomes including allergies/allergic sensitization, and dermal allergic measures had inconsistent findings.

Animal Studies

Seven short-term exposure studies in rodents evaluated PFNA effects on endpoints relevant to immune system responses after oral dosing, including a *high* confidence 28-day exposure study by NTP (2018), five *medium* confidence 14-day exposure studies by Fang and coauthors (Fang et al., 2012b; Fang et al., 2012c; Fang et al., 2010; Fang et al., 2009; Fang et al., 2008) and one *low* confidence study by Lee and Kim (2018) (see Table 3-30). The available studies provide relevant information for the consideration of immunosuppression and sensitization and allergic response. There is no animal evidence to address the potential for autoimmunity following PFNA exposure; therefore, this category is not discussed further.

Direct measures of disease resistance or immune function are the most sensitive and predictive endpoints for the identification of immunotoxicants (IPCS, 2012; Luster et al., 2005). Such measures include assays of host resistance and T-cell-dependent antibody responses. Observational parameters evaluated in the resting immune system, such as changes in immune organ weights or immune cell populations, may also indicate potential immunotoxicity and support functional data, but these are less sensitive and predictive (Luster et al., 2005). Therefore, more weight is given to results of functional assays.

The PFNA animal evidence base is limited to one *low* confidence short-term immune function assay on sensitization and allergic responses, with the remaining studies evaluating potential immunosuppressive effects based on changes in observational parameters. The lack of functional assays and longer duration exposures represent significant evidence base limitations. The short-term testing with PFNA was generally sound for the immunotoxicity endpoints examined, except most histopathological results that were found to be deficient due to insufficient reporting of methods employed and presentation of quantitative results. The cytokine data reported in Fang et al. (2012b) were judged to be *medium* confidence. The NTP 28-day study also reported high mortality in adult male and female rats at the two highest doses tested (see Section 3.2.1, "Adult rodent mortality"). Thus, this synthesis considers PFNA treatments at 2.5 mg/kg-day in males and 6.5 mg/kg-day in females to be the highest dose with reportable results for all outcomes except the histopathological results where tissue analyses were performed at the time of early death (NTP, 2018). However, it should be noted that males receiving 2.5 mg/kg-day PFNA showed significant body weight loss, averaging 44% (NTP, 2018), and thus interpretation of immune outcomes at this dose level were considered to be inconclusive. It is possible that any PFNA immunomodulation at doses causing substantial body weight decreases or mortality could be a secondary stress response to overt systemic toxicity (IPCS, 2012; U.S. EPA, 2012a). Thus, as discussed in the synthesis below, effects reported at higher doses with coinciding substantial body weight loss are less interpretable as direct PFNA immunotoxicity than effects observed at doses that did not result in overt toxicity. Notably, body weight reductions exceeding 10%, which is considered biologically significant (see Section 3.2.1, "Adult rodent body weight"), were observed in male rodents at doses ≥1.25 mg/kg-day in the NTP (2018) study, and generally at ≥3 mg/kg-day in

some of the 14-day studies (Fang et al., 2012c; Fang et al., 2009; Fang et al., 2008). Results at these doses are discussed below with the acknowledgment of some uncertainty due to the observed weight loss.

Table 3-30. Overall evaluation results of immunotoxicity studies examining the effects of PFNA exposures in rodents[a]

Author (year)	Species, strain (lifestage/sex)	Exposure design	Exposure route and dose range	Organ weight	Histopathology	Immune response markers
Functional assays, hypersensitivity, or allergic response						
Lee and Kim (2018)	Mouse, ICR (adult male)	Short-term 14 d (d 9, 11, 13)[b]	Gavage 0, 100 mg/kg-d	NM	NM	-
Observational assays						
Fang et al. (2008)	BALB/c Mice (adult male)	Short-term 14 d	Gavage 0, 1, 3, 5 mg/kg-d	+	-	+
Fang et al. (2009)	Rat, Sprague-Dawley (adult male)	Short-term 14 d	Gavage 0, 1, 3, 5 mg/kg-d	+	-	++
Fang et al. (2010)	Rat, Sprague-Dawley (adult male)	Short-term 14 d	Gavage 0, 1, 3, 5 mg/kg-d	+	-	+
Fang et al. (2012b)	Rat, Sprague-Dawley (adult male)	Short-term 14 d	Gavage 0, 0.2, 1, 5 mg/kg-d	NM	NM	+
Fang et al. (2012c)	Rat, Sprague-Dawley (adult male)	Short-term 14 d	Gavage 0, 0.2, 1, 5 mg/kg-d	NM	NM	+
NTP (2018)[b]	Rat, Sprague-Dawley (Harlan)(adult male)	Short-term 28 d	Gavage 0, 0.625, 1.25, 2.5, 5, 10 mg/kg-d	++	++	++
NTP (2018)[c]	Rat, Sprague-Dawley (Harlan)(adult female)	Short-term 28 d	Gavage 0, 1.56, 3.12, 12.5, 6.2, 12, 25 mg/kg-d	++	++	++

[a]Dark green (++) = *high* confidence; light green (+) = *medium* confidence; yellow (-) = *low* confidence; red (--) = *uninformative*; NM = not measured. Study evaluation details for all outcomes are available in HAWC.

[b]The exposure time for this study was 5 days (days 9–13), although the entire study was 14 days.

[c]5 and 10 mg/kg-day dose groups not evaluated due to high mortality, except for the histopathology that was evaluated at time of death.

[d]12.5 and 25 mg/kg-day dose groups not evaluated due to high mortality, except for the histopathology that was evaluated at time of death.

Immune function assays

Hypersensitivity or allergic responses

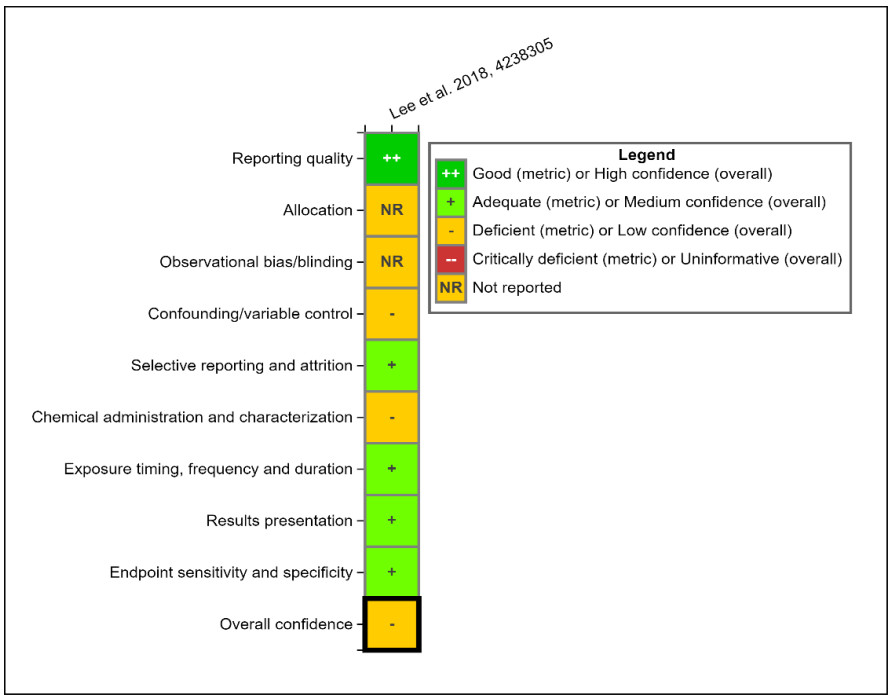


Figure 3-70. Study evaluation heat map for sensitization and allergic responses in animals. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Immune-function-assays/.

PFNA effects on allergic inflammatory responses were examined in ovalbumin (OVA)-induced, adult male ICR mice orally exposed to PFNA at 100 mg/kg 3 times over 14 days (total 5-day exposure duration), specifically days 9, 11, and 13 (Lee and Kim, 2018). This study was found to be *low* confidence overall with some deficiencies in reporting of chemical exposure methods and measures of overt toxicity (i.e., body weight loss) that may have occurred due to the high dosing level, potentially confounding results interpretation (see Figure 3-70). The high dosing level also decreased confidence in the exposure timing, frequency, and duration domain and may have affected sensitivity to the responses (e.g., the exposure did not start until after the sensitization phase, which means that the study is only able to inform potential effects on elicitation of a reaction). Nonetheless, OVA-induced active systemic anaphylaxis (ASA) is a well-accepted model for evaluating mast cell functions and immediate-type hypersensitivity reactions (Evans et al., 2014; Ribeiro-Filho et al., 2014). Short-term intermittent exposures exacerbated some responses to OVA-induced ASA as evidenced by significant hypothermia (i.e., 8% decrease in rectal temperature) and increased serum levels of the inflammatory mediators histamine (64%) and tumor necrosis factor alpha (TNFα) (55%) relative to OVA-induced controls (Lee and Kim, 2018). No statistically

significant changes in immunoglobulins (IgE and IgG1) were observed in male OVA-induced mice in comparison to the OVA-induced controls.

General/Observational immune assays

Immune response markers (WBC populations and lymphocyte proliferation)

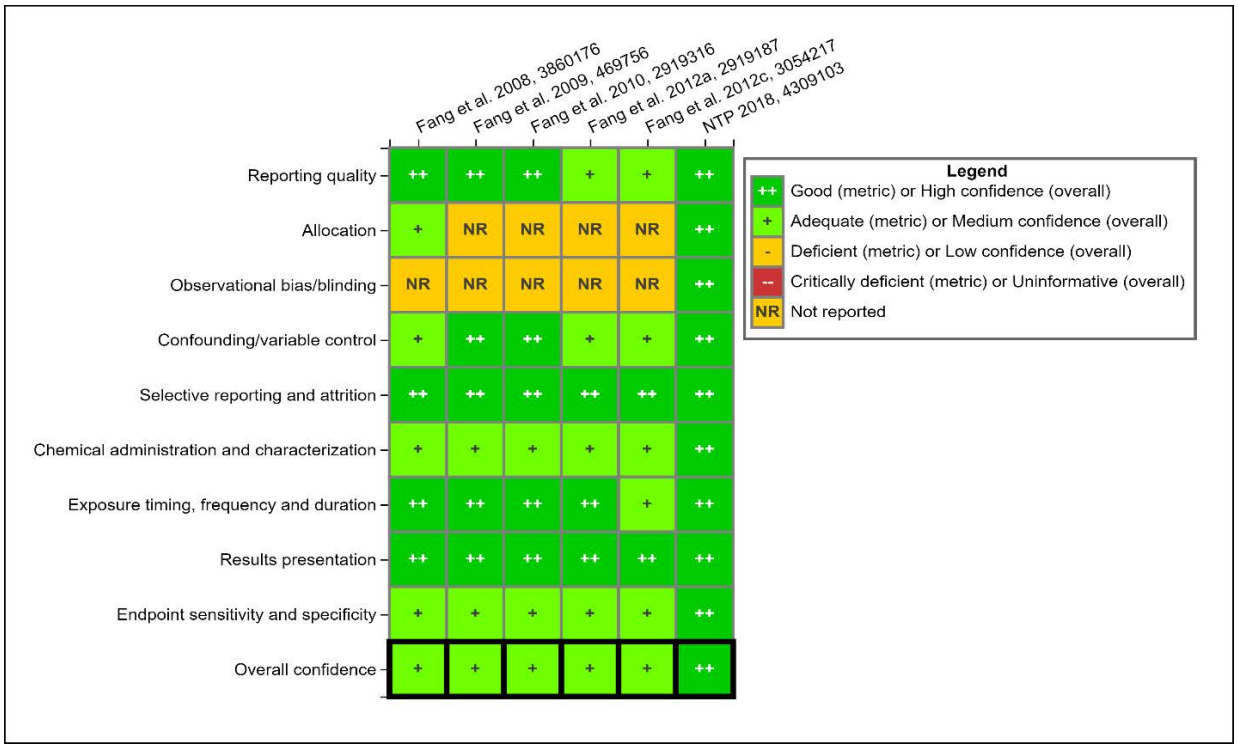


Figure 3-71. Study evaluation heat map for immune response markers in animals. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Immune-response-markers/.

Six studies examined the effects of short-term PFNA exposures on immune response markers, including WBC populations and lymphocyte proliferation in adult male and female SD rats and adult male BALB/c mice (NTP, 2018; Fang et al., 2012b; Fang et al., 2012c; Fang et al., 2010; Fang et al., 2009; Fang et al., 2008). These endpoints were found to be *high* and *medium* confidence with only minor limitations including somewhat limited samples sizes and lack of blinding or randomization (see Figure 3-71).

NTP (2018) observed significant reductions in WBC counts (eosinophils, leukocytes, lymphocytes, and neutrophils) in adult male SD rats exposed to PFNA from 1.25 to 2.5 mg/kg-day for 28-days (see Figure 3-72). This observation included statistically significant reductions in eosinophils (50%), leukocytes (48%), and lymphocytes (total and subsets, 54%) in male rats at 2.5 mg/kg-day PFNA, and significant reductions (37%) in neutrophil counts at 1.25 mg/kg-day but not higher doses. No changes in monocytes or basophils were observed in male rats. Importantly,

the effects seen at 2.5 mg/kg-day may have been affected by large reductions in body weight (44%), so these results were considered inconclusive. In adult female rats, reductions of blood neutrophils were reported, with statistically significant depressed counts (35%) at 6.25 mg/kg-day. Eosinophil, leukocyte, monocyte, basophil, and lymphocyte counts in females were unaffected by PFNA (NTP, 2018).

Fang et al. (2008) administered PFNA orally to adult male BALB/c mice for 14 days at 0, 1, 3, and 5 mg/kg-day. Statistically significant decreases in the proportions of immune cell markers for F4/80+ (macrophages, decreases of 14%, 42%, 36%, respectively) and CD49b+ (NK cells, decreases of 21%, 54%, 69%, respectively) were observed in the spleen at all doses tested (1, 3, and 5 mg/kg-day). These results suggest damage to certain splenocyte populations. Significant decreases in splenic CD11c+ dendritic cells and reductions in CD4+CD8+ T cells in the thymus were only observed in the Fang et al. (2008) study at dose levels ≥3 mg/kg-day, which was associated with overt toxicity. PFNA had no effect on splenic T-cell quantities (CD8+, CD4+, TCR+) or responses of splenic T lymphocytes to concanavalin A (Fang et al., 2008).

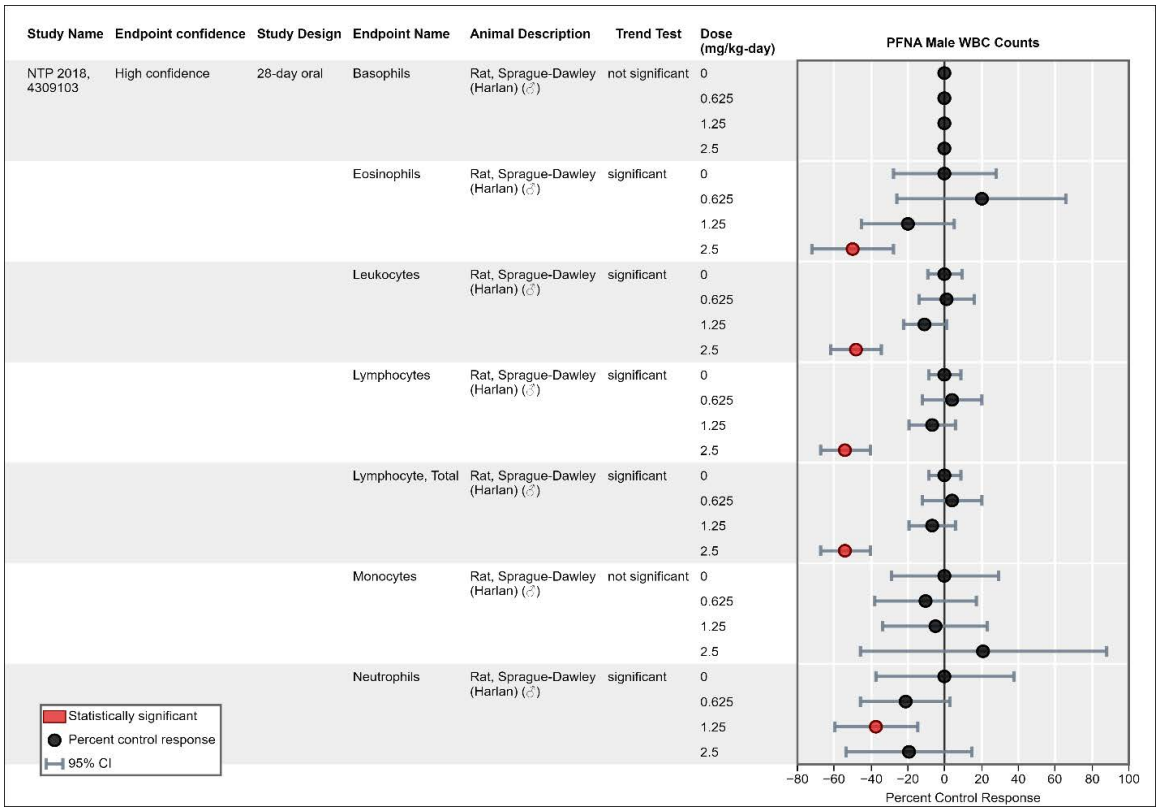


Figure 3-72. Male rat blood WBC counts after 28-days of PFNA exposure (NTP, 2018). See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-adult-male-wbc-counts/.

Organ weights

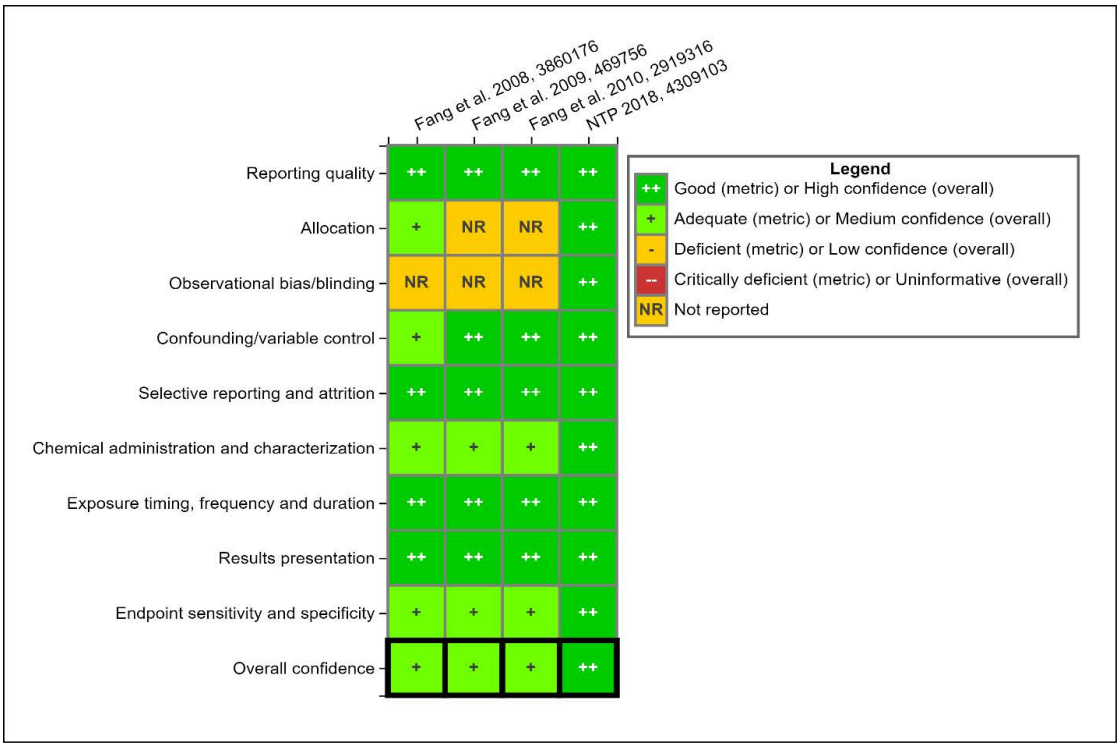


Figure 3-73. Study evaluation heat map for immune system organ weights in animals. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Immune-organ-weights/.

Four of the short-term PFNA exposure studies examined effects on spleen and thymus weights of adult rodents, specifically male and female rats (NTP, 2018), male rats (Fang et al., 2010; Fang et al., 2009), or male mice (Fang et al., 2008). These organ weight data were found to be *high* and *medium* confidence with limitations such as lack of information describing randomization and limited sample size (see Figure 3-73). Some testing in rats suggests spleen weight changes to be proportional to body weight changes and thymus to lack correlation to body weight (Nirogi et al., 2014). However, given the limited study of these relationships for immune organs, both absolute and relative spleen and thymus weights can be inferred as potentially relevant for interpreting results for these endpoints.

Organ weights in males were examined in all available studies. (NTP, 2018) reported dose-dependent reductions in absolute and relative weights of the thymus and spleen of male rats administered PFNA for 28-days, which were statistically significant at doses ≥1.25 mg/kg-day (see Figure 3-74). Significant reductions in absolute and relative spleen weights of adult males were observed at 1.25 mg/kg-day (58%, 24%, respectively) and at 2.5 mg/kg-day (70%, 35%, respectively). Absolute thymus weights were significantly reduced by 78% and 84% at 1.25 and 2.5 mg/kg-day PFNA, respectively, while relative thymus weights were reduced by 62% and 65% at

these dose levels. Consistent with NTP, 14-day exposure studies in adult male rats and mice also reported dose-dependent reductions in spleen and thymus weights, although mostly at doses with body weight loss (Fang et al., 2010; Fang et al., 2009; Fang et al., 2008). One potential exception was Fang et al. (2010), which reported statistically significant reductions in absolute spleen weight in adult male rats at 1, 3, and 5 mg/kg-day PFNA (23%, 29%, 59%, respectively), with relative spleen weight also dose dependently decreased but only statistically significant at the high dose. Fang et al. (2010) did not report on body weight but other studies by this group report significant body weight loss in adult male rats and mice at doses generally ≥3 mg/kg-day (Fang et al., 2012c; Fang et al., 2009; Fang et al., 2008). Therefore, it can be inferred that absolute spleen weight decreases at the 1 mg/kg-day dose occurred in the absence of overt toxicity. Statistically significant reductions in absolute thymus weight were reported at 3 and 5 mg/kg-day (20%, 86%, respectively), with significant reductions in relative thymus weight at the high dose (Fang et al., 2009). Similar to male rats, dose-dependent reductions in absolute and relative spleen and thymus weights were observed in adult male BALB/c mice at doses concurrent with body weight loss with statistically significant differences at 3 and 5 mg/kg-day (Fang et al., 2008). Fang et al. (2008) observed absolute spleen weight reductions by 34% and 45%, and absolute thymus weight was reduced by 34% and 64%, at 3 and 5 mg/kg-day, respectively. Relative spleen and thymus weights were reduced by 29% and 58%, respectively, at 5 mg/kg-day. Thus, altogether, the reported significant trends of reductions in spleen and thymus weights in male rodents were not possible to interpret due to body weight loss reported at higher doses.

The only animal study to examine organ weights in females was in NTP (2018). In contrast to the inconclusive results in males, significant dose-dependent reductions in absolute and relative spleen weights were reported at doses where there were no or modest (10%) reductions in body weight (see Figure 3-75). PFNA effects on spleen weights were smaller than those observed in males, and thymus weight was unaffected by exposure in females. The absolute spleen weight of adult females was statistically significantly reduced by 20% and 38% at 3.25 and 6.5 mg/kg-day, respectively, with a significant reduction in relative spleen weights (11%) at 6.5 mg/kg-day. Given that reductions in female organ weights were observed with no or modest body weight loss and one finding of spleen weight reductions in males in the absence of overt toxicity, it is plausible that similar responses in males are a direct effect of PFNA exposure, which may be exacerbated by overt toxicity.

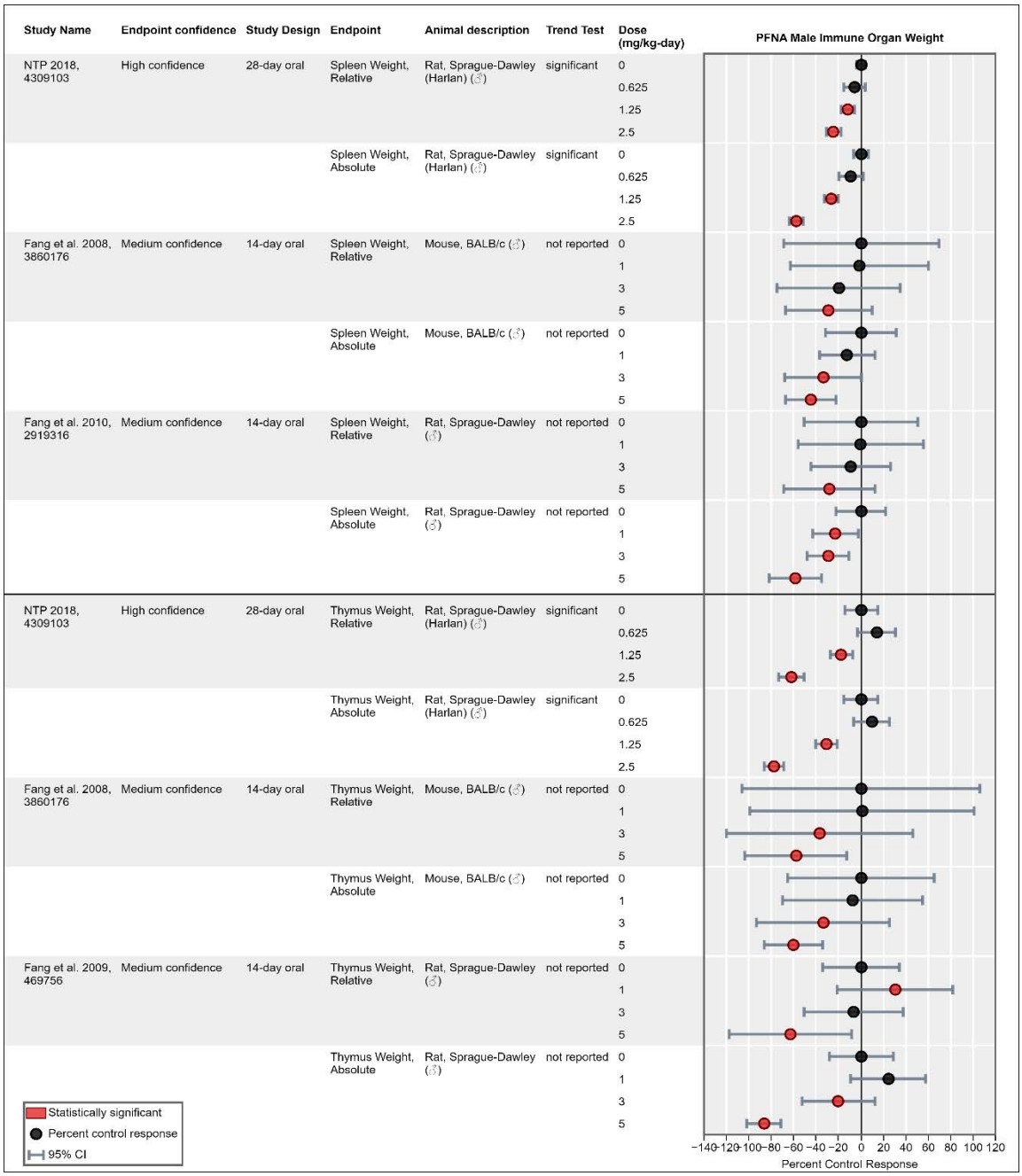


Figure 3-74. Male rodent relative and absolute spleen weights (top) and relative and absolute thymus weights (bottom). See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-male-immune-organ-weight/.

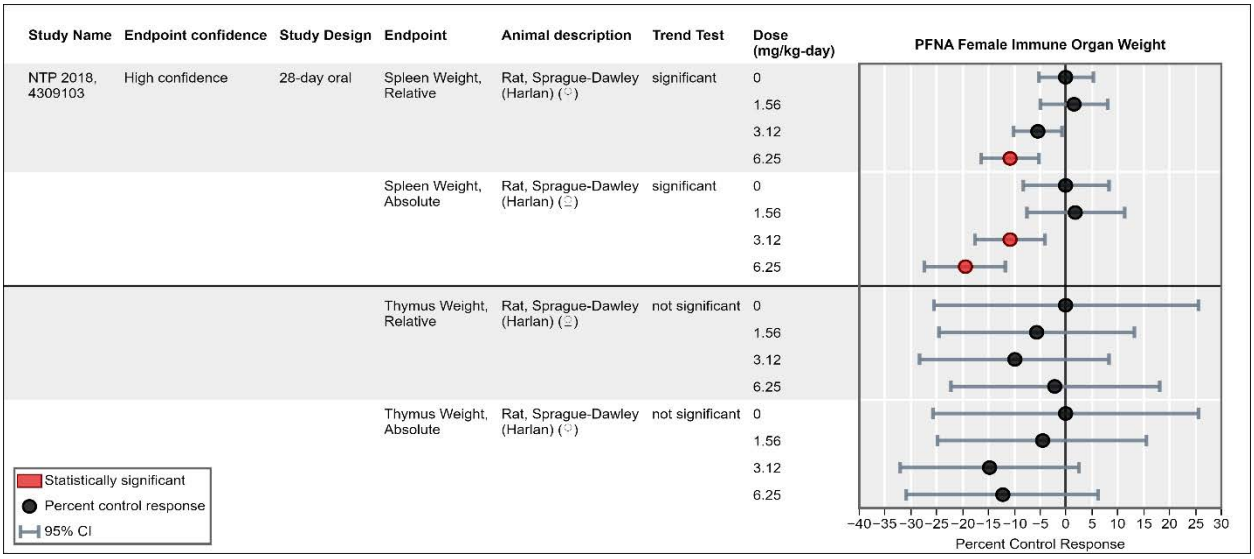


Figure 3-75. Female rodent relative and absolute spleen weights (top) and relative and absolute thymus weights (bottom). See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-female-immune-organ-weight/.

1 *Histopathology*

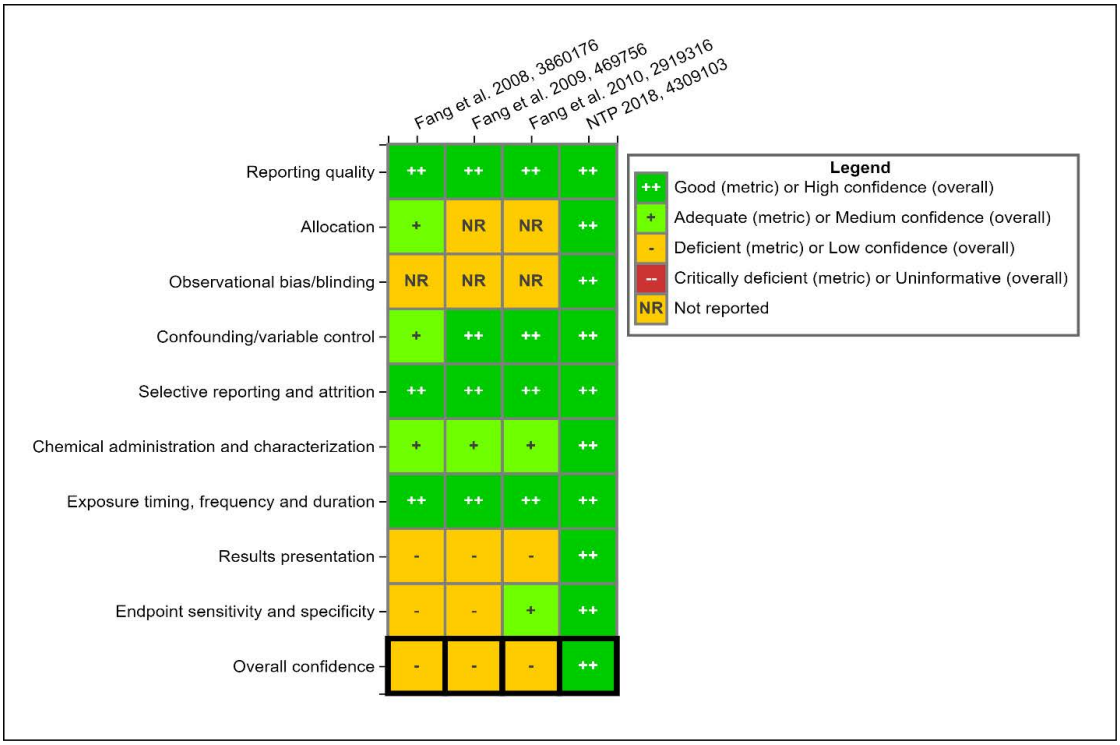


Figure 3-76. Study evaluation heat map for immune organ histopathology in animals. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Immune-histopathology/.

Four studies evaluated PFNA effects on immune organ histopathology, including the *high* confidence 28-day exposure study (NTP, 2018) and three 14-day exposure studies by Fang and coauthors (Fang et al., 2010; Fang et al., 2009; Fang et al., 2008). The histopathology data in the 14-day exposure studies were found to be *low* confidence because of deficiencies in endpoint and results presentation domains and inadequate methodological reporting (see Figure 3-76). Histopathological findings from NTP (2018) were attributed to stress and limited to PFNA doses associated with mortality and overt toxicity in both males and females, and therefore are not compelling indicators of immunotoxicity. Splenic and lymph node atrophy were characterized by reductions in the number of lymphocytes, and thymic atrophy was indicated by decreased thickness of the cortex (attributable to decreased lymphocytes) (NTP, 2019). In summary, administration of PFNA at 2.5, 5, and 10 mg/kg-day caused thymic atrophy and bone marrow hypocellularity in all male animals, with no incidences at lower doses (see Table 3-31) (NTP, 2018). In addition, significant splenic and lymph node atrophy was reported among males in the 5 and 10 mg/kg-day dose groups but with early mortality. In female rats significant splenic, thymic, and lymph node atrophy; and bone marrow hypocellularity; were observed in animals dosed with PFNA at 12.5 and 25 mg/kg-day (doses that resulted in early mortality), with no incidences reported at lower doses (NTP, 2018). Consistent with these results, the *low* confidence 14-day studies by Fang and coauthors reported spleen and thymus atrophy/apoptosis in male rodents at dose levels resulting in overt toxicity.

Table 3-31. Incidence and severity of immune organ histopathological lesions due to PFNA exposure in the (NTP, 2018) 28-day oral toxicity study[a,b]

Animal group	Dose (mg/kg-d)										
	0	0.625	1.25	1.56	2.5	3.12	5	6.25	10	12.5	25
Atrophy, spleen											
Male SD rats	0 (0/10)	0 (0/10)	0 (0/10)		0 (0/10)		***3.6 (7/8)* t = 24 d***		***4.0 (9/10)* t = 15 d***		
Female SD rats	0 (1/10)			0 (0/10)		0 (0/10)		0 (0/10)		***4.0 (8/10)* t = 15 d***	***4.0 (10/10)* t = 11 d***
Atrophy, thymus											
Male SD rats	0 (0/10)	0 (0/10)	0 (0/10)		***3.3 (10/10)* t = 29 d***		***4 (10/10)* t = 24 d***		***4.0 (8/8)* t = 15 d***		
Female SD rats	0 (0/10)			0 (0/10)		0 (0/10)		0 (0/10)		***4.0 (9/10)* t = 13 d***	***4.0 (10/10)* t = 11 d***
Atrophy, mandibular lymph node											
Male SD rats	0 (0/10)	0 (0/10)	0 (0/10)		1.5 (2/10) t = 29 d		***3.8 (5/7)* t = 24 d***		***2.2 (5/10)* t = 16 d***		
Female SD rats	0 (0/10)			0 (0/10)		0 (0/10)		0 (0/10)		***3.4 (8/8)* t = 13 d***	***3.1 (9/9)* t = 11 d***
Atrophy, mesenteric lymph node											
Male SD rats	0 (0/10)	0 (0/10)	0 (0/10)		***3.0 (3/10) t = 29 d***		***3.7 (6/8)* t = 24 d***		***3.2 (9/10)* t = 15 d***		
Female SD rats	0 (0/10)			0 (0/10)		0 (0/10)		0 (0/10)		***4.0 (8/9)* t = 13 d***	***2.9 (9/10)* t = 11 d***
Bone marrow hypocellularity											
Male SD rats	0 (0/10)	0 (0/10)	0 (0/10)		***3.4 (10/10)* t = 29 d***		***4.0 (10/10)* t = 24 d***		***3.1 (10/10)* t = 15 d***		
Female SD rats	0 (0/10)			0 (0/10)		0 (0/10)		0 (0/10)		***3.8 (10/10)* t = 13 d***	***3.1 (10/10)* t = 11 d***

[a]Bold-italicized font indicates statistically significant changes compared with controls; shaded cells represent doses not investigated in the individual studies. Severity averaged to four-point scale as follows: 0= not detected; 1 = minimal severity; 2 = mild severity; 3 = moderate severity; 4 = marked severity.

[b]Numbers in parenthesis indicate number of animals positive out of total animals examined; t = timepoint (in days) of first incidence; *$p < 0.001$.

Mechanistic and Supplemental Information

The mechanistic evidence base for PFNA immunomodulation included some in vivo examination of potential splenic and thymic atrophy after i.p. dosing and apoptosis, and glucocorticoid or cytokine signaling pathways (Rockwell et al., 2017; Hadrup et al., 2016; Rockwell et al., 2013; Fang et al., 2012b; Fang et al., 2012c; Fang et al., 2010; Fang et al., 2009; Fang et al., 2008), as well as in vitro testing in mast cells by Lee and Kim (2018) targeting allergic inflammatory responses and a human lymphoma B-cell line (Janssen et al., 2022).

Pleiotropic cytokines in various tissues of PFNA-treated mice and rats mostly showed modest changes (<twofold). The interpretation of cytokine responses is challenging without functional immune data from which to evaluate the strength of association between the two, and cytokine changes are not necessarily predictive of downstream effects on immune function (Tarrant, 2010). Most of the PFNA effects on cytokine levels were observed at doses that also resulted in significant body weight loss (≥3 mg/kg-day) and are not described in detail here. Effects reported at lower doses included significant reductions (15%–41%) in IL-4 in mouse spleen tissue at ≥1 mg/kg-day (Fang et al., 2008), and decreases in IL-10 (13%–40%) with increases in IL-1β (≥300%) and TNFα (≥130%) in rat liver tissue at ≥0.2 mg/kg-day PFNA (Fang et al., 2012c). Detailed information on in vivo cytokine responses to PFNA can be found in HAWC which provides summary results, including those reported in Fang et al. (2008)(https://hawc.epa.gov/ani/animal-group/100500504/); Fang et al. (2009)(https://hawc.epa.gov/ani/animal-group/100500507/); Fang et al. (2010)(https://hawc.epa.gov/ani/animal-group/100500516/); Fang et al. (2012b)(https://hawc.epa.gov/ani/animal-group/100500514/); and Fang et al. (2012c)(https://hawc.epa.gov/ani/animal-group/100500517/) .

Two i.p. injection studies in C57BL/6 mice provide mechanistic information generally consistent with results of PFNA oral dosing studies. Two weeks after receiving a single dose of 100 μM/kg, a significant decrease in relative spleen weight (60%–70%) in both males and females, respectively, was observed, but was concomitant with a 31%–38% decrease in body weight (Rockwell et al., 2013). Splenic atrophy was correlated with a decrease in spleen leukocyte counts, thymocyte viability, CD4+CD8+ cells, CD19+ (a B-cell marker), and CD14+ (a phagocyte marker) cells. Increases in serum TNFα in response to i.p dosing with lipopolysaccharide (LPS) were significantly larger in mice 2 weeks after PFNA exposure as compared with control mice not previously exposed to PFNA (Rockwell et al., 2013). A second study by the same group, using the same exposure design (i.e., a single dose of 100 μM/kg PFNA), found that none of the effects observed at 14 days post-exposure in the previous study had fully recovered by 28 days post-exposure (Rockwell et al., 2017). The splenic histology and decreases in splenocyte populations recapitulate observations in oral studies, but also reflect that effects seen at higher doses might be confounded by overt toxicity, diminishing their usefulness in interpreting PFNA's immunotoxicity potential.

Short-term in vivo studies by Fang et al. (2009); Fang et al. (2008) also suggest a possible role for glucocorticoid-mediated stress responses in PFNA immunomodulation at higher doses coinciding with weight loss where such a stress-mediated response might be expected. Both these studies reported significant increases in serum cortisol and adrenocorticotropic hormone (ACTH) in mice and rats, but only at doses ≥3 mg/kg-day that coincided with overt toxicity suggesting a stress response (see Section 3.2.7 for a more detailed discussion of the adrenal evidence base). One oral study reported a significant 169% increase in corticosterone in male Wistar rats at doses lower than those causing systemic toxicity, specifically a 169% increase at 0.0125 mg/kg-day PFNA, and a nonsignificant increase of 122% at 0.25 mg/kg-day, suggesting the possibility of immunomodulatory effects in rodents at lower doses than those tested in the immunotoxicity evidence base (Hadrup et al., 2016). Glucocorticoids are known to induce thymocyte/T-cell apoptosis (Herold et al., 2006), which was reported in PFNA studies at doses ≥3 mg/kg-day coincident with overt toxicity. Specifically, Fang and coauthors report PFNA-induced thymic and splenic atrophy (although histopathological results were judged as *low* confidence) involving stimulation of apoptotic pathways, including decreases in the anti-apoptotic protein Bcl-2 (Fang et al., 2009) and activation of p-JNK and p-p38 (Fang et al., 2010). The activated forms of these molecules are important mediators of apoptosis—for example, p38 can phosphorylate Bcl-2 and decrease its anti-apoptotic activity (De Chiara et al., 2006).

Lee and Kim (2018) used the rat basophilic leukemia RBL-2H3 mast cell line to study PFNA effects on mast cell functioning and allergic inflammation. In this study, RBL-2H3 cells were pre-sensitized with anti-dinitrophenyl (DNP) IgE, followed by a 30-minute exposure to 100 μM PFNA and challenge (or not) with DNP-human serum albumin (HSA). PFNA had no significant effects on mast cell degranulation as evidenced by a lack of increased histamine or β-hexosaminidase release and Ca^{2+} influx, with or without DNP-HSA. Nor did PFNA affect cytokine gene expression, TNFα secretion, or NF-ƙB transcription. These results contrast their 14-day in vivo results (discussed above in "Immune function assays"), showing aggravated allergic reactions (i.e., increased serum TNFα and histamine) in OVA-induced ASA male mice orally exposed to PFNA in the same study, but may be due to in vitro model limitations and involvement of other (i.e., non-basophilic) types of immune cells in the inflammation response (Passante and Frankish, 2009). Janssen et al. (2022) reported PFNA downregulated *RAG1* and *RAG2* in a B-cell lymphoma line in a time-dependent manner. These genes confer recognition of potentially infectious agents.

In vitro HTS under the ToxCast/Tox21 program examined genes involved in immune response including cytokines and genes that regulate inflammation. While it is difficult to infer the relationship of the in vitro HTS findings to in vivo responses absent additional testing, most alterations occurred at elevated doses generally exceeding cytotoxicity limits. For additional information on ToxCast results and specific AC50 concentrations as they relate to immune system pathways (see Appendix C.2 for a summary of ToxCast assays that were active). Taken together, the mechanistic evidence provides limited evidence of indirect PFNA immunomodulation at higher

doses that might involve stress response pathways and apoptosis leading to thymic and splenic atrophy. However, there is sparse evidence characterizing mechanistic pathways of potential immune system perturbations at lower PFNA doses that do not elicit overt toxicity.

Evidence Integration

Overall, there is *slight* evidence in human studies of PFNA effects on the immune system. This is based on limited evidence of immunosuppression, primarily reduced antibody response following vaccination. The antibody results, although largely imprecise, present a pattern of findings that higher prenatal and childhood concentrations of PFNA are associated with suppression of at least one measure of lower antibody response to common vaccines (see Table 3-32). The majority of analyses show a decrease in antibodies with higher PFNA exposure, with these reductions generally being fairly large (most were greater than a 10% decrease per doubling of exposure, ranging up to 35%). These associations were observed despite poor sensitivity. This evidence is reported in multiple well-conducted studies, including two birth cohorts in the Faroe Islands, a birth cohort in Norway, and a cross-sectional study in the United States, supported by a *low* confidence study of adults in Denmark (Grandjean et al., 2017b; Grandjean et al., 2017a; Kielsen et al., 2016; Stein et al., 2016b; Stein et al., 2016a; Granum et al., 2013; Grandjean et al., 2012). However, there is considerable uncertainty, in particular due to potential confounding across PFAS. The results for PFNA display a weaker pattern of results than other correlated PFAS, with less consistency and smaller magnitude of effect. The available data to inform potential confounding across PFAS cannot rule out that the results for PFNA are driven by associations with these other PFAS. Other uncertainties include variability in the response by age of exposure and outcome measurements, as well as vaccination exposure type (initial and boosters).

The strongest epidemiological evidence for hypersensitivity is based on one well-conducted study that reported a clear positive association with asthma incidence and an exposure-response gradient across quartiles (Dong et al., 2013), with some coherent mechanistic changes (increased IgE, eosinophils) in this and another study (Stein et al., 2016b). Other studies examining associations between PFNA exposure and asthma were largely null and had more uncertainties, including greater potential for outcome misclassification and reduced sensitivity based on exposure levels. The lack of findings supportive of the results from Dong et al. (2013) limit the interpretation of whether PFNA exposure might cause or exacerbate asthma. Other hypersensitivity outcomes including allergies/allergic sensitization, and dermal allergic measures had inconsistent findings.

There was *indeterminate* animal evidence to inform potential PFNA effects on the immune system. All but one *low* confidence study focused on observational endpoints, and there are no functional studies on immunosuppression to aid in interpreting the human evidence. This represents a clear data gap and introduces significant uncertainty in the animal evidence base. The only functional evaluation in animals was a *low* confidence study of hypersensitivity conducted in mice by Lee and Kim (2018) that was difficult to interpret due to dosing at a rate much higher than that associated with overt toxicity in other studies. The absence of long-term or chronic exposure

studies examining functional and observational immune measures is also a significant source of uncertainty.

Observational endpoints in animals, while not as sensitive or predictive of immunotoxicity as functional assays, can be useful to support a weight of evidence conclusion for immunosuppression observed in epidemiological studies (IPCS, 2012). Importantly, immunosuppressive effects (i.e., lymphoid organ atrophy, reduced bone marrow function, or changes in hematological endpoints) may be sufficient to categorize a substance as an immunotoxicant, but this evidence is considered equivocal if these effects occur with overt toxicity (IPCS, 2012). Most PFNA effects on observational endpoints coincided with significant decreases in body weight in excess of 10%, which became increasingly severe with dose and study duration (i.e., less extreme changes were noted in 14-day studies compared with the 28-day study, and generally worsened with increasing dose). Effects associated with potential overt toxicity included significant dose-dependent reductions in some innate and adaptive immune cell markers, as well as dose-dependent reductions in absolute and relative spleen and thymus weights, histopathological lesions, and cytokine changes (NTP, 2018; Fang et al., 2012b; Fang et al., 2012c; Fang et al., 2010; Fang et al., 2009; Fang et al., 2008). Effects that occurred at lower doses were limited to significant decreases in markers for macrophages and NK cells in male mice spleens (Fang et al., 2008), and significant dose-dependent reductions in neutrophil counts and absolute and relative spleen weights of female rats with no coherent histopathological evidence.

The mechanistic evidence base for PFNA immunotoxicity was mostly limited to high dose testing. Potential MAPK pathway activation and apoptotic signaling provides some potential insight on how PFNA may contribute to immunosuppression at higher doses by triggering cell death in target tissues (Fang et al., 2009). These molecular pathways are generally coherent with the high dose increases in apoptosis and atrophy, and organ weight decreases seen across studies in male rats and mice. Overall, however, the available data are too limited to meaningfully inform a MOA, especially at dose levels that do not induce overt toxicity.

Taken together, the currently available ***evidence suggests*** but is not sufficient to infer that PFNA exposure may cause immunosuppression in humans given sufficient exposure conditions (see Table 3-32).[16] This judgment is based on epidemiological studies providing evidence of reduced antibody response at PFNA levels of approximately 0.6 ng/mL, and possible evidence for effects on asthma and asthma-related outcomes from a single well-conducted study.

[16]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).

Table 3-32. Evidence profile table for immune effects

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.6. Immune Effects, Human Studies)					⊕⊙⊙ ***Evidence suggests***
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	*Primary basis:* Human studies reporting immunosuppression, primarily from reduced antibody responses in children at mean PFNA exposures as low as 0.6 ng/mL in serum *Human relevance:* Judgment is based on human evidence *Cross-stream coherence:* N/A (the animal evidence is *indeterminate*) *Susceptible populations:* Children and fetuses may be at higher risk of adverse effects due to
<u>Immunosuppression (antibody response)</u> Six *medium* and 1 *low* confidence studies in children and 1 *medium* and Two *low* confidence studies in adults	• Most studies in children observed inverse associations between PFNA exposure and antibody levels following vaccination in at least some analyses	• Generally *consistent* direction of association across vaccine type, timing of vaccination, and age at antibody response measurement • *M*edium confidence studies • Large *magnitude* of association despite poor sensitivity	• Potential for residual confounding across PFAS (weaker pattern of effects than seen for other correlated PFAS and available data on potential confounding is uncertain) • Imprecision of majority of findings	⊕⊙⊙ *Slight* Generally consistent but imprecise evidence for immunosuppression with PFNA exposure based on lower antibody responses in multiple *medium* confidence studies, with important residual uncertainty. Some evidence of hypersensitivity based primarily on a single well-conducted study of asthma	
<u>Infectious disease</u> Five *medium* and 7 *low* confidence studies	• In children, 5 of 10 studies reported positive associations with at least one infectious disease with higher PFNA exposure, including gastroenteritis, throat infection, ear infection, common cold, and lower respiratory infection, but there is considerable inconsistency	• No factors noted	• Unexplained inconsistency • High risk of bias from potential outcome misclassification in most studies		

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
<u>Sensitization or allergic response</u> Fifteen *medium* confidence studies	• The only study of asthma incidence reported a large, positive association • Of the other studies of asthma, only 1 study of "current" asthma and two studies of "ever" asthma reported higher odds of asthma in at least one sub-population, but these outcome definitions are less specific • Results for allergies/allergic sensitization, and dermal allergic measures were inconsistent	• Large effect size and exposure-response gradient observed for asthma incidence in one study with highest specificity	• Potential for residual confounding across PFAS • Inconsistent associations for all hypersensitivity outcomes		evidence that prenatal and childhood PFNA exposures may be associated with immunosuppression and a single study supporting an association with childhood asthma
Evidence from in vivo animal studies (see Section 3.2.6. Immune Effects, Animal Studies)					
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Sensitization or allergic response (functional)</u> One *low* confidence study, adult male mice • 14 d intermittent, with 3 exposures over 5 d	• One functional assay provided some evidence of allergic hypersensitivity in OVA-induced adult male mice exposed intermittently (3× over 5 d) at 100 mg/kg-d	• *Magnitude* of increase in serum levels of inflammatory mediators as high as 64% for histamine and 55% for TNFα	• *Low* confidence functional study at dose levels associated with overt toxicity in observational studies (see below)	⊙⊙⊙ *Indeterminate*	
<u>Immunosuppression (observational)</u>	• Three *high/medium* confidence studies reported reductions in spleen and	• *Consistent* and dose-response gradient for reductions in spleen	• Indirectness of observational immunoassays		

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
One *high* confidence study • 28 d, adult male and female rats Five *medium* confidence studies, adult male mice and rats (with some *low* confidence endpoints) • 14 d (×5) One *low* confidence study (with some *medium* confidence endpoints), adult male rats • 14 d	thymus weights in adult male rodents, generally at ≥1.25 mg/kg-d, and reductions in spleen weights in adult female rats at ≥3.12 mg/kg-d (null effects on thymus weight) • Histological lesions of spleen, thymus, and lymph nodes reported in male rats, generally at ≥2.5 mg/kg-d and female rats at ≥12.5 mg/kg-d • *High/medium* confidence studies reported reductions in WBC counts in adult male rats at ≥1.25 mg/kg-d, and decreases in some immune cell markers (macrophages, NK cells, dendritic cells, T cells), in adult male mice, generally at ≥1 mg/kg-d	and thymus weights in 2 adult male species, as well as for reductions in spleen weights in both rat sexes • *Coherence* of reductions in spleen and thymus weights with reductions in splenic and thymic immune cell markers in adult male rodents • Effects observed in *high/medium* confidence studies	• Potential confounding in male rodents due to overt toxicity at doses generally ≥2.5 mg/kg-d (Immunotoxicity could be secondary to stress responses) • Histopathology in male and female rats at doses associated with overt toxicity		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Summary of key findings and interpretation**			**Evidence stream judgment**	
Molecular events – Apoptosis Pathways	Activated JNK and p38 (MAPK pathways) in thymus and spleen of male rats at doses ≥1 mg/kg-d. Coherent with the increased apoptosis, atrophy, and decreased organ weight at higher doses; Anti-apoptotic Bcl-2 decreased, pro-apoptotic AIF increased, suggesting a caspase-independent apoptosis pathway.			Activation of MAPK cascades may be initiating or amplifying signaling events in pro-apoptotic pathways, supporting apical	
Molecular events – Stress Responses; adrenal stimulation	At PFNA doses associated with overt toxicity, glucocorticoid-mediated stress responses may indirectly cause immune effects.				

Summary of human, animal, and mechanistic evidence			Evidence integration summary judgment
	Increases in serum cortisol and ACTH in adult male rats and mice at doses ≥3 mg/kg-d.	effects seen in animals at higher doses. I.p. study results recapitulated effects seen after oral dosing and did not recover	
Organ-level effects – Decreased spleen weight/atrophy	I.p. injection at a high PFNA dose (100 µMol/kg) in mice caused splenic atrophy and other effects generally consistent and coherent with effects observed at doses causing effects in oral toxicity studies. Decreased spleen weight and WBC counts in both males and females, concomitant with decreased body weight, and thymocyte viability 14 d after a single, high-dose injection of PFNA, with no recovery 28-d post-exposure.		

3.2.7. Endocrine Effects

This section describes and integrates the PFNA evidence informative to assessing the potential for endocrine effects of the thyroid and adrenal glands. The epidemiological evidence is focused on associations of PFNA exposures to thyroid perturbations. The animal toxicological evidence includes studies that examined PFNA effects on the thyroid and adrenal glands. Thyroid hormones (THs) are key regulators of neurodevelopment and somatic growth, so this section is also relevant to the evidence synthesis and integration summary of developmental and neurotoxicity effects (see Sections 3.2.2 and 3.2.8, respectively). PFNA effects on reproductive hormones (e.g., testosterone, estradiol) and organs (e.g., testes, ovaries) that are also part of the endocrine system are discussed in the sections on male reproductive effects (see Section 3.2.4) and female reproductive effects (see Section 3.2.5).

Human Studies – Thyroid Effects

Forty studies (45 publications) have investigated the relationship between PFNA exposure and THs and/or thyroid disease in humans. All the included thyroid studies examined the association between PFNA exposures measured in blood and levels of THs (see Figure 3-77).

Multiple outcome-specific considerations for study evaluation were influential to the study evaluation 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 ideal for measurement of THs to avoid misclassification due to potential diurnal variation (van Kerkhof et al., 2015). Studies that did not consider these factors (e.g., by study design or adjustment) were considered deficient for the outcome ascertainment domain, primarily for TSH, which is more affected by these issues than are T4 or T3. However, this was not considered a major source of bias, and thus studies were not downgraded in overall study confidence if lack of fasting and consideration of diurnal variation were the primary limitations identified. This possible outcome misclassification was expected to be non-differential and thus likely a bias toward the null; the domain ratings were used to assess possible sources of inconsistency in the results. 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 changes to THs can be a short-term response, and the estimated half-life of PFNA in humans is long (see Section 3.1).[17] All of the available studies analyzed PFNA in serum or plasma using appropriate methods as described in the systematic review protocol. THs were analyzed using standard methods (e.g., immunoassays, HPLC-

[17]Reverse causation was considered based on binding of lipophilic chemicals (such as PBDEs) to serum lipids (Chevrier, 2013) and the association of thyroid disease with fat mass. However, this was unlikely to significantly bias the results because PFAS, including PFNA, do not appear to preferentially bind to serum lipids, so concurrent exposure measurement was adequate for this outcome.

MS/MS) in all studies. Overall, while most studies were considered *medium* confidence, nearly all of them had limitations in outcome ascertainment and several had concerns for study sensitivity as well (primarily due to limited PFNA exposure contrast in the study populations). These issues and other non-differential sources of measurement error are likely to bias the results toward the null, and thus null associations are difficult to interpret. The *low* confidence studies generally had additional concerns such as selection bias or confounding.

In summary, 26 studies were *medium* confidence (Cakmak et al., 2022; Gallo et al., 2022; Guo et al., 2021; Sarzo et al., 2021; Aimuzi et al., 2020; Kim et al., 2020a; Lebeaux et al., 2020; Aimuzi et al., 2019; Caron-Beaudoin et al., 2019; Inoue et al., 2019; Reardon et al., 2019; Blake et al., 2018; Dufour et al., 2018; Kang et al., 2018; Liu et al., 2018; Preston et al., 2018; Wen et al., 2018; Berg et al., 2017; Crawford et al., 2017; Tsai et al., 2017; Yang et al., 2016a; Wang et al., 2014a; Webster et al., 2014; Wang et al., 2013; Lopez-Espinosa et al., 2012), and 11 were *low* confidence (Liu et al., 2021b; Itoh et al., 2019; Byrne et al., 2018; Heffernan et al., 2018; Khalil et al., 2018; Zhang et al., 2018b; Shah-Kulkarni et al., 2016; Lewis et al., 2015; Lin et al., 2013b; Ji et al., 2012; Bloom et al., 2010). Three studies were *uninformative* in study evaluation due to substantial concerns for potential residual confounding (Seo et al., 2018; Kim et al., 2016) or serious concerns in multiple domains (Kim et al., 2011a). A small number of *medium* confidence studies reported different effect estimate types than the other studies (e.g., correlation coefficients rather than regression coefficients); results from these studies are described in text and figure footnotes but are not included in forest plots. Results are described separately for adults and children, including infants; when effect estimates were combined across age groups in the studies, the results are reported with studies of adults.

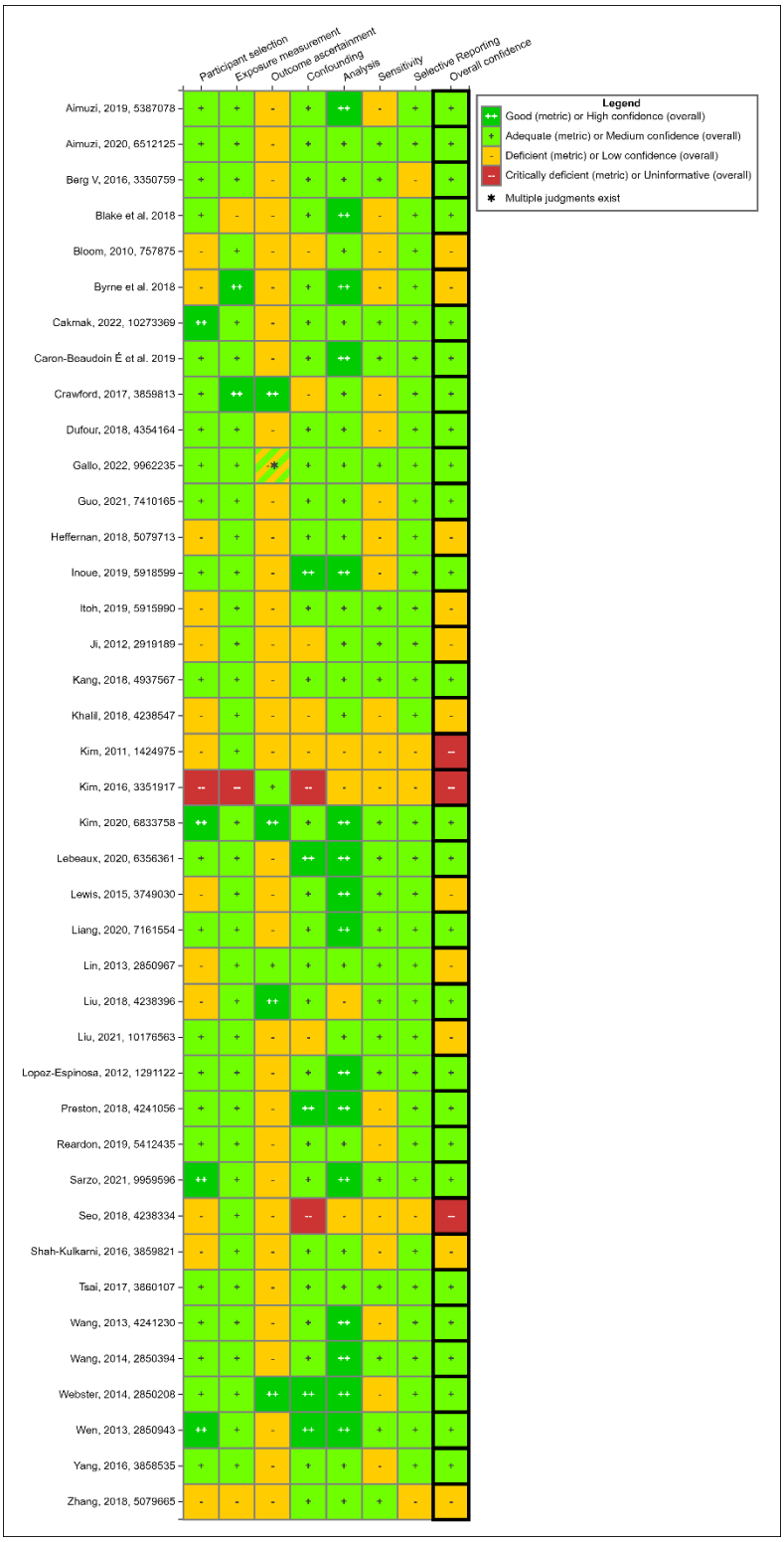


Figure 3-77. Human studies examining relationships between PFNA exposures and thyroid effects, along with heat map to summarize study evaluations. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500202/.

Thyroid effects in adults

Twenty-six studies examined associations with THs in adults, including 10 focused on pregnant women. For T4, out of 24 studies, the results are mixed, with no consistent direction of association across studies and imprecision for several of the estimates. In the 16 *medium* confidence studies (see Figure 3-78, both free and total T4), while statistically significant inverse associations were observed in a few studies, there were a similar number with positive associations and most results were close to null. There was no clear pattern of association by exposure levels. Effect sizes and the direction of association within some studies differed by sex (Wen et al., 2018; Lewis et al., 2015), but there was no consistent pattern of direction across studies. The *low* confidence studies were also inconsistent in direction of association (Liu et al., 2021b; Byrne et al., 2018; Heffernan et al., 2018; Zhang et al., 2018b; Lewis et al., 2015; Lin et al., 2013b; Ji et al., 2012; Bloom et al., 2010).

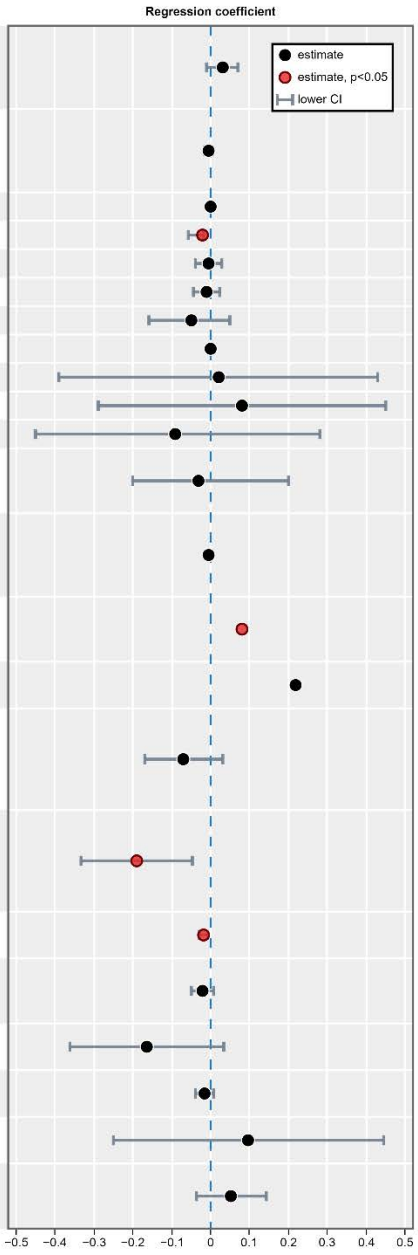
Sixteen studies examined associations with T3. In the 10 *medium* confidence studies, 2 studies (Crawford et al., 2017; Wen et al., 2013) reported higher levels of total T3 with higher exposure to PFNA in women (statistically significant in Crawford et al. (2017)). One of these studies (Wen et al., 2013), also reported an inverse association (i.e., in the opposite direction) with total T3 in men. Another study reported an inverse though not statistically significant association in pregnant women (Sarzo et al., 2021). The other *medium* confidence studies reported no association. Results in the five *low* confidence studies (Liu et al., 2021b; Itoh et al., 2019; Byrne et al., 2018; Heffernan et al., 2018; Zhang et al., 2018b; Lewis et al., 2015) were inconsistent.

Twenty-five studies reported on TSH (see Figure 3-79), and findings were mixed across the 13 *medium* confidence studies. Specifically, one study reported statistically significantly higher TSH with higher exposure (Webster et al., 2014) while one reported a significant inverse association (Yang et al., 2016a). Wang et al. (2013) and Inoue et al. (2019) also reported positive associations, although not statistically significant, and in Inoue et al. (2019), the association was non-monotonic across quartiles (inverse in 2nd quartiles, positive in 3rd and 4th). Inverse but non-statistically significant associations were observed in Cakmak et al. (2022) and in men but not women in Gallo et al. (2022). The remaining studies reported no clear association. In the *low* confidence studies, most reported non-statistically significant positive associations with TSH in at least some subgroups (Liu et al., 2021b; Byrne et al., 2018; Heffernan et al., 2018; Zhang et al., 2018b; Lewis et al., 2015; Lin et al., 2013b; Ji et al., 2012), with the exception of Bloom et al. (2010), which was essentially null. One statistically significant association was observed in men and boys aged 12–20 years in Lewis et al. (2015). However, the lack of consistency among the *medium* confidence studies reduced the overall certainty in the relationship between PFNA exposure and serum TSH in adults. Most studies did not report sex-stratified results or only included women, so it is not possible to draw conclusions about differences in the association by sex.

To focus on associations in pregnant women, the results of the relevant 10 studies (9 *medium* confidence) are shown across hormones in Figure 3-80. The inconsistency remains when

looking at this more focused group of studies. Within most studies that look at more than one hormone, two studies do show opposite directions of association between T4 and TSH (Wang et al., 2014a; Webster et al., 2014), with inverse associations for T4 and positive associations for TSH, but with the exception of T4 in Wang et al. (2014a), these estimates are imprecise. A positive association (not statistically significant) with TSH was also observed in Wang et al. (2013), but this study did not examine T4. In contrast, in Yang et al. (2016a), an inverse correlation was observed with TSH but no association was observed with T4, while in Sarzo et al. (2021), the direction of association was positive for both T4 and TSH (though results for TSH were essentially null). In Aimuzi et al. (2020), Preston et al. (2018), and Berg et al. (2017), there was no clear association for TSH or T4. Taken together, there is not sufficient consistency or precision to draw conclusions about a pattern of changes in T4 and TSH (not presupposing any specific relationship between the two) in pregnant women or adults more broadly.

Study	Population	Median exposure	Sub-population	Outcome	Exposure group
Blake et al. 2018	Fernald Community Cohort (1990-2008), U.S., 210 adults (81 men, 129 women)	0.5	Men and women	T4 (total)	Continuous
Preston, 2018, 4241056	Project Viva (1999-2002), U.S., 732 pregnant women and 480 neonates	0.6	Pregnant women (n=726)	T4 (free, ln)	Continuous
					Q1
					Q2
					Q3
					Q4
				T4 (total)	Continuous
					Q1
					Q2
					Q3
					Q4
Webster, 2014, 2850208	CHirP (2007-2008), Canada, 152 women	0.6	Pregnant women (n=150)	T4 (free)	Continuous
Reardon, 2019, 5412435	Alberta Pregnancy Outcomes and Nutrition cohort (2009-2012), 494 women	0.69	Pregnant women (n=478)	Free T4 (Log)	Continuous
Crawford, 2017, 3859813	Time to Conceive study (2008-2009), U.S., 99 women	0.8	Women	T4 (free)	Continuous (Log)
				T4 (total)	Continuous (Log)
Lebeaux, 2020, 6356361	Health Outcome and Measures of the Environment (2003-2006), 355 mother-infant pairs	0.9	Pregnant women (n=171)	T4 (log2)	Continuous
Wang, 2014, 2850394	Taiwan Maternal and Infant Cohort Study (2000-2001), Taiwan, 285 pregnant women and 116 neonates	1.5	Pregnant women (n=274)	T4 (total)	Continuous
			Pregnant women (n=285)	T4 (free)	Continuous
Wen, 2013, 2850943	NHANES (2007-2010), U.S., 1,181 adults (672 men, 509 women)	1.5	Men	T4 (free, ln)	Continuous (Ln)
				T4 (total)	Continuous (Ln)
			Women	T4 (free, ln)	Continuous (Ln)
				T4 (total)	Continuous (Ln)
Aimuzi, 2020, 6512125	Shanghai Birth Cohort (2013-2016), China, 1,885 pregnant women	1.6	Pregnant women (n=1885)	Free T4	Continuous (Ln)



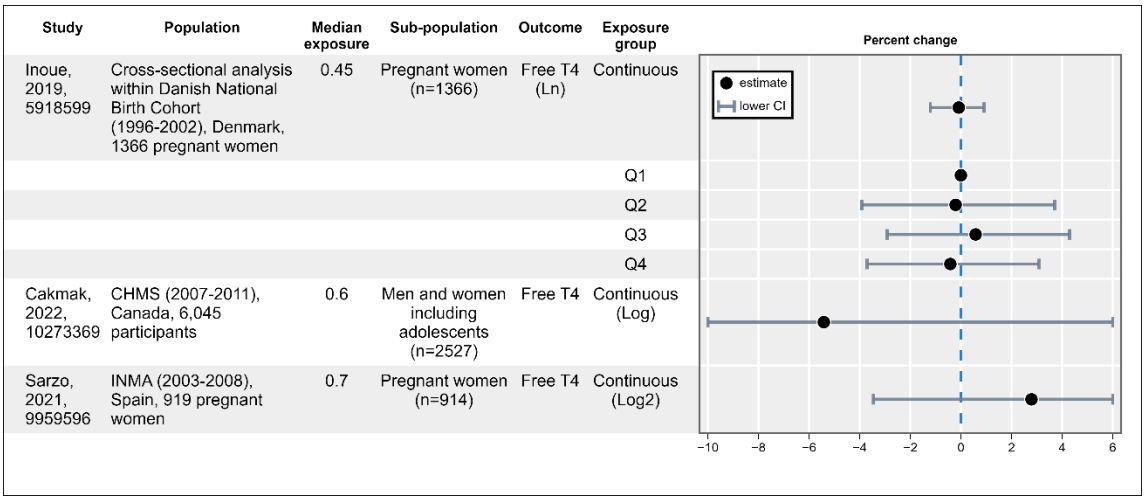


Figure 3-78. Associations between *medium* confidence studies of PFNA exposure and T4 levels in adults, including (a) results expressed as regression coefficient and (b) results expressed as percent change.[a-e] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-t4-adults/. https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-T4-in-Adults-percent-change/.

[a]Three studies are not displayed because they used a different effect estimate type (Liu et al., 2018; Yang et al., 2016a) or only presented quantitative results for significant associations (Berg et al., 2017). None reported an association between PFNA exposure and T4.

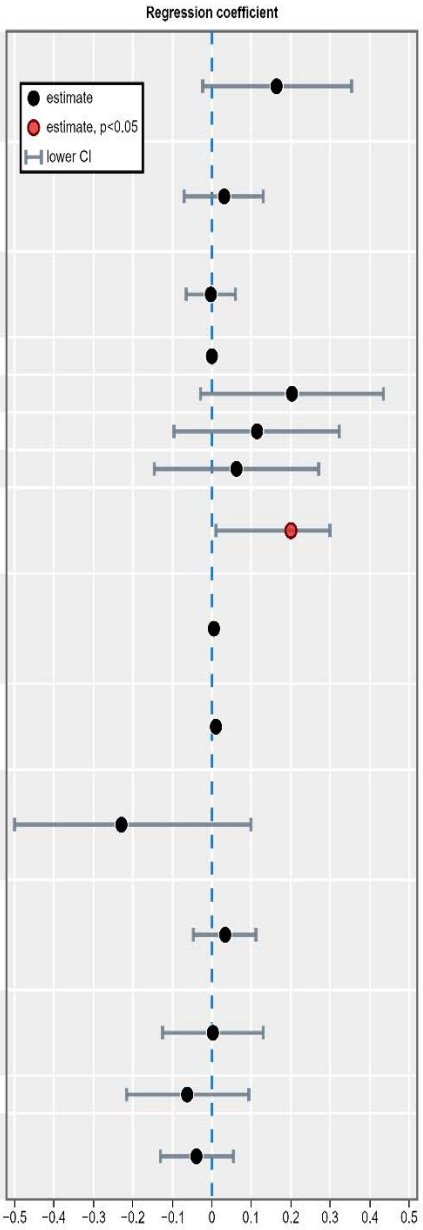
[b]Regression coefficient of zero represents no/null association.

[c]Studies are sorted by median exposure level (in ng/mL).

[d]Regression coefficients are expressed as the increase in T4 levels per 1 ng/mL unit increase in exposure unless otherwise noted. Units for the outcome varied across studies.

[e]Effect estimates for Preston et al. (2018) were recalculated from percent difference to regression coefficient for consistency with the other studies.

Study	Population	Median exposure	Sub-population	Outcome	Exposure group
Wang, 2013, 4241230	Norwegian Mother and Child Cohort Study (2003-2004), Norway, 903 pregnant women	0.4	Pregnant women (n=903)	TSH (ln)	Continuous
Blake et al. 2018	Fernald Community Cohort (1990-2008), U.S., 210 adults (81 men, 129 women)	0.5	Men and women	TSH	Continuous
Preston, 2018, 4241056	Project Viva (1999-2002), U.S., 732 pregnant women and 480 neonates	0.6	Pregnant women (n=718)	TSH (ln)	Continuous
					Q1
					Q2
					Q3
					Q4
Webster, 2014, 2850208	CHirP (2007-2008), Canada, 152 women	0.6	Pregnant women (n=151)	TSH	Continuous
Reardon, 2019, 5412435	Alberta Pregnancy Outcomes and Nutrition cohort (2009-2012), 494 women	0.69	Pregnant women (n=478)	TSH (Log)	Continuous
Crawford, 2017, 3859813	Time to Conceive study (2008-2009), U.S., 99 women	0.8	Women	TSH	Continuous (Log)
Lebeaux, 2020, 6356361	Health Outcome and Measures of the Environment (2003-2006), 355 mother-infant pairs	0.9	Pregnant women (n=171)	TSH (log2)	Continuous
Wang, 2014, 2850394	Taiwan Maternal and Infant Cohort Study (2000-2001), Taiwan, 285 pregnant women and 116 neonates	1.5	Pregnant women (n=283)	TSH	Continuous
Wen, 2013, 2850943	NHANES (2007-2010), U.S., 1,181 adults (672 men, 509 women)	1.5	Men	TSH (ln)	Continuous (Ln)
			Women	TSH (ln)	Continuous (Ln)
Aimuzi, 2020, 6512125	Shanghai Birth Cohort (2013-2016), China, 1,885 pregnant women	1.6	Pregnant women (n=1885)	TSH	Continuous (Ln)



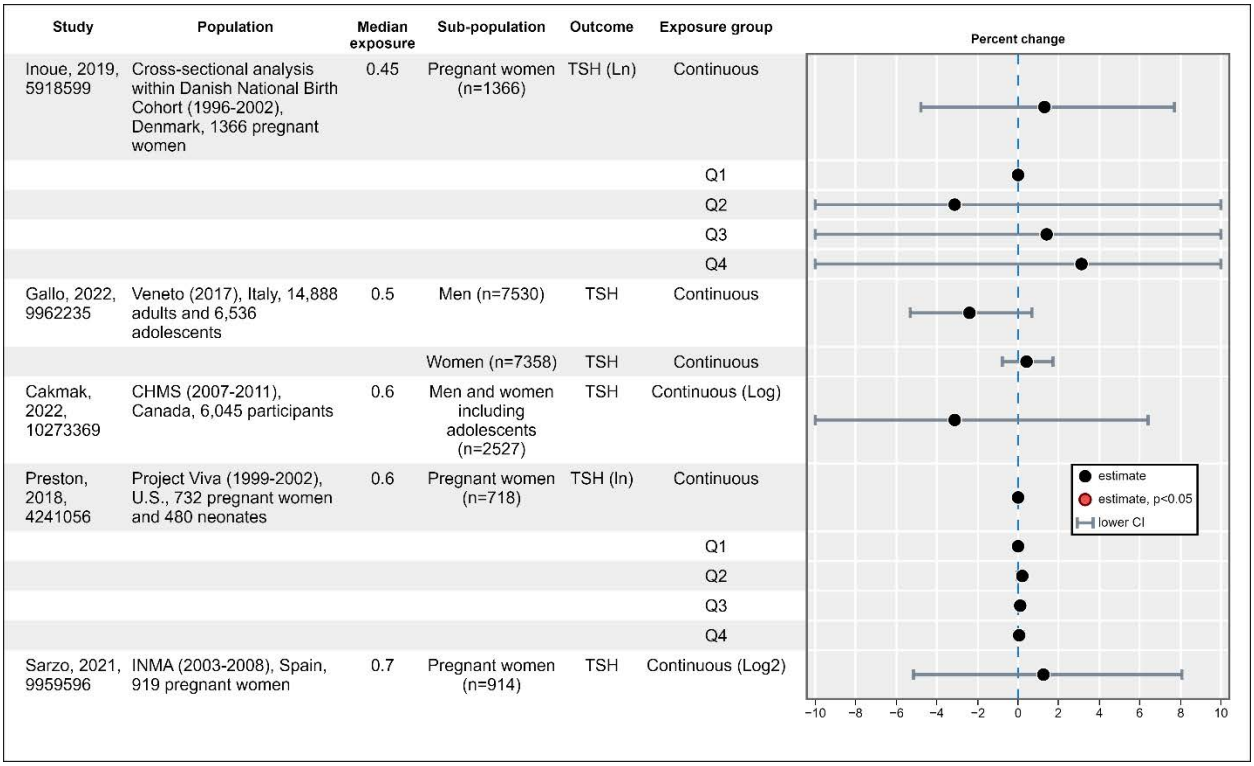


Figure 3-79. Associations between *medium* confidence studies of PFNA exposure and TSH levels in adults, including (a) results expressed as regression coefficient and (b) results expressed as percent change.[a-e] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-and-tsh-adults/ https://hawc.epa.gov/summary/data-pivot/assessment/100500071/PFNA-and-TSH-in-Adults-percent-change/.

[a]Two studies are not displayed because they used a different effect estimate type (Yang et al., 2016a) or only presented quantitative results for significant associations (Berg et al., 2017). Yang et al. (2016a) reported a statistically significant inverse association (Spearman correlation coefficient: −0.17, $p < 0.05$). Berg et al. (2017) reported no association between PFNA and TSH.

[b]Regression coefficient of zero represents no/null association.

[c]Studies are sorted by median exposure level (in ng/mL).

[d]Regression coefficients are expressed as the increase in TSH levels per 1 ng/mL unit increase in exposure unless otherwise noted. Units for the outcome varied across studies.

[e]Effect estimates for Preston et al. (2018) were recalculated from percent difference to regression coefficient for consistency with the other studies.

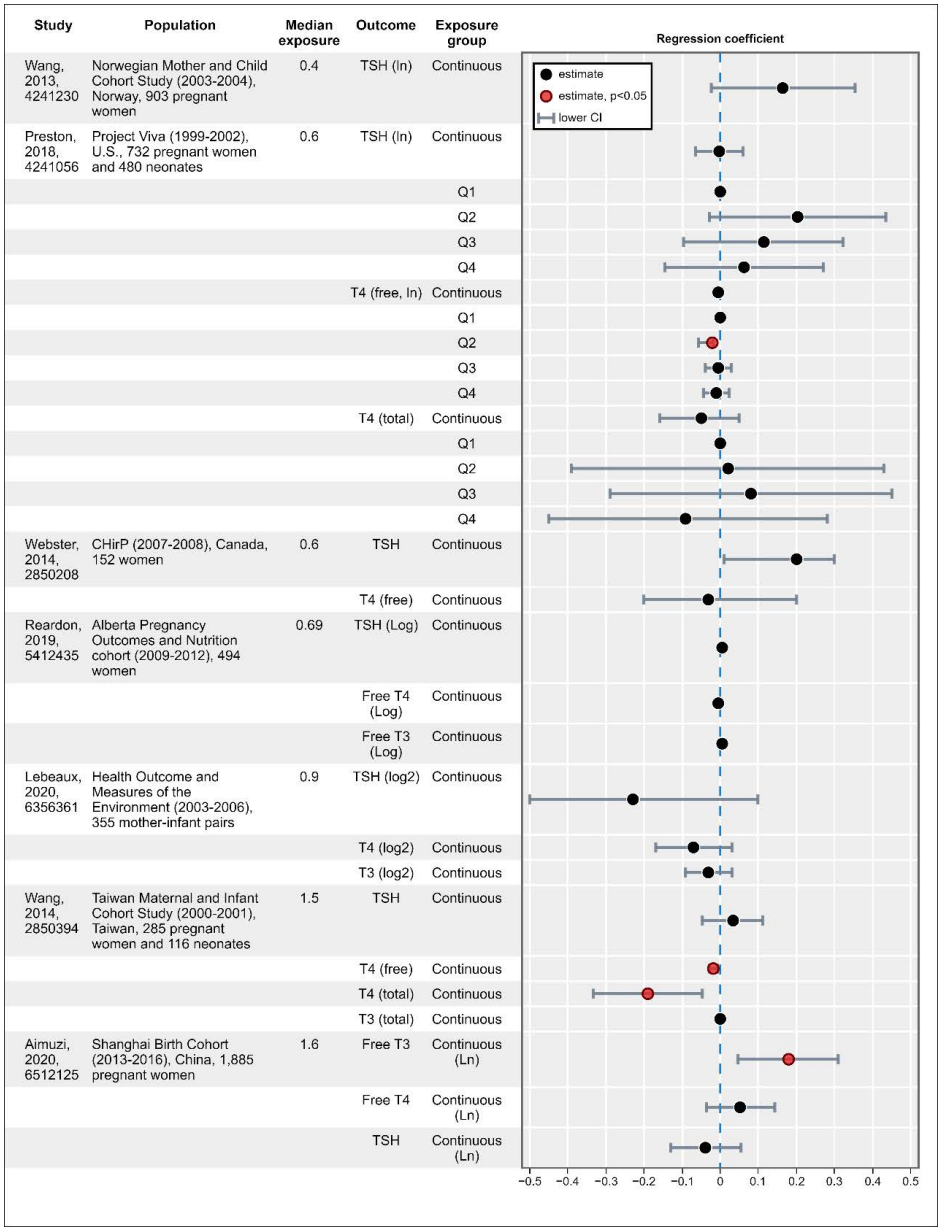


Figure 3-80. Associations between *medium* confidence studies of PFNA exposure and thyroid hormones levels in pregnant women.[a-c] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-and-thyroid-hormones-pregnant-women/.

[a]Four studies are not displayed because they used a different effect estimate type (Sarzo et al., 2021; Inoue et al., 2019; Yang et al., 2016a) or only presented quantitative results for significant associations (Berg et al., 2017). Yang et al. (2016a) reported a statistically significant inverse association (Spearman correlation coefficient: −0.17, $p < 0.05$) for TSH but no association with T4 or T3. Inoue et al. (2019) reported a positive association ($p > 0.05$) with TSH when exposure was modeled continuously and in Q3 and Q4 when modeled in quartiles; no association with T4. Sarzo et al. (2021) reported a positive but not statistically significant association with total T4 (2.79% change, 95% CI −3.46, 9.45), an inverse association with total T3, and no association with TSH. Berg et al. (2017) and Liu et al. (2018) reported no association between PFNA and TSH, T4, or T3.

[b]Regression coefficient of zero represents no/null association.

[c]Studies are sorted by median exposure level (in ng/mL).

Thyroid effects in children and adolescents

Six studies examined associations with THs in children and/or adolescents (Gallo et al., 2022; Kim et al., 2020a; Caron-Beaudoin et al., 2019; Kang et al., 2018; Khalil et al., 2018; Lopez-Espinosa et al., 2012), in addition to one *medium* and three *low* confidence studies of adults that included a substantial number of adolescents but did not analyze the lifestages separately, which were described above (Cakmak et al., 2022; Lewis et al., 2015; Lin et al., 2013b; Ji et al., 2012). Five of the studies in children and/or adolescents were *medium* confidence. Four *medium* confidence studies reported statistically significant positive associations between PFNA exposure and T4 levels (see Figure 3-81) in at least some subgroups. Among the *low* confidence studies, a positive association, though not statistically significant, was also observed in Lin et al. (2013b), while an inverse association was observed in Lewis et al. (2015). Three studies (Caron-Beaudoin et al., 2019; Lewis et al., 2015; Lopez-Espinosa et al., 2012) examined sex-stratified associations. Results were similar in boys and girls. Results for TSH were primarily null (see Figure 3-81). In the *low* confidence studies, one study also reported a positive association (Lin et al., 2013b), while two reported inverse associations (Khalil et al., 2018; Lewis et al., 2015), all non-statistically significant. Results were similar in boys and girls.

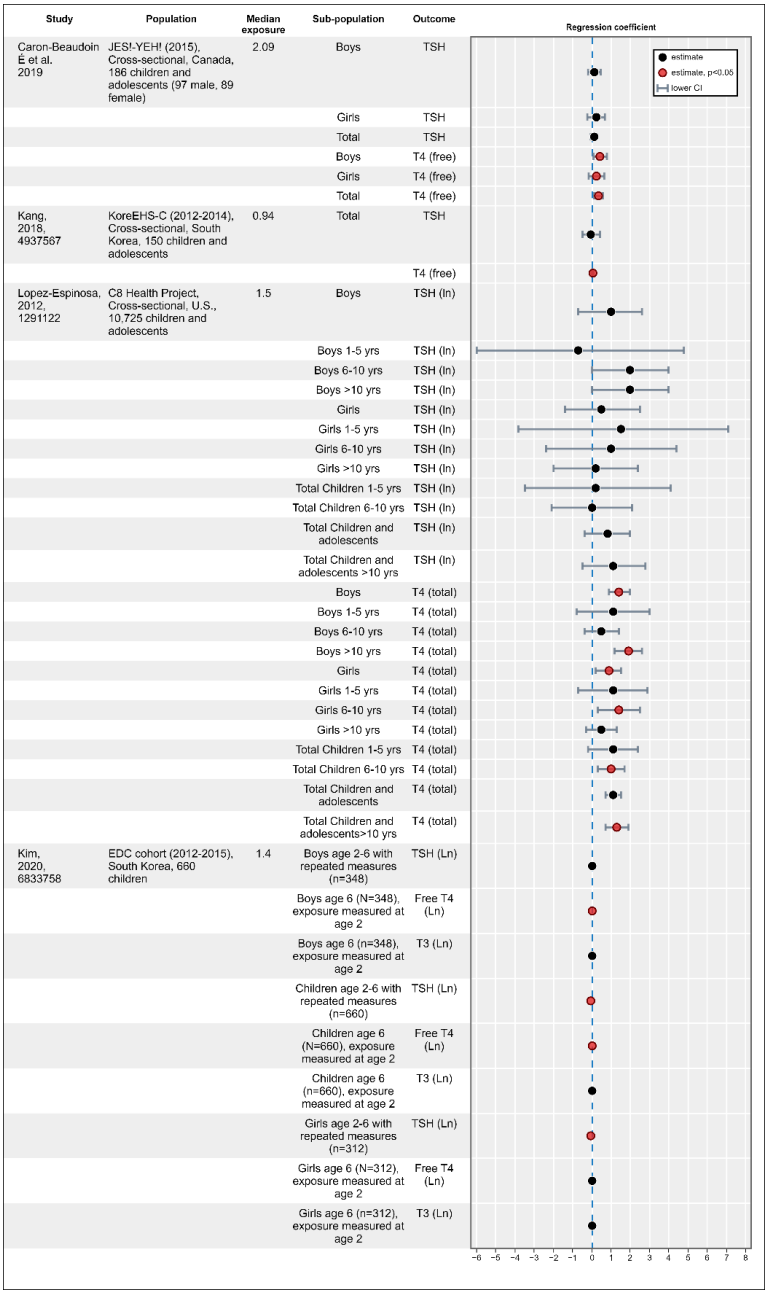


Figure 3-81. Associations between *medium* confidence studies of PFNA exposure and thyroid hormone levels (T4 and TSH) in children and adolescents.[a-d] See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/pfna-t4-children-adolescents/.

[a]One study is not displayed because a different effect estimate type was used. In adolescents, Gallo et al. (2022) reported no association with TSH in boys and an inverse but not statistically significant association in girls (−3.6% change, 95% CI −9.6, 2.9).
[b]Effect estimates in Lopez-Espinosa et al. (2012) are presented as percent change in thyroid hormone for IQR change in PFNA. Effect estimates in Caron-Beaudoin et al. (2019) and Kang et al. (2018) are regression coefficients for a ln-unit change in PFNA.
[c]Regression coefficient of zero represents no/null association.
[d]Median exposures are ng/mL.

Thyroid effects in infants

Eleven studies examined associations with THs in infants. For T4, there were 10 studies available, including nine of *medium* confidence (see Figure 3-82). Four of the nine *medium* confidence studies (Liang et al., 2020; Preston et al., 2018; Tsai et al., 2017; Wang et al., 2014a) reported inverse associations between PFNA exposure and T4 levels, with statistical significance in total T4 in Wang et al. (2014a), Liang et al. (2020) and in boys in Tsai et al. (2017). In two studies, this association was primarily driven by boys (Preston et al., 2018; Tsai et al., 2017). An exposure-response gradient was observed across quartiles in Preston et al. (2018) for boys, although the estimates were imprecise. The remaining studies reported no association. Aimuzi et al. (2019) and the *low* confidence study by Shah-Kulkarni et al. (2016) also examined sex-stratified associations. Aimuzi et al. (2019) did not observe associations in either sex, while Shah-Kulkarni et al. (2016) observed an inverse association with T4 in boys only. The other three studies did not examine sex-stratified results. Across studies, the studies with the highest exposure levels (Liang et al., 2020; Tsai et al., 2017) also reported the largest effect sizes, suggesting that study sensitivity may be an explanation for the inconsistent results.

Nine studies examined T3 in infants, including eight that were *medium* confidence (see Figure 3-83). The evidence of an association between PFNA exposure and T3 is limited, with most studies reporting null results. There was a statistically significant (though small) inverse association in Wang et al. (2014a), and an exposure-response gradient across quartiles in Tsai et al. (2017). As with T4, there was an indication of a stronger association in boys than girls, with non-statistically significant inverse associations observed for boys in two studies (one *medium* and one *low* confidence) that examined sex-stratified associations (Tsai et al., 2017; Shah-Kulkarni et al., 2016), though no sex differences were observed in the *medium* confidence study by Aimuzi et al. (2019), the third study examining sex differences. Because most studies did not report sex-stratified results and the combined estimates were primary null, it is difficult to draw conclusions about the association in boys.

Eleven studies examined the association between TSH and PFNA exposure in infants, including 10 that were *medium* confidence (see Figure 3-84). Five *medium* confidence studies (Guo et al., 2021; Liang et al., 2020; Aimuzi et al., 2019; Dufour et al., 2018; Wang et al., 2014a) and one *low* confidence study (Shah-Kulkarni et al., 2016) reported inverse associations, with statistical significance in most, though effect sizes were small. There were some indications of a positive association in Caron-Beaudoin et al. (2019), Tsai et al. (2017), and in boys in Shah-Kulkarni et al. (2016), (Tsai et al., 2017; Shah-Kulkarni et al., 2016) but the effect estimates were imprecise.

Looking across hormones, no clear pattern in the results could be deduced, although observed associations for both T4 and TSH tended to be in the inverse direction. Similarly, a pattern is not clear within individual studies.

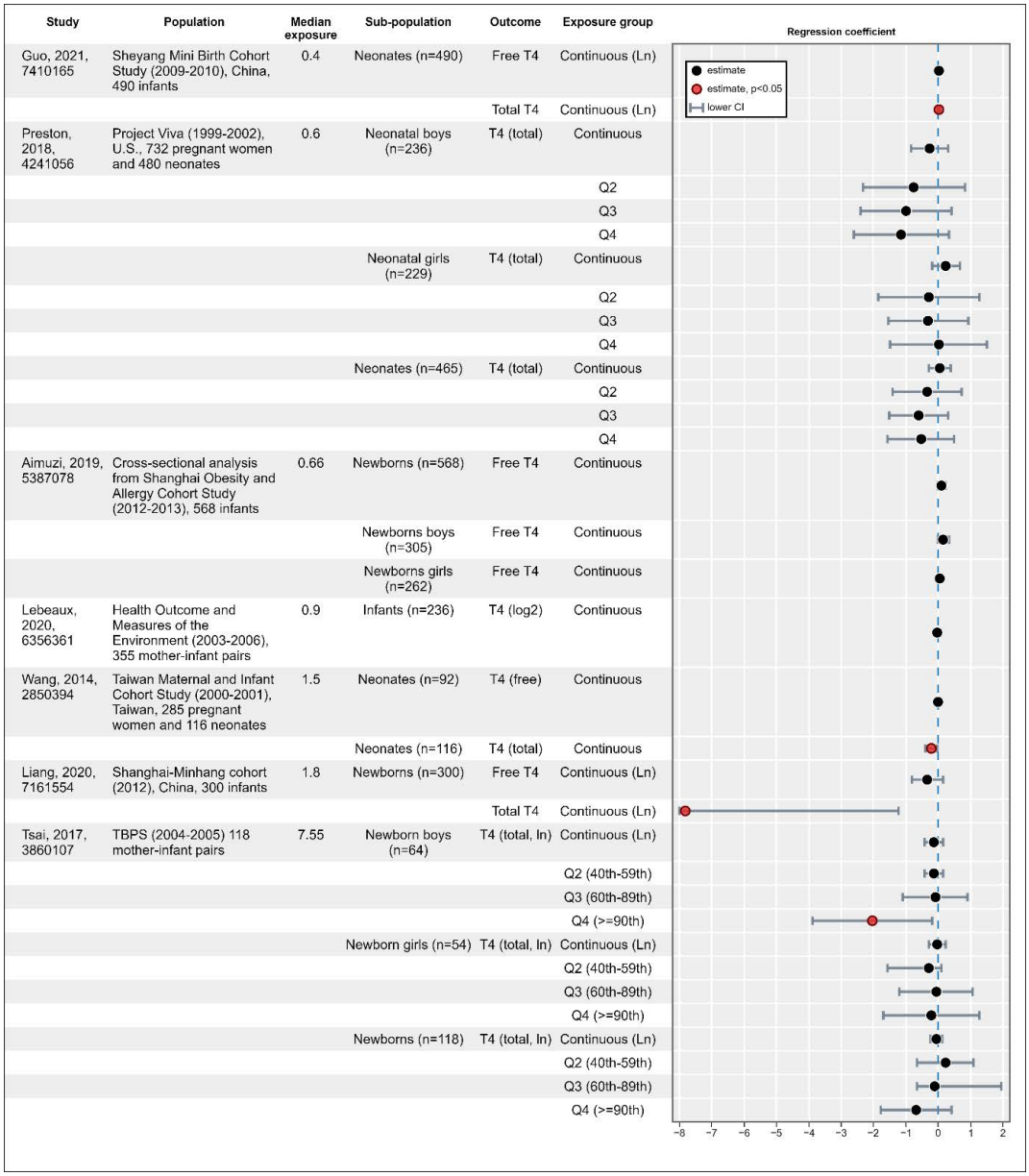


Figure 3-82. Associations between *medium* confidence studies of PFNA exposure and T4 levels in infants.[a-e] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-and-t4-infants/.

[a]Two studies are not displayed on the figure because they used a different effect estimate type (Yang et al., 2016a) or only presented quantitative results for significant associations (Berg et al., 2017). Neither reported an association between PFNA exposure and T4.

[b]Regression coefficient of zero represents no/null association.

[c]Studies are sorted by median exposure level (in ng/mL).

[d]Regression coefficients are expressed as the increase in T4 levels per 1 ng/mL unit increase in exposure unless otherwise noted. Units for the outcome varied across studies.

[e]Effect estimates for Preston et al. (2018) were recalculated from percent difference to regression coefficient for consistency with the other studies.

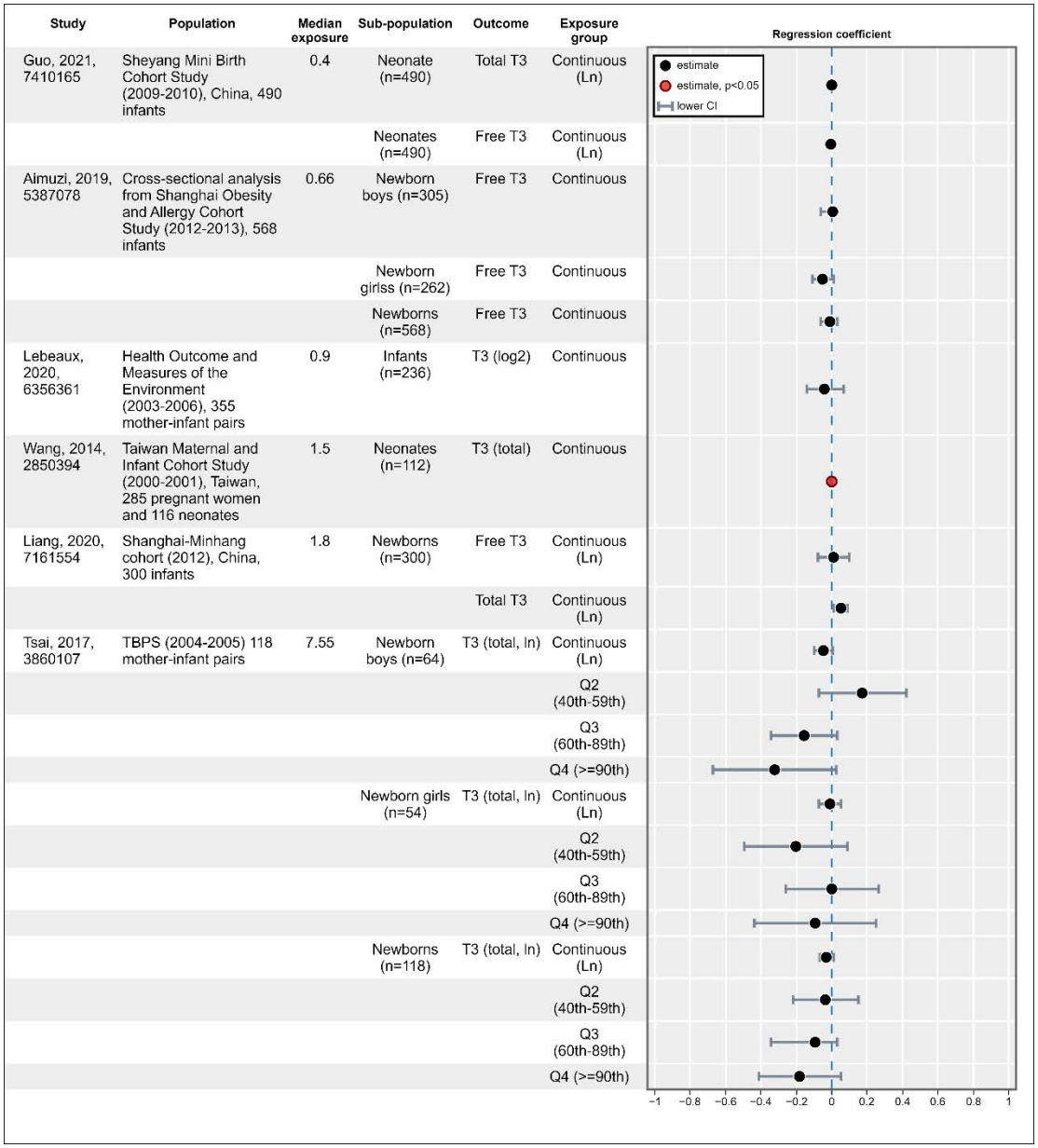


Figure 3-83. Associations between *medium* confidence studies of PFNA exposure and T3 levels in infants.[a-e] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-and-t3-infants/.

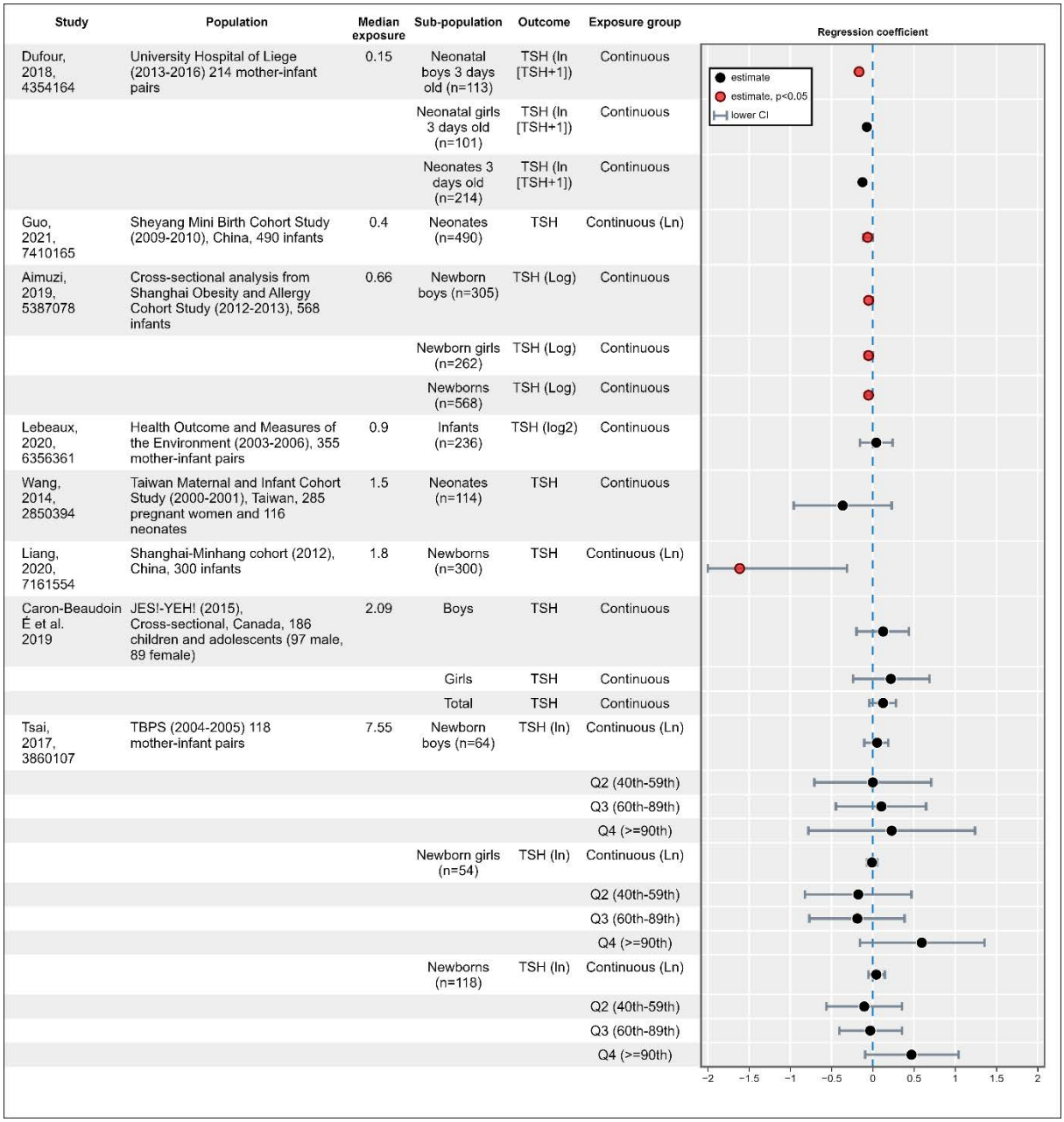
[a]Two studies are not displayed on the figure because they used a different effect estimate type (Yang et al., 2016a) or only presented quantitative results for significant associations (Berg et al., 2017). Neither reported an association between PFNA exposure and T3.

[b]Regression coefficient of zero represents no/null association.

[c]Studies are sorted by median exposure level (in ng/mL).

[d]Regression coefficients are expressed as the increase in T3 levels per 1 ng/mL unit increase in exposure unless otherwise noted. Units for the outcome varied across studies.

[e]If a study presented results for both free T3 and total T3 and the effect estimates were similar, both were extracted, but only free T3 is presented on the figure.



Study	Population	Median exposure	Sub-population	Outcome	Exposure group
Dufour, 2018, 4354164	University Hospital of Liege (2013-2016) 214 mother-infant pairs	0.15	Neonatal boys 3 days old (n=113)	TSH (ln [TSH+1])	Continuous
			Neonatal girls 3 days old (n=101)	TSH (ln [TSH+1])	Continuous
			Neonates 3 days old (n=214)	TSH (ln [TSH+1])	Continuous
Guo, 2021, 7410165	Sheyang Mini Birth Cohort Study (2009-2010), China, 490 infants	0.4	Neonates (n=490)	TSH	Continuous (Ln)
Aimuzi, 2019, 5387078	Cross-sectional analysis from Shanghai Obesity and Allergy Cohort Study (2012-2013), 568 infants	0.66	Newborn boys (n=305)	TSH (Log)	Continuous
			Newborn girls (n=262)	TSH (Log)	Continuous
			Newborns (n=568)	TSH (Log)	Continuous
Lebeaux, 2020, 6356361	Health Outcome and Measures of the Environment (2003-2006), 355 mother-infant pairs	0.9	Infants (n=236)	TSH (log2)	Continuous
Wang, 2014, 2850394	Taiwan Maternal and Infant Cohort Study (2000-2001), Taiwan, 285 pregnant women and 116 neonates	1.5	Neonates (n=114)	TSH	Continuous
Liang, 2020, 7161554	Shanghai-Minhang cohort (2012), China, 300 infants	1.8	Newborns (n=300)	TSH	Continuous (Ln)
Caron-Beaudoin É et al. 2019	JES!-YEH! (2015), Cross-sectional, Canada, 186 children and adolescents (97 male, 89 female)	2.09	Boys	TSH	Continuous
			Girls	TSH	Continuous
			Total	TSH	Continuous
Tsai, 2017, 3860107	TBPS (2004-2005) 118 mother-infant pairs	7.55	Newborn boys (n=64)	TSH (ln)	Continuous (Ln)
					Q2 (40th-59th)
					Q3 (60th-89th)
					Q4 (>=90th)
			Newborn girls (n=54)	TSH (ln)	Continuous (Ln)
					Q2 (40th-59th)
					Q3 (60th-89th)
					Q4 (>=90th)
			Newborns (n=118)	TSH (ln)	Continuous (Ln)
					Q2 (40th-59th)
					Q3 (60th-89th)
					Q4 (>=90th)

Figure 3-84. Associations between *medium* confidence studies of PFNA exposure and TSH levels in infants.[a-d] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-and-tsh-infants/.

[a]Two studies are not displayed in the figure because they used a different effect estimate type (Yang et al., 2016a) or only presented quantitative results for significant associations (Berg et al., 2017). Neither reported an association between PFNA exposure and TSH.

[b]Regression coefficient of zero represents no/null association.

[c]Studies are sorted by median exposure level (in ng/mL).

[d]Regression coefficients are expressed as the increase in TSH levels per 1 ng/mL unit increase in exposure unless otherwise noted. Units for the outcome varied across studies.

Hyperthyroidism and hypothyroidism

Six studies (Dufour et al., 2018; Lin et al., 2013b; Wen et al., 2013; Lopez-Espinosa et al., 2012) examined associations between PFNA and dichotomous hyper- and hypothyroidism outcomes in addition to previously described evaluation of continuous THs. All of these but Lin et al. (2013b) were *medium* confidence. There were no statistically significant associations between PFNA exposure and hyper- or hypothyroidism or "thyroid disease" (see Figure 3-85), but higher odds were observed with hyperthyroidism in Wen et al. (2013), hypothyroidism in Lin et al. (2013b), and subclinical hypothyroidism in Wen et al. (2013). Subclinical hypothyroidism was defined as TSH > 5.43 mIU/L, and hyperthyroidism was defined as TSH < 0.24 mIU/L. Because of the small number of studies for each outcome and the imprecise estimates, these findings are difficult to interpret.

Outcome	Study	Population	Median exposure	Sub-population	Exposure group	Odds Ratio
Hyperthyroidism (subclinical)	Lopez-Espinosa, 2012, 1291122	C8 Health Project, Cross-sectional, U.S., 10,725 children and adolescents	1.5	Children 1-17 yrs (n=10725)	Continuous (Ln)	0.78
	Wen, 2013, 2850943	NHANES (2007-2010), U.S., 1,181 adults (672 men, 509 women)	1.5	Men	Continuous (Ln)	2.41
				Women	Continuous (Ln)	1.91
Hypothyroidism	Lin, 2013, 1332458	Young Taiwanese Cardiovascular Cohort Study (2006-2008), Taiwan, 511 12-30 year olds (214 men, 337 women)	1	Men and women 12-30 years old	T2 (60th-90th)	1.93
					T3 (>90th)	1.75
	Lopez-Espinosa, 2012, 1291122	C8 Health Project, Cross-sectional, U.S., 10,725 children and adolescents	1.5	Children 1-17 yrs (n=10725)	Continuous (Ln)	1.11
	Dufour, 2018, 4354164	University Hospital of Liege (2013-2016) 214 mother-infant pairs	0.15	Pregnant women (n=214)	Q2	1.78
					Q3	1.86
					Q4	1.17
Hypothyroidism (subclinical)	Lopez-Espinosa, 2012, 1291122	C8 Health Project, Cross-sectional, U.S., 10,725 children and adolescents	1.5	Children 1-17 yrs (n=10725)	Continuous (Ln)	0.99
	Kim, 2020, 6833758	EDC cohort (2012-2015), South Korea, 660 children	1.4	Boys age 6 (n=348)	Continuous (Ln)	0.73
				Children age 6 (n=660)	Continuous (Ln)	0.82
				Girls age 6 (n=312)	Continuous (Ln)	0.91
	Wen, 2013, 2850943	NHANES (2007-2010), U.S., 1,181 adults (672 men, 509 women)	1.5	Men	Continuous (Ln)	1.3
				Women	Continuous (Ln)	2.54
Thyroid disease	Lopez-Espinosa, 2012, 1291122	C8 Health Project, Cross-sectional, U.S., 10,725 children and adolescents	1.5	Children 1-17 yrs (n=10725)	Continuous (Ln)	1.05
Thyroid disease or medication use	Gallo, 2022, 9962235	Veneto (2017), Italy, 14,888 adults and 6,536 adolescents	0.5	Female adolescents (n=3160)	Continuous	0.91
				Female adults (n=7358)	Continuous	1.05
				Male adolescents (n=3376)	Continuous	1.13
				Male adults (n=7530)	Continuous	1.08

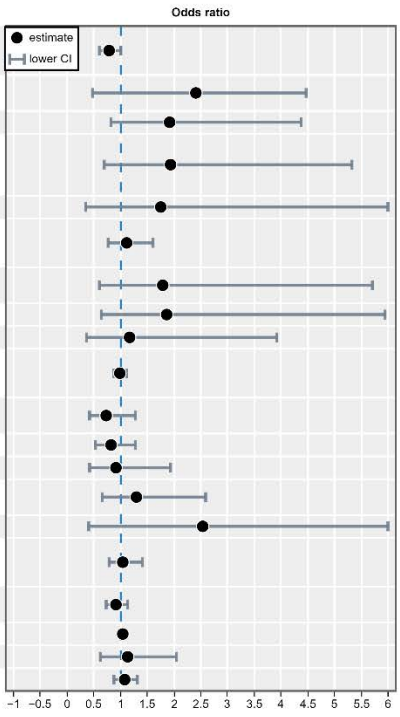


Figure 3-85. Associations between PFNA exposure and thyroid disease effects in adults and children. See interactive HAWC link: https://hawc.epa.gov/summary/data-pivot/assessment/100500071/pfna-and-thyroid-disease/.

Animal Studies – Thyroid and Adrenal Effects

The animal evidence base for PFNA includes a *high* confidence 28-day exposure study of PFNA effects on thyroid endpoints and adrenal gland weight of adult rats (NTP, 2018), and three *medium* confidence 14-day exposure studies in adult male rats evaluating adrenal hormones (Hadrup et al., 2016; Fang et al., 2009; Fang et al., 2008) (see Figure 3-86). For the *high* confidence

NTP study (NTP, 2018) thyroid hormones (TSH, T3, and T4) were evaluated in blood and determined by standard automated methods.

Another two studies were not experimental in design but were rather observational studies of house cats that examined PFNA (and other PFAS) associations with thyroid health (Weiss et al., 2021; Wang et al., 2018b). The study by Weiss et al. (2021) was judged to be overall *low* confidence due to deficiencies in reporting of methods employed for measuring serum THs and control of potential confounders (unknown diet, serum sampling timing, and fasting status), and concerns for endpoint sensitivity due to the use of a single point estimate of serum THs in a highly mixed population of adult males and females of wide age ranges and reproductive status. The study by Wang et al. (2018b) was considered *uninformative* for hazard characterization and is not considered further because there were several critical deficiencies, including substantial missing reporting on study methods, how potentially confounding variables were controlled, and major concerns for endpoint sensitivity related to the use of subjective physical appearance to indicate thyroid disease (Wang et al., 2018b).

The NTP 28-day study reported overt toxicity with increased mortality in adult male and female rats at higher PFNA doses (5 and 10 mg/kg-day in males; 12.5 and 25 mg/kg-day in females; see Section 3.2.1, "Adult rodent mortality"). This study also reported significant body weight loss in male rats at 1.25 mg/kg-day (17%) and 2.5 mg/kg-day (44%), and in female rats at 6.25 mg/kg-day (10%). THs are central modulators of basal metabolism and adaptive thermogenesis, and dietary restriction studies provide some insights into effects of body weight loss on TH economy, although there is uncertainty regarding the relevance of this paradigm to interpreting the changes resulting from PFNA exposure as the mechanism for body weight loss differs. In female rats, serum concentrations of T4, T3, and TSH were unaffected in a 20-day dietary restriction that led to decreases in body weight up to 19% (Laws et al., 2007). However, in male rats, effects of short-term body weight loss on serum THs are inconsistent in magnitude, with reductions in T4, T3, and TSH starting at ≥9% weight loss (Laws et al., 2007) or ≥15% weight loss (O'Connor et al., 1999) after 30-day feed restrictions, or with serum THs unaffected after 30 days in pair-fed control rats at up to 15% weight loss (Stoker et al., 2000). Thus, PFNA treatments at 0.625 mg/kg-day for males and up to 6.25 mg/kg-day PFNA in females are considered the most reliable indicators of potential treatment-related thyroid alterations, i.e., serum TH changes evident in the absence of weight loss. Alterations in serum hormones of male rats at the higher doses of 1.25 and, most notably, 2.5 mg/kg-day dose levels are discussed below and interpreted with caution. The 14-day exposure studies that evaluated PFNA effects on adrenal hormones also reported body weight loss exceeding 10% in the rodent testing at ≥3 mg/kg-day (Hadrup et al., 2016; Fang et al., 2009; Fang et al., 2008). These endpoints, summarized below, are likewise interpreted with caution.

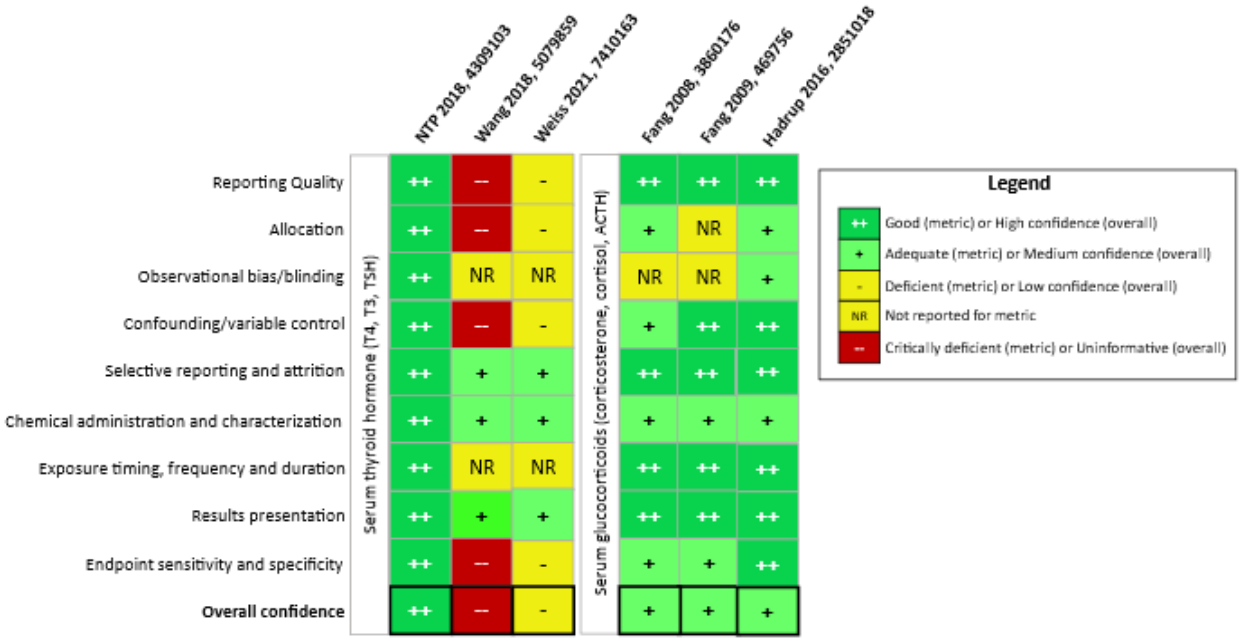


Figure 3-86. Heat map of that examined PFNA effects on thyroid and adrenal hormone levels. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Animal-thyroid-hormones/; https://hawc.epa.gov/summary/visual/assessment/100500071/Adrenal-hormones/.

Thyroid and adrenal hormones

PFNA effects on circulating levels of THs (T4, T3, TSH) have been evaluated in the *high* confidence 28-day exposure study (NTP, 2018), and the *low* confidence environmental surveillance study of house cats (Weiss et al., 2021). Three short-term exposure studies have also examined PFNA effects on serum levels of the glucocorticoids, corticosterone, cortisol, and ACTH (Hadrup et al., 2016; Fang et al., 2009; Fang et al., 2008). The serum TH data were *high* confidence and the adrenal hormone data were judged to be *medium* confidence with some minor limitations, although the evaluations were generally well conducted.

The NTP 28-day study showed patterns of TH perturbation characterized by consistent reductions in serum T4 in adult male and female rats. Serum TSH remained unchanged in females and there was a statistically significant decreasing trend in TSH in males. In females, significant dose-dependent declines in free and total T4 were observed with significant reductions ranging from 36% (both free and total) to 40% and 53% respectively at ≥3.12 mg/kg-day PFNA. There were no significant changes in TSH (or T3) reported, presenting a pattern different from what is seen in hypothyroidism (hypothyroidism is characterized by elevated TSH as a compensatory response of the hypothalamic-pituitary-thyroid axis to reductions in T4) (see Table 3-33; Figure 3-87). This pattern has been observed for several other PFAS that have been tested to date and is consistent with a clinical condition in humans known as hypothyroxinemia that is characterized by low

concentrations of serum T4 in the presence of TSH concentrations within normal physiological ranges [see additional discussion in (U.S. EPA, 2018b)]. In male rats, PFNA caused dose-dependent reductions in both serum T4 and in TSH at ≥0.625 mg/kg-day. However large TSH decrease of 33% at 0.625 mg/kg-day were not reported as statistically significant (higher doses showed statistical significance). This pattern of decreased T4 and decreased TSH is difficult to interpret without additional data, such as more extensive hormone testing and closer examination of pituitary functioning. However, serum T4 declines in males were notably more severe than in females, which may in part be explained by the longer half-life of PFNA in males (see Section 3.1). Specifically, serum total T4 in males was reduced by 91% and 84% at 0.625 and 1.25 mg/kg-day, respectively, while free T4 was reduced by 75%–85% at doses ≥0.625 mg/kg-day. While reductions in free and total T4 in male rats at 1.25 mg/kg-day co-occurred with significant weight loss of approximately 17%, large reductions in free and total T4 were also observed at the lowest dose of 0.625 mg/kg-day where there was no significant body weight loss. The total T4 response did lack dose dependency with less severe reductions at the higher 2.5 mg/kg-day dose level, a dose that caused an increase in serum T3 but that was likely confounded by high weight loss (44%).

Some uncertainties and observations in the NTP 28-day TH data are worth noting. One uncertainty is that the free T4 was measured by NTP (2018) using analog RIA assay methods that could have potentially over-estimated reductions in serum free T4. For measurement of free T4, the reference method is to use equilibrium dialysis followed by RIA to avoid potential negative bias from interference with serum proteins (NTP, 2019; Chang et al., 2007). As discussed below (see "Mechanistic and Supplemental Information"), PFNA has been shown to competitively bind the serum TH transport protein transthyretin. It cannot be ruled out that this binding could have interfered with the performance of the analog RIA contributing to artificially low free T4 values. Regarding the total T4 analysis in males, serum measurements for most samples fell below the assay LOQ (0.5 µg/dl) at the two lowest doses, 0.625 and 1.25 mg/kg-day. Therefore, the magnitude of the declines in serum total T4 in males resulted in T4 measurements approaching or falling below reported LOQs in most cases. When serum T4 levels were <LOQ in individual samples, the LOQ was reported to have been assigned for statistical testing. While this is standard practice and does not detract from the results as reported, it does reinforce the analytical challenges of measuring low levels of T4 and the severity of reported declines in T4 of males.

A second observation in the male hormone data related to the significant trend of reduced TSH, with statistically significant decreases at ≥1.25 mg/kg-day (see Table 3-33 and Figure 3-87) (NTP, 2018). As noted above, this pattern of change (decreases in both T4 and TSH) is generally different from what has been observed for other PFAS to date where TSH is unresponsive to reductions in serum T4 (note: stereotypical goitrogens that disrupt the hypothalamic-pituitary-thyroid axis of rats cause decreases in T4 with compensatory increases in TSH). Similar to the changes in serum T4, it is possible that the body weight loss associated with PFNA doses ≥1.25 mg/kg-day in males was a contributing factor. However, given the added support from

nonsignificant reductions in TSH (33%) at 0.625 mg/kg-day, a dose without significant body weight deficits, this also suggests the possibility of different mechanisms of PFNA effects on thyroid signaling, at least in males, which are currently unknown. It is also plausible that sex-specific pharmacokinetic differences could be a contributing factor with the longer half-life of PFNA in males (see Section 3.1).

Although it is difficult to interpret the TSH reductions in males and patterns of TH changes in animals may not translate directly to human clinical definitions, the observed decreases in total or free T4 in females in the absence of increases in TSH indicate thyroid perturbations and are considered biologically relevant to humans (Crofton, 2004; Lau et al., 2003b). TSH is an indicator that the thyroid system has been perturbed, but it does not always change when serum T4 is decreased (Hood et al., 1999). Adverse neurological outcomes have been demonstrated following decreased T4 levels during the early neonatal period with no changes in T3 or TSH (Crofton, 2004). During pregnancy and early development, even transient perturbations in thyroid function can have permanent impacts on normal growth and neurodevelopment of offspring. The more limited result in males of decreasing trends of free T4 is also considered relevant to humans, although the mechanism for the accompanying decrease in TSH is unknown and body weight loss at higher doses made the male findings difficult to interpret. Given the potential consistency of these findings with those observed for other PFAS, the availability of a single short-term study of THs represents a significant data gap for PFNA.

The reduction in serum T4 observed in the 28-day study in rats was not aligned with *low* confidence findings from the observational study in male and female house cats that reported statistically significant positive associations of total T4 with PFNA exposures from house dust (Weiss et al., 2021). However, this study was judged to be overall *low* confidence due to deficiencies in reporting the analytical methods for THs, how some potential confounders were controlled, and endpoint sensitivity concerns due to the use of a single point estimate of serum THs.

Table 3-33. PFNA effects on serum thyroid hormones in adult female and male Sprague-Dawley rats reported as absolute values and percent reductions relative to controls in a *high* confidence 28-day study (NTP, 2018)[a]

Dose (mg/kg-d)	Body weight change (%)	TSH		T3		Free T4		Total T4	
		Absolute level (ng/mL; mean ±SE)	Relative change (%; CI 95)	Absolute level (ng/dl; mean ±SE)	Relative change (%; CI 95)	Absolute level (ng/dl; mean ±SE)	Relative change (%; CI 95)	Absolute level (µg/dl; mean ±SE)	Relative change (%; CI 95)
Female rats									
0		14.637 ± 1.663		93.700 ± 6.025		1.702 ± 0.199*		4.370 ± 0.409*	
1.56	−3.0	15.515 ± 1.531	+6.0 (−25,+37)	84.100 ± 4.148	−10 (−25,+4.0)	1.473 ± 0.154	−14 (−40,+13)	3.570 ± 0.283	−18 (−38,+1.3)
3.125	−5.8	14.108 ± 1.126	−3.6 (−30,+23)	83.260 ± 2.674	−11 (−24,+1.4)	***1.096 ± 0.097***	***−36 (−54,−17)***	***2.810 ± 0.166***	***−36 (−50,−22)***
6.25	−10	14.331 ± 1.077	−2.1 (−28,+24)	89.900 ± 6.819	−4.1 (−23,+15)	***0.797 ± 0.096***	***−53 (−69,−38)***	***2.610 ± 0.237***	***−40 (−55,−25)***
Male rats									
0		20.330 ± 2.312*		78.210 ± 4.542*		2.157 ± 0.152*		2.360 ± 0.266	
0.625	−3.3	13.702 ± 1.265	−33 (−52, −13)	58.540 ± 2.11	−25 (−35, −15)	***0.546 ± 0.024***	***−75 (−79,−75)***	***0.210 ± 0.069 (<LOQ)***	***−91 (−97,−85)***
1.25	−17	***10.967 ± 1.233(9)***	***−46 (−62,−29)***	84.930 ± 2.941	+8.6 (−5.8,+23)	***0.328 ± 0.009***	***−85 (−87,−83)***	***0.380 ± 0.066 (<LOQ)***	***−84 (−90,−77)***
2.5	−44	***10.160 ± 3.348(4)***	***−50 (−84,−16)***	***111.789 ± 10.157(9)***	***+43 (+12,+73)***	***0.302 ± 0.001(7)***	***−86 (−88,−84)***	1.490 ± 0.129	−36 (−54, −19)

LOQs: TSH = 1 ng/mL; T3 = 50 ng/mL; free T4 = 0.3 ng/dl; total T4 = 0.5 µg/dl.

*Author-reported statistically significant trend at $p < 0.01$.

[a]Bold-italicized font indicates author-reported significant differences observed between control and PFNA treatments; n = 10 unless otherwise noted (in parentheses). Results at higher doses not included due to mortality and small sample sizes; in males at 5 mg/kg-d and females at 12.5 mg/kg-d two or one animals were examined with all dying at the highest dose.

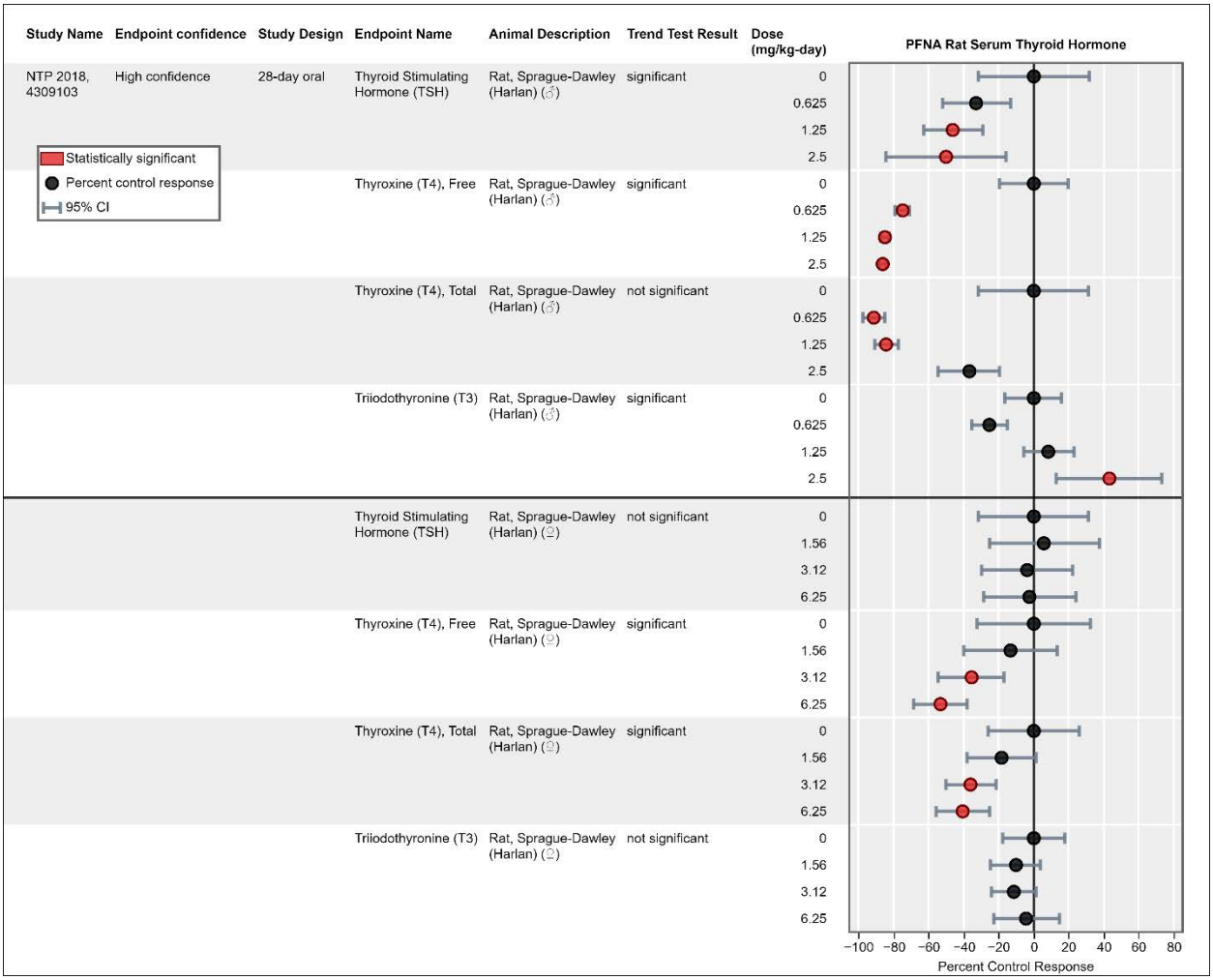


Figure 3-87. PFNA effects on serum thyroid hormones of adult male (top) and female (bottom) Sprague-Dawley rats in the *high* confidence 28-day study displayed as percent control response. See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-rat-TH/.

With respect to adrenal hormones, dose-dependent increases in serum cortisol were reported in adult male mice after a 14-day exposure at 3 and 5 mg/kg-day PFNA (35% and 50%) increase, respectively (see Figure 3-88) (Fang et al., 2008). In addition, significant increases in serum cortisol (67%) and ACTH (102%) were observed in adult male SD rats exposed to 5 mg/kg-day PFNA for 14 days (Fang et al., 2009). However, the concurrent body weight loss in mice and rats at ≥3 mg/kg-day reduce certainty in these adrenal hormone perturbations as direct effects of PFNA. Hadrup et al. (2016) measured elevated plasma corticosterone in male rats at 0.013 mg/kg-day PFNA but results did not follow a dose-response gradient, with null findings for ACTH alterations. Together, changes in glucocorticoids were difficult to interpret because of the small evidence base and lack of dose-dependent changes in the absence of overt toxicity.

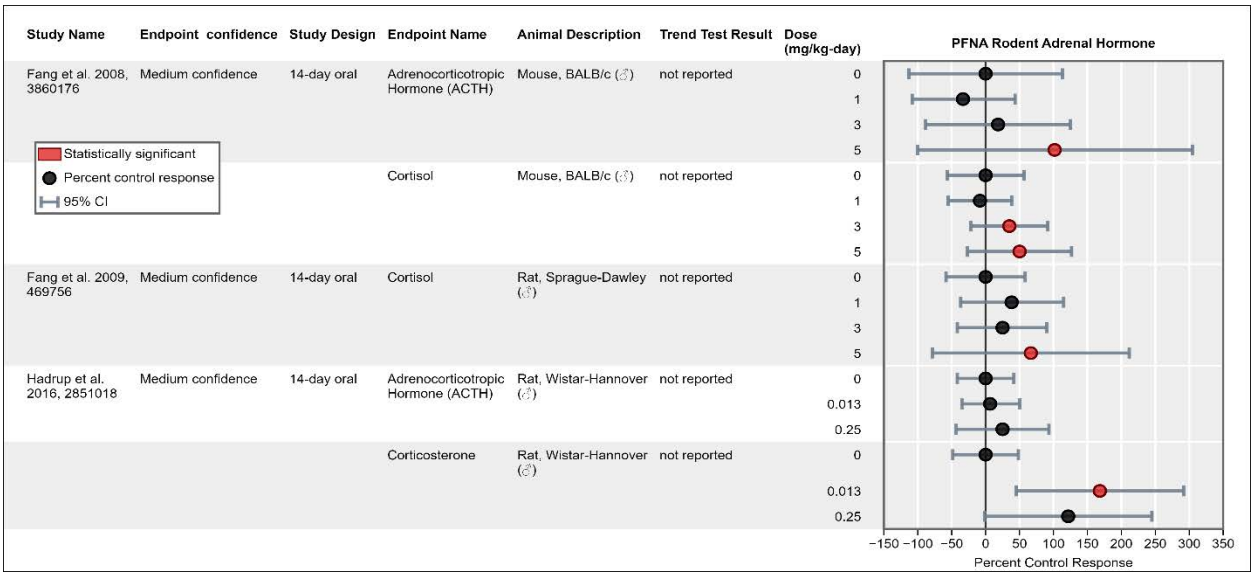


Figure 3-88. PFNA effects on serum adrenal hormones of adult male rats and mice from short-term studies displayed as percent control responses. See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/PFNA_adrenal_rodent/.

1 Organ weights and histopathology

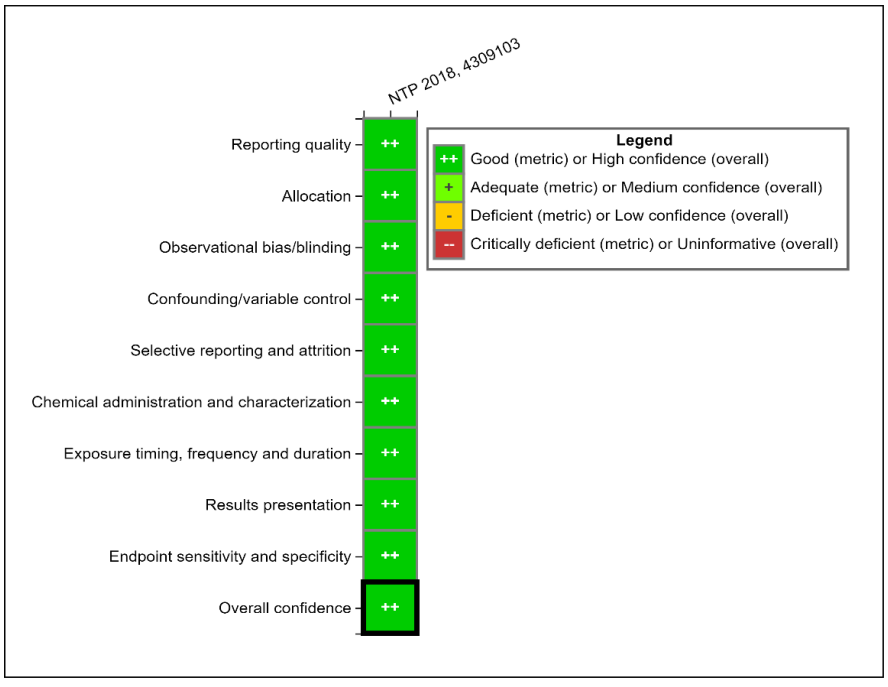


Figure 3-89. Heat map of the NTP (2018) 28-day study of PFNA-induced effects on thyroid gland weight and histopathology, and adrenal gland weight. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Thyroid-and-adrenal-organ-weight-and-histopat-6e51/.

The effects of PFNA exposures on adult male and female thyroid and adrenal gland weights, and thyroid, parathyroid, and pituitary histology, were evaluated in the NTP 28-day study. These endpoints were found to be *high* confidence (see Figure 3-89). The parathyroid and pituitary tissue histopathology was evaluated at ≥2.5 mg/kg-day in males and ≥6.25 mg/kg-day in females, and not at the lower two doses. Histological examinations were undertaken in animals from the two higher PFNA dose groups that died early prior to terminal sacrifice as a result of chemical-induced death (termed natural death as distinguished from moribund sacrifice). Routine checks for morbidity and mortality (twice daily) mitigated concerns for tissue degradation in non-surviving animals.

There were no significant changes reported in absolute or relative thyroid gland weights in female or male rats at doses that did not reduce body weight (see Figure 3-90) (NTP, 2018). Consistent with the lack of effects on thyroid weights, there was no evidence of follicular cell hypertrophy or hyperplasia reported in either adult male or female thyroid glands. The parathyroid and pituitary were also negative for histopathological changes at the higher dose levels examined, although there were some generally minor reductions in sample sizes of the parathyroid due to missing tissues. Neither was histopathology evident in the thyroid and parathyroid glands of animals from the two highest PFNA dose groups with mortality prior to terminal sacrifice. Although consistent with findings at lower doses, this preterm mortality clearly truncated the window of exposure, possibly obviating the progression and manifestation of morphological changes in these glands.

Dose-dependent reductions in absolute adrenal weights were observed in adult males in the 28-day exposure study (see Figure 3-90). Studies report conflicting results that adrenal gland weight is proportional (Nirogi et al., 2014) and not proportional (Bailey et al., 2004) to body weight. Thus, both absolute and relative to body weight are considered potentially relevant for this endpoint. No histopathological changes were reported in the adrenal or pituitary glands of either sex.

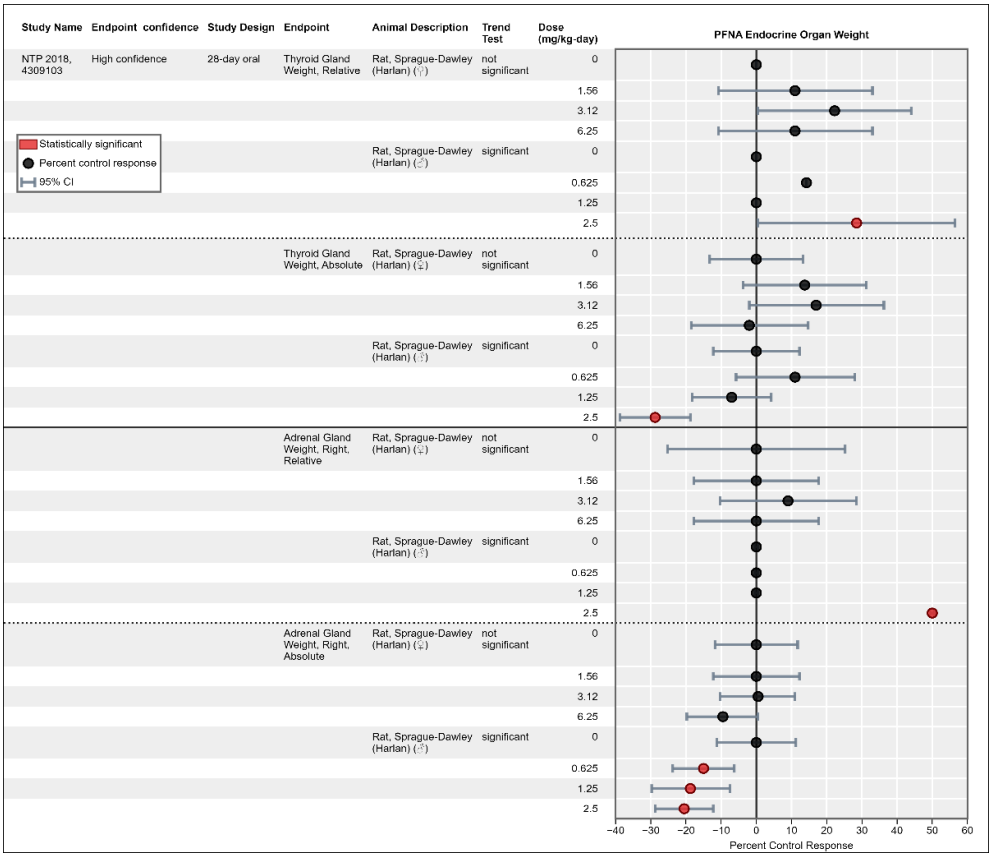


Figure 3-90. PFNA effects on thyroid (top) and adrenal gland (bottom) weight of adult male and female Sprague-Dawley rats from a 28-day study displayed as percent control responses. See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-animal-endocrine-organ-weight/.

Mechanistic and Supplemental Studies

A handful of studies have examined molecular mechanisms underlying PFNA effects on the thyroid as presented in the thyroid adverse outcome pathway (AOP) network described in Noyes et al. (2019), with no studies identified that are directly relevant to adrenal signaling. Observations of reduced serum T4 with unchanged TSH levels are reported in adult and neonatal rodent models exposed to other short-chain PFAS (e.g., PFBA; PFBS; PFHxA) (U.S. EPA, 2023h, 2022a, 2021b) and long-chain PFAS (e.g., PFOA, PFOS; PFHxS) (U.S. EPA, 2023e, f). One pathway of chemical-induced dysregulation in the thyroid AOP involves MOAs of hepatic CAR/PXR activation that favor metabolism and excretion of free T4. There is some evidence with the PFAS suggesting this MOA pathway may be operating (Davidsen et al., 2022; Dong et al., 2016; Yu et al., 2009; 2007; Luebker et al., 2005; Lau et al., 2003a; Thibodeaux et al., 2003). For PFNA, in vivo transcriptional profiling in CAR-null and wild type mice treated with PFNA (Rosen et al., 2017), as well as in vitro testing in rodent liver cell lines (Zhang et al., 2017), show PFNA activation of CAR and PXR, suggesting possibly shared MOAs of TH catabolism with other PFAS.

Additional mechanistic testing of targets in the thyroid pathway show PFNA competitive binding with the TH serum transporter protein, transthyretin (TTR) but not thyroid binding globulin (TBG) (Kar et al., 2017; Ren et al., 2016; Weiss et al., 2009). While TBG (and not TTR) appears to be the major serum TH transporter in adult humans (Larsson et al., 1985; Snyder et al., 1976), this action could potentially displace native T4, increasing unbound T4 for tissue uptake or elimination (which could lead to reductions in serum T4). Additionally, TTR is critical during early human development in regulating T4 transport across the blood-placenta (2009), blood-brain (Schreiber et al., 1990; Herbert et al., 1986), and cerebrospinal fluid-brain (Richardson et al., 2018) barriers. Therefore, although currently unstudied for this chemical, it is possible that early lifestages could be at increased susceptibility to competition by PFNA for T4:TTR binding. Other data suggest that PFNA can bind serum human albumin but the potency to competitively displace TH is unclear (Bischel et al., 2010; Hebert and Macmanus-Spencer, 2010). Taken together, it remains unknown the extent to which metabolic activation and serum transporter binding affinities by PFNA may be contributing to the observed reductions in serum T4.

Other in vitro screening assays for PFNA interactions with other molecular targets in the thyroid pathway were mixed. PFNA was inactive in some Toxcast/Tox21 HTS assays that examined several molecular initiating events in the thyroid AOP network (Noyes et al., 2019), including TR agonism, thyroid stimulating hormone (TSH) receptor binding, thyrotropin releasing hormone (TRH) interaction, and sodium-iodide symporter (NIS) inhibition. In alignment with published studies, PFNA elicited activity in the in vitro HTS assay evaluating competitive binding to transthyretin (TTR). The in vitro HTS assays were also weakly active for thyroid peroxidase (*TPO*), iodothyronine deiodinase type 2 (*Dio2*), and iodotyrosine deiodinase (IYD) inhibition, TBG competitive binding and TH receptor (*TRβ*) antagonism, the latter results being equivocal due to potential nonspecific effects (Paul-Friedman et al., 2019) (see Appendix C.2). Dose-dependent TR antagonisms were also reported in T-screens with rat primary pituitary cells (Long et al., 2013). T-screen results, however, are also difficult to interpret due to nonspecific cell proliferation that can occur through TH-independent pathways (OECD, 2014). Finally, in vitro biochemical testing and in silico molecular docking reported PFNA is capable of binding TRα ligand binding domain (Ren et al., 2015). While not definitive, it appears from the totality of current screening-level data that PFNA does not act as a direct ligand for TR.

Evidence Integration

Thyroid

There is *slight* evidence in humans suggesting a potential association between PFNA exposures and thyroid effects, with considerable uncertainty in the results overall. The strongest evidence came from positive associations with T4 in children/adolescents, with most studies showing a consistent direction of association in both sexes, though effect sizes were small. There was also an indication of an inverse association with T4 and TSH in infants (primarily boys for T4);

however, there was considerable inconsistency, which reduces certainty. Across age groups, the direction of association was inconsistent as the studies suggested a positive association with T4 in children/adolescents and an inverse association in infants. Studies in adults, including among pregnant women, were even less consistent. Thus, there was some evidence that PFNA exposure might interfere with TH homeostasis, especially in the young, but it was not possible to identify the underlying physiological basis for the change in direction of responses that varies with age. At all assessed lifestages, associations for T3 and TSH, or for diagnosed thyroid disease, with PFNA were generally inconsistent. Overall, there was concern for bias toward the null across the body of evidence, therefore the null results are interpreted with caution.

With uncertainties, the animal data provides *moderate* evidence of thyroid toxicity. This conclusion is based on consistent and dose-dependent effects observed in a short-term *high* confidence exposure study showing pronounced declines in serum free and total T4 in both adult female and male rats, although the effects in males were difficult to interpret and this also influenced the interpretation of findings in females. In females, serum free and total T4 were dose dependently reduced at ≥3.12 mg/kg-day PFNA. This effect was not accompanied by an increase in TSH, reductions in serum T3, altered thyroid weights, or thyroid histopathology that are characteristic of overt hypothyroidism, but it is consistent with findings for several other PFAS. A similar pattern of T4 reductions was evident in males at lower dose levels (0.625 and 1.25 mg/kg-day) where there were no or only modest concurrent effects on body weight. However, PFNA effects on serum total T4 in males lacked dose-dependence and were accompanied by a dose-dependent and large decline in serum TSH. The biological basis for this response in males is hard to decipher. However, overall, concern for potential adversity remains, particularly given the large reductions in serum T4 in both sexes (75% reduction in free T4 and 91% reduction in total T4 in males at 0.625 mg/kg-day, a dose level unaffected by weight loss, and 36%–53% in females at ≥3.12 mg/kg-day). The greater severity of effects on serum T4 of males may be related to pharmacokinetic differences as the serum half-life of PFNA in male rats is substantially longer than in females (see Section 3.1). Underlying mechanisms of PFNA effects on TH signaling pathways are not well characterized with the evidence base comprising mostly in vitro screening-level assays that were generally equivocal and unclear (e.g., active TTR competitive binding and weak TPO inhibition). Some in vitro testing with PFNA in rodent liver cell lines also provides a plausible hypothesis that PFNA-induced activation of CAR/PXR enzymatic pathways may upregulate T4 metabolism and elimination, consistent with some evidence for other long-chain PFAS (PFOA, PFOS) that are also shown to reduce serum TH in rodents, but this is currently unknown for PFNA.

Although the short-term testing with PFNA provides evidence of adversity in T4 homeostasis in adults, uncertainties remain concerning the data from the only available study, including questions surrounding the reliability of methods used for measuring free T4 and difficult to decipher responses in males that may relate in part to body weight loss at higher doses. The lack of data in younger animals or for longer duration exposures also represent important data gaps.

The evidence of reduced serum free and total T4 in adult female rats is of concern particularly because of the potential susceptibility of the developing fetus with maternal/gestational exposures to PFNA. T4 insufficiencies during development, including moderate or transient reductions in T4 alone absent compensatory increases in TSH, can cause irreversible sensory, motor, and cognitive decrements, with the timing, duration, and severity of reductions influencing the types of neurological deficits manifested (e.g., see reviews by (O'Shaughnessy and Gilbert, 2019; Bernal, 2015; Gilbert et al., 2012; Crofton and Zoeller, 2005; Morreale de Escobar et al., 2004; Zoeller and Rovet, 2004)). For PFNA, the human neurotoxicity evidence base provides *slight* evidence of developmental neurotoxicity with considerable uncertainty, and with some studies reporting associations of PFNA exposures to ADHD and related behavioral disorders (see Section 3.2.8, "ADHD and related behavior"). More reliably, developmental toxicity studies in mice with PFNA (Das et al., 2015; Wolf et al., 2010) show dose-dependent delays in eye opening, a well-characterized responses in postnatal rodents with chemically induced TH insufficiency (Gilbert, 2011; Dong et al., 2009; Goldey et al., 1995) (see Section 3.2.2, "Postnatal developmental landmarks"). Delays of 1 day in eye opening due to hypothyroxinemia during a critical period of retinal development has been shown to reduce the time available for visual cortex development related to orientation selectivity by approximately 20% (Espinosa and Stryker, 2012). Whether TH perturbations play a mechanistic role in the developmental effects of PFNA remains poorly studied.

Taken together, the available ***evidence suggests*** but is not sufficient to infer that PFNA exposure may cause thyroid toxicity in humans given sufficient exposure conditions (see Table 3-34).[18] This was a complex evidence base to interpret as the human evidence was *slight* and the only animal study available provided *moderate* evidence but also had some uncertainties related to the free T4 analytical method used and body weight loss in males at higher doses. Despite these uncertainties, the large, dose-dependent reductions in free and total T4 in female rats and free T4 in male rats suggest some level of concern.

Adrenal

The human and animal evidence are *indeterminate* for PFNA effects on the adrenal gland as there are no human studies and only limited testing in animals with inconclusive results. Thus, the available ***evidence is inadequate*** to inform the potential for PFNA exposure to cause adrenal toxicity in humans. An evidence profile table for adrenal effects is not presented.

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

Table 3-34. Evidence profile table for thyroid effects

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.7. Human Studies – Thyroid Effects)					⊕⊙⊙ ***Evidence suggests but is not sufficient to infer*** *Primary basis:* *A high* confidence short-term exposure study in adult rats of reduced serum T4, with the most reliable findings in females at doses ≥3.12 mg/kg-d. The human and animal evidence are *indeterminate* for PFNA effects on the adrenal gland *Human relevance:* Thyroid effects in rats are considered potentially relevant to humans based on conserved biological processes *Cross-stream coherence:* Studies in humans were *slight* and neither increase nor decrease confidence in the animal findings *Susceptible populations and lifestages:* The developing fetus and children are susceptible to
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Hormone levels</u> Twenty-six *medium* confidence and 11 *low* confidence studies	• Inconsistent direction of associations between PFNA exposure and T4, T3, and TSH in adults, including pregnant women • Generally consistent positive associations with T4 in children • 4 of 9 studies reported inverse associations with T4 and 5 of 10 with TSH in infants. Sex-stratified results indicated stronger associations in boys in some studies	• *Medium* confidence studies	• Direction of association with T4 not consistent across age groups. • Unexplained inconsistency • Imprecision in most studies	⊕⊙⊙ *Slight* Some evidence of an association between PFNA exposure and thyroid hormone in children with median PFNA exposure levels ranging from 0.6 to 7.6 ng/mL	
Evidence from in vivo animal studies (see Section 3.2.7. Animal Studies – Thyroid Effects)					
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Hormone levels</u> One *high* confidence study in rats • 28 d	• Reductions in serum free and total T4 in adult female rats exposed short-term at ≥3.12 mg/kg-d and in serum free and total T4 in adult male rats exposed short-term at ≥0.625 mg/kg-d, including at a dose below those causing substantial body weight loss	• *Consistent* reductions in serum free and total T4 in females and males • *Consistency* with other PFAS • *Dose-response* in free and total T4 in females and free T4 in males • *Magnitude of effect*, up to 53% in females and 91% in males	• Analytical methods for measuring free T4 (potentially some overestimation of reductions)	⊕⊕⊙ *Moderate* Dose-dependent effects on reduced serum free and total T4 absent increases in TSH, thyroid weight, or tissue pathology in female rats in a single, short-term	

Summary of human, animal, and mechanistic evidence					Evidence integration summary judgment
Histopathology One *high* confidence study in rats • 28 d	• No evidence of thyroid follicular hypertrophy or hyperplasia in adult female and male rats exposed short-term	• No factors noted	• No factors noted	study, with some supportive findings in male rats and from evidence on other PFAS	altered thyroid hormone status and thus represent potentially susceptible lifestages. Developmental toxicity testing in mice shows delayed eye opening in rodent offspring, which is a well-described outcome in hypothyroid rodents (see Section 3.2.1: Animal Evidence) *Other inferences:* The T4 reductions absent increases in TSH are consistent with effects of other short (PFBS, PFBA, PFHxA) and long (PFOA, PFOS, PFHxS) chain PFAS. However, although there has been some screening of thyroid MOAs for PFNA, the evidence base findings are generally equivocal and unclear
Organ weight One *high* confidence study in rats • 28 d	• No evidence of thyroid weight changes in adult female and adult male rats exposed short-term	• No factors noted	• No factors noted		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Summary of key findings and interpretation**			**Evidence stream judgment**	
Molecular events – Receptor-ligand binding; TRH, TSH, TR	PFNA did not appear to interact with TRH, TSH, or TR receptors, although results were inconclusive for TR antagonism.			In vitro HTS assays were generally null or just weakly active, with some evidence of binding the serum transport protein, TTR, as well as potential upregulated T4 catabolism by activation of CAR/PXR that are unknown	
Molecular events – Serum transport proteins; TTR	PFNA appeared capable of acting as a competitive inhibitor of T4-TTR binding, although the biological relevance herein is unstudied.				
Molecular events – TH synthesis; TPO, IYD	ToxCast screening-level assays indicated some potential for weak inhibition of TPO and IYD activity.				
Cellular events – TH metabolism; DIO, Hepatic CAR/PXR activation	PFNA appeared capable of activating CAR/PXR pathways in vitro in murine and human liver cell lines but effects of these inductions in increasing hepatic T4 catabolism are unstudied. Toxcast screening-level assays also showed some weak inhibition of Dio2.				

3.2.8. Nervous System Effects (Developmental Neurotoxicity)

This section describes evidence informative to assessing potential neurotoxicity following exposure to PFNA. There are no epidemiological studies in adults that have examined neurological outcomes. A single short-term study in adult animals was available and reported null results for changes in brain weight or lesions, and no clinical observations that suggested impaired neurological function (NTP, 2018); thus, the animal evidence for adult neurotoxicity is *indeterminate* and no studies of developmental neurotoxicity were identified. Therefore, this section describes the epidemiological evidence currently available to inform the assessment of potential developmental neurotoxicity in early lifestages exposed maternally and/or postnatally to PFNA. Readers are referred to Section 3.2.2 for the assessment of evidence of effects on other developmental endpoints, such as fetal and offspring survival, growth, and developmental progression.

Human Studies

Twenty-five studies (34 publications) examined associations between PFNA exposure (measured in blood) and neurodevelopmental outcomes. Neurodevelopment is typically assessed with a wide array of neurobehavioral or neuropsychological tests, and it is difficult to define clear-cut divisions of neuropsychological categories. For example, longer mean reaction time (a measure of response time after a stimulus is introduced) on a continuous performance test typically indicates inattention but may also be impacted by slower information processing or issues with motor control. For the purposes of this review, and based partly on data availability, tests were categorized into those evaluating cognition, ADHD and related behaviors, social behavior and autism, and other outcomes. Cognition was evaluated in 10 studies using a variety of endpoints including IQ, executive function, language development, and intellectual disability. ADHD and related behaviors were evaluated in 10 studies, with endpoints including: ADHD diagnosis, inattention, impulsivity, hyperactivity, externalizing problems, and total behavior problems. Social behavior was evaluated in nine studies with endpoints including: autism spectrum disorder diagnosis, and three different autism screening scores. Outcomes outside these categories included motor effects (four studies) and cerebral palsy (one study). Within each of these categories, it can be difficult to assess consistency due to heterogeneity in the tools and age ranges used in the studies.

Several considerations were specific to neurodevelopment. For outcome ascertainment, tests used in a study should be appropriate for the age range being studied and to the culture and language of the study participants. Any relevant factors such as time of day of test administration or computer use should have been considered in the analysis, and some description of the testing environment provided. Consideration should also have been given if there were multiple raters (e.g., statistical adjustment for rater, or analysis of interrater reliability). Studies preferably reported results for subscales as well as summary scales. Evaluation of confounding included both

the approach used to identify potential confounders, as well as consideration of key covariates. Confounders that were considered potentially relevant across studies included child age and sex, maternal age, socioeconomic status, quality of caregiving environment, prenatal tobacco exposure, and maternal mental health and IQ. It was considered preferable for analyses to use the outcome scales as continuous variables to minimize misclassification and improve statistical power (Sagiv et al., 2015), though this does not apply to clinical diagnosis of conditions such as autism spectrum disorder and ADHD.

The majority of available studies were birth cohorts with maternal exposure measurement (Dalsager et al., 2021; Oh et al., 2021; Skogheim et al., 2021; Luo et al., 2020; Skogheim et al., 2020; Spratlen et al., 2020a; Weng et al., 2020; Niu et al., 2019; Harris et al., 2018; Høyer et al., 2017; Jeddy et al., 2017; Lien et al., 2016; Oulhote et al., 2016; Vuong et al., 2016; Wang et al., 2015b; Liew et al., 2014). In some cohort studies, childhood exposure was measured as well (Dalsager et al., 2021; Harris et al., 2018; Oulhote et al., 2016; Vuong et al., 2016) or exposure was measured in cord blood only (Yao et al., 2022). There were two case-control studies with measurement of banked maternal samples (Lyall et al., 2018; Ode et al., 2014) and one case-control study with maternal samples collected concurrent with outcome ascertainment (Shin et al., 2020). In addition, there were three cross-sectional studies that were based on data from NHANES (Hoffman et al., 2010), the C8 Health Project (Stein and Savitz, 2011), and a survey in the United States (Gump et al., 2011). The cross-sectional studies were rated as deficient for exposure measurement and *low* confidence overall due to concerns that current exposure during late childhood and adolescence may not be a reasonable proxy of exposure during an etiologically relevant period (e.g., interpreted as likely to be earlier than 6 years of age for most cases of ADHD) given the half-life of PFNA (generally in the range of 2–5 years). The study evaluations are summarized in Figure 3-91.

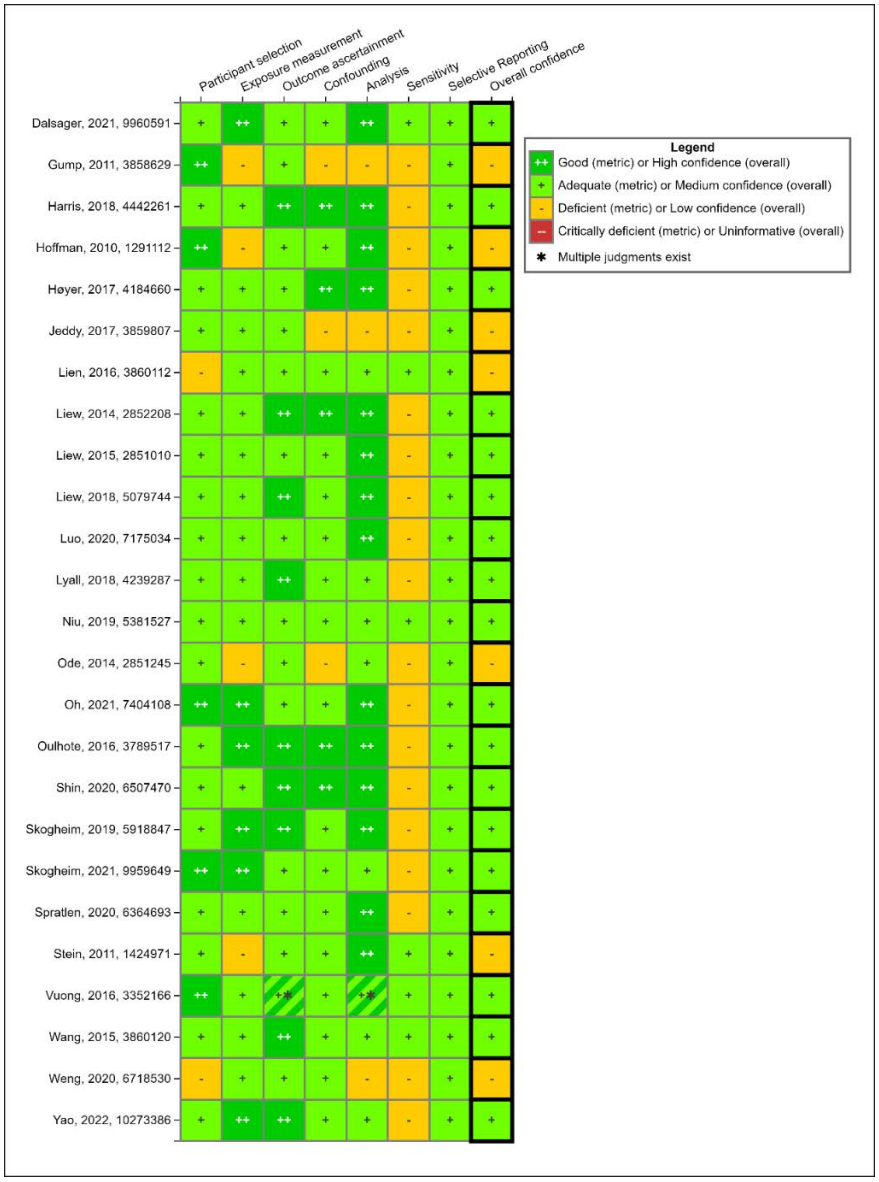


Figure 3-91. Summary of study evaluation for epidemiology studies of neurodevelopment.[a] See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/pfna-and-neurodevelopment-epidemiology-study-evalu/.

[a]Multiple publications of the same study: HOME study: Vuong et al. (2016) also includes Vuong et al. (2018b), Vuong et al. (2018a), Vuong et al. (2019), Vuong et al. (2021a), Vuong et al. (2020), Vuong et al. (2021b), Braun et al. (2014), and Zhang et al. (2018a); Project Viva: Harris et al. (2018) also includes Harris et al. (2021). Four publications with data from the Danish National Birth Cohort were evaluated separately due to significantly different procedures but should not be considered independent: Liew et al. (2014), Liew et al. (2015), Liew et al. (2018b), Luo et al. (2020). 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). Two publications with data from the Taiwan Maternal and Infant Cohort Study were evaluated separately due to different sub-samples: Wang et al. (2015b) and Weng et al. (2020).*Ratings for this domain varied by outcome.

Cognition

Ten studies reported on cognition endpoints, including nine *medium* confidence, and one *low* confidence study. The *medium* confidence studies are presented in Table 3-35. Results were inconsistent across the studies. One *medium* confidence study by (Wang et al., 2015b) reported a statistically significant decrease in verbal IQ at 8 years with maternal PFNA exposure, and inverse associations were also observed in full-scale intelligence quotient (FSIQ) and performance IQ at that age, though not statistically significant. However, no association with visual or FSIQ was observed at 5 years. Another *medium* confidence study reported statistically significant positive associations between childhood exposure and both FSIQ and global executive function when PFNA was measured in the children but not when measured in maternal samples taken during pregnancy (Vuong et al., 2019; Vuong et al., 2016); another analysis in this study population found a positive association with reading scores with maternal samples (Vuong et al., 2020). The remaining *medium* confidence studies of IQ and other cognitive effects reported non-monotonic associations across quartiles (including changing directions in some studies) or different directions of associations by age, which do not clearly support an association in either direction (Yao et al., 2022; Skogheim et al., 2020; Spratlen et al., 2020a; Niu et al., 2019; Harris et al., 2018; Liew et al., 2014). The single *medium* confidence study of intellectual disability (Lyall et al., 2018) indicated reduced odds of intellectual disability with higher PFNA exposure. Lastly, the *low* confidence study (Jeddy et al., 2017), which examined communication skills at 15 and 38 months, also reported associations in both positive and inverse directions, without a clear explanation. Overall, there is no clear evidence of an association between PFNA exposure and cognition. Sensitivity is a concern for many of the available studies, so null results are difficult to interpret, but the inconsistency across studies that do report an association reduces confidence.

Table 3-35. Summary of results of *medium* and *high* confidence epidemiology studies of PFNA exposure and cognitive effects

Study name, country, reference(s)	Measured Endpoint	Exposure measurement timing	Estimate type (adverse direction[a])	N	Group or unit change	Exposure median (IQR) or range (quartiles) in ng/L	Effect estimate	CI LCL	CI UCL
Danish National Birth Cohort, Denmark Liew et al. (2018b)	FSIQ at 5 yr with WPPSI-R	Maternal (mean 8.7-wk gestation)	Mean Difference vs. Q1 (↓)	Boys (n = 831)	Q1	<LOQ–0.36	Ref		
					Q2	0.37–0.46	2.1	−1.9	6.2
					Q3	0.47–0.57	3.2	−0.7	7.2
					Q4	≥0.58	−0.5	−5.2	4.1
		Maternal (mean 8.7-wk gestation)	Mean Difference vs. Q1 (↓)	Girls (n = 761)	Q1	<LOQ–0.36	Ref		
					Q2	0.37–0.46	1.0	−2.8	4.8
					Q3	0.47–0.57	2.8	−0.5	6.2
					Q4	≥0.58	0.7	−2.8	4.3
Health Outcomes and Measures of the Home Environment (HOME), U.S. Vuong et al. (2016) Vuong et al. (2019) Vuong et al. (2018b) Vuong et al. (2020)	FSIQ at 8 yr with WISC-IV	3 yr	Regression Coefficient (↓)	221	Ln-unit increase in exposure	NR	**3.0***	**0.5**	**5.6**
		Maternal (16 ± 3-wk gestation)	Regression Coefficient (↓)	221	Ln-unit increase in exposure	GM 10.9	3.5	−0.4	7.4
	Global executive function score at 8 yr with BRIEF	Maternal (16 ± 3-wk gestation)	Mean Difference (↑)	219	ln-unit increase in exposure	0.9 (0.7–1.1)	2.01	−0.89	4.92
		3 yr	OR (↑)	208	ln-unit increase in exposure	NR	1.43	0.87	2.33
		8 yr	OR (↑)			NR	**3.07***	**1.60**	**5.90**

Study name, country, reference(s)	Measured Endpoint	Exposure measurement timing	Estimate type (adverse direction[a])	N	Group or unit change	Exposure median (IQR) or range (quartiles) in ng/L	Effect estimate	CI LCL	CI UCL
	Reading composite scores at 8 yr	Maternal	Regression Coefficient (↓)	161	Log10-unit increase in exposure	GM 1.0	**16.7***	**4.8**	**28.6**
Project Viva, U.S. Harris et al. (2018) Harris et al. (2021)	Peabody Picture Vocabulary Test early childhood	Maternal (5- to 21-wk gestation)	Mean Difference vs. Q1 (↓)	948	Q1	<0.1–0.4	Ref		
					Q2	0.5–0.6	1.0	−1.4	3.3
					Q3	0.7–0.9	0.2	−2.2	2.6
					Q4	1.0–6.0	−0.4	−3.0	2.3
	Verbal IQ mid-childhood with KBIT	Maternal (5- to 21-wk gestation)	Mean Difference vs. Q1 (↓)	851	Q1	<0.1–0.4	Ref		
					Q2	0.5–0.6	0.0	−2.5	2.4
					Q3	0.7–0.9	−0.05	−3.0	2.0
					Q4	1.0–6.0	2.0	−0.9	4.8
		Mid-childhood (6–10 yr)	Mean Difference vs. Q1 (↓)	631	Q1	<0.1–1.0	Ref		
					Q2	1.1–1.5	−0.9	−3.6	1.9
					Q3	1.6–2.3	0.9	−2.1	3.9
					Q4	2.4–25.7	−2.2	−5.1	0.8
	Non-Verbal IQ mid-childhood with KBIT	Maternal (5- to 21-wk gestation)	Mean Difference vs. Q1 (↓)	862	Q1	<0.1–0.4	Ref		
					Q2	0.5–0.6	0.0	−2.5	2.4
					Q3	0.7–0.9	−0.05	−3.0	2.0
					Q4	1.0–6.0	2.0	−0.9	4.8
		Mid-childhood (6–10 yr)		640	Q1	<0.1–1.1	Ref		
					Q2	<0.1–1.0	−2.1	−5.6	1.3

Study name, country, reference(s)	Measured Endpoint	Exposure measurement timing	Estimate type (adverse direction[a])	N	Group or unit change	Exposure median (IQR) or range (quartiles) in ng/L	Effect estimate	CI LCL	CI UCL
			Mean Difference vs. Q1 (↓)		Q3	1.1–1.5	−0.9	−4.6	2.8
					Q4	1.6–2.3	−0.2	−3.9	3.5
	Global executive function score at 6–10 yr (BRIEF)	Maternal (5- to 21-wk gestation)	Mean Difference vs. Q1 (↑)	921	Q1	0.6 (0.5–0.9)	Ref		
					Q2		−0.4	−2.1	1.3
					Q3		−0.3	c.0	1.4
					Q4		−0.6	−2.4	1.3
Taiwan maternal and infant cohort study, Taiwan Wang et al. (2015b)	FSIQ at 5 yr with WPPSI-R	Maternal (3rd trimester)	Regression Coefficient (↓)	120	Doubling of exposure	1.6 (0.8–2.4)	−0.2	−2.1	1.7
	VIQ at 5 yr with WPPSI-R						0.7	−1.3	2.7
	PIQ at 5 yr with WPPSI-R						−1.4	−3.4	0.6
	FSIQ at 8 yr with WISC-III	Maternal (3rd trimester)	Regression Coefficient (↓)	120	Doubling of exposure	1.6 (0.8–2.4)	−1.5	−3.4	0.4
	VIQ at 8 yr with WISC-III						**−2.1***	**−3.9**	**−0.2**
	PIQ at 8 yr with WISC-III						−1.5	−3.5	0.4
WTC cohort, U.S. Spratlen et al. (2020a)	MDI at 1 yr with Bayley	Cord blood/ maternal 1 d post-delivery	Regression Coefficient (↓)	302	Log-unit increase	GM 0.4 (range <LOQ–10)	−0.14	−2.30	2.02
				Girls 150			−0.38	−2.91	2.15
				Boys 152			0.23	−2.43	2.89
	MDI at 3 yr with Bayley			302			2.05	−0.80	4.89
				Girls 150			2.38	−0.87	5.63

Study name, country, reference(s)	Measured Endpoint	Exposure measurement timing	Estimate type (adverse direction[a])	N	Group or unit change	Exposure median (IQR) or range (quartiles) in ng/L	Effect estimate	CI LCL	CI UCL
				Boys 152			1.51	−2.96	5.98
	FSIQ at 4 yr with WPPSI			302			−0.23	−3.37	2.91
				Girls 150			0.1	−3.62	3.82
				Boys 152			−0.76	−5.14	3.62
	FSIQ at 6 yr with WPPSI			302			1.55	−2.12	5.22
				Girls 150			1.98	−2.21	6.17
				Boys 152			0.85	−3.56	5.26
Norwegian Mother, Father and Child cohort, Norway Skogheim et al. (2020)	Verbal working memory at 42 mo with CDI	Maternal (17-wk gestation)	Regression coefficient (↓)	768	Q2	0.4 (0.3–0.5)	0.15	−0.08	0.37
					Q3		0.20	−0.03	0.43
					Q4		−0.01	−0.24	0.21
					Q5		0.34	0.10	0.57
	Non-verbal working memory at 42 mo with CDI			934	Q2		−0.06	−0.26	0.15
					Q3		−0.23	−0.44	−0.02
					Q4		−0.12	−0.33	0.09
					Q5		−0.15	−0.37	0.06
Shanghai-Minhang cohort, China Niu et al. (2019)	Communication at 4 yr with ASQ–3	Maternal (12- to 16-wk gestation)	RR for problems (↑)	533	Ln-unit increase in exposure	1.8 (1.3–2.5)	0.85	0.61	1.17
				Girls 236			0.63	0.35	1.12
				Boys 297			1.04	0.70	1.54
				533			0.92	0.60	1.39
				Girls 236			0.93	0.45	1.91

Study name, country, reference(s)	Measured Endpoint	Exposure measurement timing	Estimate type (adverse direction[a])	N	Group or unit change	Exposure median (IQR) or range (quartiles) in ng/L	Effect estimate	CI LCL	CI UCL
	Problem solving at 4 yr with ASQ–3			Boys 297			0.95	0.57	1.57
Laizhou Wan Birth Cohort, China Yao et al. (2022)	Adaptive Development Quotient at 1 yr	Cord serum	Regression coefficient (↓)	274	Log10-unit increase in exposure	0.4	−0.54	−3.90	2.81
				Girls 135			0.66	−4.92	6.25
				Boys 139			−1.88	−6.50	2.73
	Language Development Quotient at 1 yr			274			0.66	−2.63	3.95
				Girls 135			1.38	−3.92	6.68
				Boys 139			0.57	−4.08	5.23
Early Markers for Autism (EMA), U.S. Lyall et al. (2018)	Intellectual disability	Maternal (mid-pregnancy)	OR (↑)	189	Ln-transformed exposure	GM 0.6	**0.62***	**0.42**	**0.92**
			OR vs. Q1 (↑)	83	Q1	<0.4 ng/mL	1.0		
				60	Q2	0.4–<0.6 ng/mL	0.92	0.58	1.46
				22	Q3	0.6–<0.8 ng/mL	0.57	0.30	1.06
				24	Q4	≥0.8 ng/mL	0.68	0.35	1.31

FSIQ = full-scale intelligence quotient; OR = odds ratio; RR = risk ratio; VIQ = verbal IQ; PIQ = performance IQ; WPPS-R = Wechsler Primary and Preschool Scales of Intelligence-Revised; WISC = Wechsler Intelligence Scale for Children; BRIEF = Behavior Rating Inventory of Executive Function; KBIT = Kaufman Brief Intelligence Test; ASQ = Ages and Stages Questionnaire; CDI = Child Development Inventory; GM = geometric mean.

*$p < 0.05$.

[a]The arrows indicate the direction the effect estimate will be if there is an association between PFNA and reduced cognitive performance. For some tests, a higher score means better performance, while for other tests, a higher score means more problems.

ADHD and related behavior

Fourteen studies reported on endpoints related to ADHD, including eight *medium* confidence and six *low* confidence studies. The results for *medium* confidence studies of behavior as well as *low* confidence studies of ADHD diagnosis (because of the small number of studies available) are presented in Tables 3-36 and 3-37. Looking at studies of ADHD diagnosis, two studies examined associations with doctor diagnosis with or without medication use for ADHD as reported by participants. Stein and Savitz (2011) reported higher odds of ADHD diagnosis plus medication, with an exposure-response gradient observed across quartiles, but no association was observed with diagnosis alone. Hoffman et al. (2010) reported higher odds of both diagnosis and diagnosis plus medication. ADHD diagnosis plus medication is considered a more specific outcome than diagnosis alone and may focus on the more severe forms of ADHD, which may explain the inconsistency in results in Stein and Savitz (2011), but the estimates in both studies are imprecise. Another *low* confidence study of ADHD diagnosis Ode et al. (2014) reported slightly higher odds (OR 1.1, 95% CI: 0.8, 1.7), also imprecise. The two *medium* confidence studies, Liew et al. (2014) and Skogheim et al. (2021), examined ADHD cases identified from registries. In the case of Liew et al. (2014), the registry was limited to hospital and psychiatric admissions, which likely represent severe cases. These two studies did not observe a positive association with ADHD diagnosis. Given the difference in outcome measures (registry vs. direct information on diagnosis), it is difficult to determine whether the inconsistency between *medium* and *low* confidence studies is due to timing of exposure measurement or outcome ascertainment, but the lack of association in *medium* confidence studies decreases certainty in the association. These studies adjusted for sex but did not examine associations by sex.

The remaining studies focused on behaviors potentially consistent with ADHD. Externalizing problems (consisting of hyperactivity and conduct subscales on the Strengths and Difficulties Questionnaire [SDQ]) were examined in five studies (four *medium* confidence). The SDQ is a validated instrument, but its sensitivity for ADHD has been inconsistent in different populations (Hall et al., 2019; Pritchard, 2012; Ullebo et al., 2011). Three of the *medium* confidence studies reported positive associations (i.e., more behavior problems with higher exposure (Luo et al., 2020; Høyer et al., 2017; Oulhote et al., 2016)), including statistically significant associations between exposure at 5 years and total SDQ score (Oulhote et al., 2016) and maternal exposure, externalizing problems at 7 years and maternal exposure (Luo et al., 2020), and SDQ hyperactivity and total scores (Høyer et al., 2017). No associations were observed with internalizing problems. The *low* confidence study that examined SDQ hyperactivity scores reported non-monotonic associations across quartiles, with null results in the fourth quartile (Lien et al., 2016). This *low* confidence study also administered two other questionnaires, the SNAP-IV tests and the Child Behavior Checklist and found a statistically significant inverse association with inattention and hyperactivity (Lien et al., 2016). A *medium* confidence study also using the Child Behavior Checklist found no association (Dalsager et al., 2021). Another *medium* confidence study used multiple tools and found a positive

association with hyperactivity with maternal but not childhood exposure (Vuong et al., 2021a) and no association with impulsivity and inattention (Vuong et al., 2018a). Skogheim et al. (2020) reported results only for a mixture including PFNA, PFDA, and PFUnDA (exposure levels were highest for PFNA) and reported no association with ADHD symptoms assessed using the Preschool Age Psychiatric Assessment. Finally, a *low* confidence study examined inter-response time (IRT) and found a statistically significant decrease in IRT, which indicates poor response inhibition as the test is designed to reward longer response times (Gump et al., 2011). A few studies examined the possibility of an interaction with sex. In Oulhote et al. (2016), there is an indication of stronger associations in girls in stratified analyses. However, Vuong et al. (2018a) and Høyer et al. (2017) did not report sex-specific differences with PFNA exposure. There is not adequate evidence to fully assess differences in the association with ADHD and potentially related behaviors by sex.

Taken together, there is some evidence of an association between PFNA exposure and ADHD symptoms, but there is considerable uncertainty. Several available studies of both ADHD diagnosis and behavior problems related to ADHD suggest an association with PFNA exposure in at least one analysis. However, results for ADHD diagnosis (and diagnosis plus medication), the most specific and reliable outcomes, were imprecise and associations were limited to cross-sectional studies. It is possible that exposure levels/contrast were not adequate to reach statistical significance, but pattern is not clear between exposure levels/contrast and detecting an association.

Table 3-36. Summary of results for epidemiology studies of PFNA exposure and attention-deficit/hyperactivity disorder (ADHD)

Study name, country, confidence, reference	Measured endpoint	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
C8 Health Project, USA, *low* Stein and Savitz (2011)	ADHD diagnosis	Cross-sectional	OR vs. Q1 (↑)	Children 5–18 yr (n = 10,546)	Q1	0.25–<1.2	1.0		
					Q2	1.2–<1.5	1.00	0.84	1.19
					Q3	1.5–<2.0	0.94	0.79	1.11
					Q4	2.0–24.1	0.99	0.84	1.18
	ADHD diagnosis+ medication	Cross-sectional	OR vs. Q1 (↑)	Children 5–18 yr (n = 10,546)	Q1	0.25–<1.2	1.0		
					Q2	1.2–<1.5	1.02	0.78	1.34
					Q3	1.5–<2.0	1.06	0.82	1.36
					Q4	2.0–24.1	1.16	0.90	1.49
NHANES (1999–2000, 2003–2004), USA, *low* Hoffman et al. (2010)	ADHD diagnosis	Cross-sectional	OR (↑)	571	1 unit increase in exposure	0.6 (0.5)	1.32	0.86	2.02
	ADHD diagnosis+ medication						1.57	0.67	3.64
Danish National Birth Cohort, Denmark, *medium* Liew et al. (2018b)	ADHD diagnosis	Maternal (1st trimester)	RR (↑)	770	Ln-unit increase	Controls 0.4 (0.4–0.6)	0.80	0.62	1.03
					Q1	<LOQ–0.35	1.0		
					Q2	0.36–0.43	1.08	0.90	1.30
					Q3	0.44–0.56	1.12	0.93	1.33
					Q4	>0.56	0.85	0.69	1.04
			OR (↑)	1801	Q1	0.3 (0.1– 0.4)	Ref		

Study name, country, confidence, reference	Measured endpoint	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 Child Cohort, Norway, *medium*	ADHD diagnosis (national registry)	Maternal (2nd trimester, 18-wk gestation)			Q2		0.93	0.71	1.23
					Q3		0.91	0.69	1.21
Skogheim et al. (2021)					Q4		0.82	0.62	1.11

OR = odds ratio; RR = risk ratio.
*$p < 0.05$.
[a]The arrows indicate the direction the effect estimate will be if there is an association between PFNA and reduced behavior. For all the tests included here, higher scores indicate more difficulties/behavior problems/ADHD diagnosis.

Table 3-37. Summary of results for medium confidence epidemiology studies of PFNA exposure and behavior

Study name, country, confidence, reference	Measured endpoint	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
Faroe Island cohort, Denmark, *medium*	Externalizing problems	5 yr	Mean Difference (↑)	508	Per doubling of exposure	1.0 (0.8–1.2)	0.5	0	1
Oulhote et al. (2016)		Maternal (32-wk gestation)		539		0.6 (0.5–0.8)	−0.27	−0.96	0.42
	Total SDQ score at 7 yr	5 yr	Mean Difference (↑)	508	Per doubling of exposure	1.0 (0.8–1.2)	**0.78***	**0.01**	**1.55**
		Maternal (32-wk gestation)		539		0.6 (0.5–0.8)	−0.11	−1.2	0.98
INUENDO (Biopersistent organochlorines in	SDQ hyperactivity score at 5–9 yr	Maternal (second	Regression Coefficient (↑)	1023	ln-unit increase in exposure	0.6 (10th–90th 0.4–1.4)	**0.25***	**0.00**	**0.50**

Study name, country, confidence, reference	Measured endpoint	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
diet and human fertility), Greenland, Ukraine, Poland, *medium* [Høyer et al. (2017)]		trimester median)			Low exposure	0.1–0.5	Ref		
					Medium exposure	0.5–0.8	0.27	−0.03	0.57
					High exposure	0.8–5.7	0.24	−0.08	0.55
	Total SDQ score at 5–9 yr	Maternal (second trimester median)	Regression Coefficient (↑)	1,023	ln-unit increase in exposure	0.6 (10th–90th 0.4–1.4)	**0.72***	**0.13**	**1.31**
					Low exposure	0.1–0.5	Ref		
					Medium exposure	0.5–0.8	0.37	−0.35	1.09
					High exposure	0.8–5.7	0.58	−0.17	1.32
Project Viva, U.S., *medium* [Harris et al. (2021)]	Externalizing problems at 6–10 yr (SDQ)	Maternal (5- to 21-wk gestation)	Mean Difference vs. Q1 (↑)	921	Q1	0.6 (0.5–0.9)	Ref		
					Q2		0.2	−0.4	0.8
					Q3		0.1	−0.5	0.7
					Q4		0.2	−0.5	0.8
	Internalizing problems at 6–10 yr (SDQ)				Q1		Ref		
					Q2		−0.1	−0.6	0.3
					Q3		−0.2	−0.7	0.3
					Q4		0.2	−0.3	0.7

Study name, country, confidence, reference	Measured endpoint	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
	Total SDQ score at 6–10 yr				Q1		Ref		
					Q2		0.1	−0.8	0.9
					Q3		−0.1	−1.0	0.8
					Q4		0.4	−0.6	1.3
Danish National Birth Cohort, Denmark, *medium* [Luo et al. (2020)]	Externalizing problems at 7 yr	Maternal (1st trimester)	OR (↑) (odds of elevated score)	2,421	Per doubling of exposure	0.4 (0.4–0.6)	**1.35***	**1.13**	**1.62**
	Internalizing problems at 7 yr						0.90	0.77	1.06
	Total SDQ score at 7 yr						1.03	0.88	1.21
Odense Child Cohort, Denmark, *medium* [Dalsager et al. (2021)]	Behavior problems (CBC) at 2–5 yr	Maternal (8- to 16-wk gestation)	Incidence rate ratio (↑)	1,138	Doubling of exposure	0.6 (95th percentile 1.2)	1.03	0.96	1.12
			OR (↑)				1.06	0.80	1.39
		18 mo	Incidence rate ratio (↑)	817			0.99	0.90	1.08
			Odds ratio (↑)				1.11	0.84	1.46
Health Outcomes and Measures of the Home Environment (HOME), *USA, medium* [Vuong et al. (2018a)] [Vuong et al. (2021a)]	Impulsivity – Comissions	3 yr	Regression Coefficient (↑)	204	ln-unit increase in exposure	1.2 (1.0–1.8)	−0.4	−2.6	1.7
		Maternal (16 ± 3 wk gestation)				0.9 (0.7–1.1)	−0.6	−3.1	1.8
	Inattention – Omissions	3 yr	Regression Coefficient (↑)		ln-unit increase in exposure	1.2 (1.0–1.8)	0.4	−3.6	4.5
		Maternal (16 ± 3 wk gestation)				0.9 (0.7–1.1)	−1.2	−6.2	3.8

Study name, country, confidence, reference	Measured endpoint	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
Vuong et al. (2021b)	Externalizing problems (BASC-2) at 5 and 8 yr	Maternal (16 ± 3 wk gestation)	OR (↑)	241	ln-unit increase in exposure	GM 0.9	2.1	0.7	5.7
	Hyperactivity (BASC-2)						**3.2***	**1.3**	**8.0**
	Attention (BASC-2)						1.4	0.6	3.1
	Internalizing problems (BASC-2)						1.4	0.5	3.7
	Externalizing problems (BASC-2)	3 yr	Regression Coefficient (↑)	208	Ln-unit increase in exposure	GM1.4	−0.7	−2.5	1.1
	Hyperactivity (BASC-2)						−0.8	−1.4	3.0
	Attention (BASC-2)						**−1.8***	**−3.4**	**−0.2**
	Conduct problems (BASC-2)						−0.4	−2.2	1.4

SDQ = Strengths and Difficulties Questionnaire; OR = odds ratio.

*$p < 0.05$.

Externalizing problems calculated from conduct and hyperactivity subscales; internalizing problems calculated from emotional and peer subscales.

[a]The arrows indicate the direction the effect estimate will be if there is an association between PFNA and reduced behavior. For all the tests included here, higher scores indicate more difficulties/behavior problems/ADHD diagnosis.

Social behavior and autism spectrum disorder

Nine studies examined social behaviors or autism spectrum disorder. Of the five studies of autism spectrum disorder diagnosis, only one reported higher odds of diagnosis with higher exposure (Oh et al., 2021), while the other four studies reported inverse but not statistically significant associations (Skogheim et al., 2021; Shin et al., 2020; Lyall et al., 2018; Liew et al., 2015). Four studies examined screening questionnaires for social behavior. Niu et al. (2019) examined the Ages and Stages Questionnaire at 4 years of age and reported a statistically significant elevated risk ratio (RR) for personal social skills problems with higher exposure (RR 1.92, 95% CI: 1.21, 3.05 per ln-unit increase in exposure). Braun et al. (2014) used the Social Responsiveness Scale and reported a nonsignificant positive association (more problem behaviors) with maternal exposure (β: 1.4, 95% CI: −0.6, 3.4), while Oulhote et al. (2016) calculated an autism screening score using the peer problems and prosocial behavior subscales on the SDQ and also reported a nonsignificant positive association (mean difference: 0.27, 95% CI: −0.09, 0.63) with childhood (5-year) exposure (no association with maternal exposure). Yao et al. (2022) used the Gesell Development Schedules (GDS) and found no association with SDQ. Overall, there is some largely imprecise evidence of an association between PFNA exposure and social behaviors, but not with autism diagnosis.

Other outcomes

Four *medium* confidence studies reported on motor effects (Yao et al., 2022; Spratlen et al., 2020a; Niu et al., 2019; Harris et al., 2018). There was a decrease in the visual-motor score from the Wide Range Assessment of Visual Motor Abilities (WRAVMA) test in mid-childhood with maternal exposure, but this was non-monotonic and not statistically significant (mean difference (95% CI) vs. Q1: Q2: −0.9 (−4.6, 2.8); Q3: 1.0 (−2.9, 5.0), Q4: −3.0 (−6.9, 0.9). No association was observed between the WRAVMA total score in early childhood and maternal exposure. In Spratlen et al. (2020a), positive associations (better motor function on Motor Development Index on Bayley Scales of Infant Development) were observed with PFNA exposure at 2 and 3 years, but no association was observed at 1 year. No association with gross or fine motor skills was observed in Niu et al. (2019) using the Ages and Stages Questionnaire or with gross motor DQ using the GDS in Yao et al. (2022). There were some sex-specific differences, with girls having greater gross motor skill problems but fewer fine motor skills problems compared with boys, but the results were imprecise. Given the lack of consistency across studies, there is no clear evidence of an association with motor effects.

One *medium* confidence study examined associations with cerebral palsy (Liew et al., 2014). There was a positive association ($p > 0.05$) with congenital cerebral palsy in boys (RR 1.2, 95% CI: 0.6, 2.5, exposure-response gradient across quartiles). An inverse association ($p > 0.05$) was observed in girls (RR 0.6, 95% CI: 0.3, 1.2). Given the lack of additional studies, inconsistency across sexes, and imprecision in the estimates, there is no clear evidence of an association between PFNA exposure and cerebral palsy.

Lastly, one study examined MRI data to assess functional development in the teenage brain based on activity in specific areas of the brain during a resting state (Weng et al., 2020). This study was considered *low* confidence due to lack of information on participant selection and analysis. They found that maternal PFNA exposure was inversely associated with activity in the left putamen, right putamen, and left caudate nucleus.

Animal and Mechanistic Studies

While the MOA for developmental effects, including neurodevelopmental effects are unknown, screening assays in embryonic zebrafish and *Xenopus*, are relevant to the mechanistic discussion of neurological effects (see Section 3.2.2. "Mechanistic and Supplemental Information"). While the majority of the embryotoxicity assays did not target specific neurological endpoints, they show elevated mortality, delayed hatching, and malformations that involve putative effects on neurological signaling. In one of the studies in embryonic zebrafish, PFNA disrupted synaptogenesis by decreasing acetylcholine, dopaminergic, noradrenaline, 5-hydroxytryptamine, glutamate, gamma-aminobutyric acid neurotransmitters, and facilitating synaptic dysfunction through gene downregulation (Liu et al., 2023). Two ToxCast in vitro screening assays also show some activity on neurological targets (see Appendix C.2.5. for summary information on these screening results and a link to null ToxCast assays).

Evidence Integration

The available human epidemiological studies provide *slight* evidence of developmental neurotoxicity, with considerable uncertainty. The strongest signal for a potential neurodevelopmental effect is some evidence of an association between PFNA exposure and diagnosis of ADHD and potentially related behaviors. Several *medium* confidence studies reported higher ADHD diagnosis or ADHD-related behaviors with higher PFNA exposure. However, there is inconsistency across studies of ADHD diagnosis, the most specific outcome, with associations observed only in cross-sectional studies. Associations with other neurodevelopmental outcomes were inconsistent across studies (cognition, social behavior) or imprecise, inconsistent across sexes, and only observed in a single study (cerebral palsy) and thus the evidence is uncertain. Studies assessing neurological function in adults are a data gap.

No animal toxicological studies or mechanistic studies in mammalian models are available, making the animal evidence base *indeterminate* for developmental neurotoxicity. A handful of non-mammalian mechanistic studies indicated developmental and behavioral effects in embryonic zebrafish (see Section 3.2.2, "Mechanistic and Supplemental Information"). However, the MOA for PFNA-induced effects on development, including on nervous system development, is currently unknown, and these studies were not interpreted to inform the biological plausibility of the findings related to developmental neurotoxicity.

Overall, as summarized in Table 3-38, the currently available ***evidence suggests*** but is not sufficient to infer, that PFNA may cause developmental neurotoxicity in humans given sufficient

exposure conditions.[19] This conclusion is based on studies of humans exposed at PFNA median blood levels of 0.6–1.2 ng/mL. Neurotoxicological studies in adults and in experimental animals at any lifestage are a data gap.

Table 3-38. Evidence profile table for developmental neurotoxicity[a]

Summary of human evidence					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.8. Nervous System Effects, Human Studies)					⊕⊙⊙ ***Evidence suggests but is not sufficient to infer***
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
Neurodevelopment Eighteen *medium* confidence studies Seven *low* confidence studies	• Some evidence of a positive association between PFNA exposure and ADHD diagnosis (2/4 studies) and potentially related behaviors (4/7 studies) • Evidence of association with other outcomes was inconsistent and imprecise	• *Medium* confidence studies observing effects	• Inconsistency for ADHD, diagnosis, cognition (across studies) and cerebral palsy (across sexes) • Imprecision	⊕⊙⊙ *Slight*	*Primary basis:* Human studies report generally consistent, but imprecise, associations between PFNA exposure and ADHD and potentially related behaviors, with maternal or childhood PFNA median blood levels of 0.6–1.2 ng/mL *Human relevance:* N/A *Cross-stream coherence:* N/A; animal evidence *Indeterminate* (no data) *Susceptible populations and lifestages:* Infants and children appear to be susceptible although the specific developmental window of susceptibility is unknown

[a]No animal or mechanistic evidence.

3.2.9. Cardiometabolic Effects

This section describes and integrates the evidence informative for the assessment of cardiometabolic effects following exposure to PFNA. Cardiometabolic risk refers to the likelihood of developing diabetes, heart disease, or stroke. Contributors to this risk include a combination of

[19]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).

metabolic dysfunctions mainly characterized by insulin resistance, dyslipidemia, hypertension, adiposity, and changes in heart weight and histopathology. There is some overlap in this section with the evidence synthesis and integration summary of liver effects as some PFNA studies examined hepatic concentrations of triglycerides and cholesterol (see Section 3.2.3, "Mechanistic and Supplemental Information").

Human Studies

Serum lipids

High cholesterol, specifically low-density lipoprotein (LDL) cholesterol, is one of the major controllable risk factors for cardiovascular disease including coronary heart disease, myocardial infarction (heart attack), and stroke. Cholesterol levels are typically measured in the blood (most often in serum). Forty-one studies (49 publications) report on the association between PFNA exposure and blood lipids (i.e., cholesterol, LDL cholesterol, and triglycerides).

Multiple outcome-specific considerations for study evaluation were influential to the ratings. First, for outcome ascertainment, collection of blood during a fasting state is preferred for all blood lipid measures but lack of fasting was considered deficient only for triglycerides and LDL cholesterol (which is typically calculated using triglycerides) (NIH, 2020; Nigam, 2011). This downgrading is because triglyceride levels remain elevated for several hours after a meal (Nigam, 2011), which is likely to result in substantial outcome misclassification if there is not standardization across study participants. Self-reported high cholesterol was also considered deficient because of the high likelihood of misclassifying cases as controls (Natarajan et al., 2002). It was also important for studies 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 available studies analyzed PFNA in serum or plasma and serum lipids using standard, appropriate methods. As described in Section 3.2.7, reverse causation was considered but is unlikely to significantly bias the results because PFAS, including PFNA, do not preferentially bind to serum lipids, so concurrent measurement of exposure and outcome was considered adequate for this outcome.

A summary of the study evaluations is presented in Figure 3-92, and additional details can be obtained from HAWC. Five studies were excluded from further analysis because of *critical deficiencies* in confounding—lack of consideration of potential confounding (Zhang et al., 2019; Seo et al., 2018; Yang et al., 2018; Tao et al., 2008) and lack of adjustment for significant co-exposures from the World Trade Center disaster (Tao et al., 2008) or participant selection (Sinisalu et al., 2021). Twenty-three studies were classified as *medium* confidence (Cakmak et al., 2022; Dunder et al., 2022; Zeng et al., 2022; Averina et al., 2021; Blomberg et al., 2021; Canova et al., 2021; Gardener et al., 2021; Li et al., 2021; Papadopoulou et al., 2021; Tian et al., 2021; Jensen et al., 2020a; Liu et al., 2020a; Spratlen et al., 2020b; Yang et al., 2020b; Dong et al., 2019; Lin et al., 2019; Jain and

Ducatman, 2018; Kang et al., 2018; Mora et al., 2018; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017; Zeng et al., 2015; Starling et al., 2014b), although 10 of these were *low* confidence for triglycerides and LDL cholesterol, for the reasons described above (Zeng et al., 2022; Blomberg et al., 2021; Canova et al., 2021; Papadopoulou et al., 2021; Jensen et al., 2020a; Yang et al., 2020b; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017; Zeng et al., 2015; Starling et al., 2014b). Thirteen studies were classified as *low* confidence for all lipid endpoints (Batzella et al., 2022; Varshavsky et al., 2021; Zare Jeddi et al., 2021; Khalil et al., 2020; Lin et al., 2020c; Chen et al., 2019; Graber et al., 2019; Khalil et al., 2018; Koshy et al., 2017; Christensen et al., 2016; Fu et al., 2014; Lin et al., 2013a; Mundt et al., 2007).

Figure 3-92. Study evaluation results for epidemiology studies of PFNA and blood lipids.[a] See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500131/.

[a]Multiple publications of single study: Dong et al. (2019) includes Nelson et al. (2010), Jain (2014, 2013), He et al. (2018), Christensen et al. (2019), and Jain and Ducatman (2019c). Dong et al. (2019) was considered the primary study because of more thorough consideration of outcome-specific issues (e.g., cholesterol lowering medications), and it included the full year range reported in other publications. Liu et al. (2020a) includes Liu et al. (2018) and has a more thorough analysis of lipid effects.

The results for the association between PFNA exposure and blood lipids among the *medium* confidence studies are presented in Table 3-39. Six *medium* confidence studies examined associations in adults, 4 examined associations in pregnant women, 2 examined cord blood, and 12 examined associations in adolescents and children.

In general population studies of adults, the five *medium* confidence studies examining total cholesterol all reported positive associations between PFNA exposure and total cholesterol (Cakmak et al., 2022; Dunder et al., 2022; Liu et al., 2020a; Dong et al., 2019; Lin et al., 2019), with the former three being statistically significant. In Lin et al. (2019), there was an exposure-response gradient across quartiles (not statistically significant) and an association was also observed with high cholesterol levels in both cross-sectional and prospective analyses. In Dong et al. (2019), the positive association was observed in each NHANES cycle that was analyzed (2003–2004, 2005–2006, 2007–2008, 2011–2012, 2013–2014), but not 2009–2010. Results in Dong et al. (2019) and Lin et al. (2019) were similarly positive for LDL (both studies) and triglycerides (only examined in Lin et al. (2019)). However, in Liu et al. (2020a) and Cakmak et al. (2022), the association with triglycerides was in the opposite direction (although not statistically significant).

Of eight *low* confidence studies of general population adults, four studies, including one limited to young adults and adolescents (Chen et al., 2019; Koshy et al., 2017; Fu et al., 2014; Lin et al., 2013a) reported positive associations with serum lipids, but the effect estimates in most of these studies were not large and were imprecise. Only (Koshy et al., 2017) reported a statistically significant association (total cholesterol β: 0.05 95% CI: 0.01, 0.09) per unit change in PFNA; no association was observed with triglycerides. Three *low* confidence studies of adult anglers (Christensen et al., 2016), general population adults with high exposure because of contamination (Zare Jeddi et al., 2021), and older adults (Lin et al., 2020c), reported no association with serum lipids. Three *low* confidence studies examined occupational exposure in workers, including two studies in factory workers (Batzella et al., 2022; Mundt et al., 2007) and one study in firefighters (Khalil et al., 2020). These studies each had concerns related to selection bias and confounding but provide insight into different exposure scenarios than general population studies. Batzella et al. (2022) reported a strong positive association between total cholesterol and PFNA exposure (β 10.21, 95% CI 2.65, 17.78 per IQR increase in PFNA), while Mundt et al. (2007) observed positive associations in cross-sectional, but not longitudinal analyses, and in men but not women. Khalil et al. (2020) reported no association.

In adolescents and children, 7 of 11 *medium* confidence studies reported higher total cholesterol with higher PFNA exposure in at least some analyses (Zeng et al., 2022; Averina et al., 2021; Blomberg et al., 2021; Canova et al., 2021; Jain and Ducatman, 2018; Mora et al., 2018; Zeng et al., 2015), although the results in Jain and Ducatman (2018) and Mora et al. (2018) were not statistically significant, and in some studies, the results varied based on timing of exposure measurement (Blomberg et al., 2021; Mora et al., 2018) and/or sex (Mora et al., 2018). No associations were observed in Jensen et al. (2020a), Manzano-Salgado et al. (2017b), Li et al.

(2021), or Kang et al. (2018). There is some indication that the association may be stronger in adolescents than in small children, as the null results tended to include younger participants (Jensen et al., 2020a; Kang et al., 2018; Manzano-Salgado et al., 2017b), but the evidence currently available is not adequate to establish this difference. Similarly, associations appear stronger in analyses of childhood exposure than in prenatal measurements. With the available evidence, it is unclear whether this observation represents etiologically relevant differences or greater potential for reverse causation in the analyses of childhood exposure. Exposure levels and contrast did not differ notably across studies, so there is no clear trend in associations by these factors. Sex-stratified results were available in two studies; a positive association was observed in boys in Mora et al. (2018), whereas an inverse association was observed in girls. In Manzano-Salgado et al. (2017b), the results for both sexes were essentially null. In *low* confidence studies, positive associations were observed in adolescents (Koshy et al., 2017) and children (Khalil et al., 2018), with the increase being statistically significant in Koshy et al. (2017), but both studies had serious limitations. Results for LDL cholesterol and triglycerides were less consistent.

In the four *medium* confidence studies of pregnant women, one study (Gardener et al., 2021) reported a statistically significant positive association with total cholesterol and PFNA exposure. Matilla-Santander et al. (2017) also reported a positive, although not statistically significant, association. Both studies reported monotonic exposure-response gradients across quartiles. The other two *medium* confidence studies did not provide clear evidence. Yang et al. (2020b) reported a positive but small association, whereas Starling et al. (2014b) reported a positive association in quartile 4 versus quartile 1 but inverse associations in quartiles 2 and 3 (none statistically significant). The *low* confidence study in pregnant women (Varshavsky et al., 2021) reported an inverse association with "serum lipids" that can be inferred as composed of cholesterol and triglycerides.

Two *medium* confidence studies examined serum lipids in cord blood. Altered cord blood lipid profiles have been associated with higher risk of cardiovascular disease among the offspring later in life (Spratlen et al., 2020b; Tank and Jain, 2016) reported no association with total cholesterol and a nonsignificant inverse association with triglycerides. Tian et al. (2021) reported inverse associations with total cholesterol ($p < 0.05$), LDL cholesterol, and triglycerides ($p > 0.05$).

Overall, the available studies provide some evidence that higher PFNA exposure may be associated with higher total cholesterol levels. The *medium* confidence studies in adults, support this association, although the *low* confidence studies are less consistent. Among *medium* confidence studies, there was consistency across studies in general population adults with statistically significant changes in three studies (Dunder et al., 2022; Dong et al., 2019; Lin et al., 2019) and exposure-response gradients across quartiles in one of these studies (Lin et al., 2019) and in a study of pregnant women (Gardener et al., 2021), which provides evidence of an association with this outcome. Two of these studies were in overweight and/or pre-diabetic adults, and thus the observed associations of those studies are not necessarily relevant to all adults, but the consistency

in Dong et al. (2019) indicates that the concern extends beyond those subpopulations. In pregnant women, two of four studies reported positive associations, although only one was statistically significant. Studies in adolescents also indicate a positive association with total cholesterol, however results among infants, and children are inconsistent, and interpretation of these differing results is difficult because of heterogeneity in study designs.

Table 3-39. Associations between PFNA and blood lipids in *medium* confidence epidemiology studies

Reference confidence	Population	Median exposure (IQR) or as specified (µg/mL)	Effect estimate	Total cholesterol	LDL	Triglycerides
General population, adults						
Lin et al. (2019)	Participants from randomized trial of interventions for diabetes prevention, U.S.; 888 overweight and pre-diabetic adults in the placebo and lifestyle intervention arms	0.6 (0.4–0.8)	Mean diff (95% CI) for twofold increase	**2.86 (0.70, 5.01)***	**2.09 (0.13, 4.05)***	3.39 (−2.45, 9.24)
			quartiles vs. Q1	Q2: 4.45 (−2.17, 11.08) Q3: 5.77 (−0.94, 12.50) Q4: 5.84 (−1.35, 13.04)	Q2: 3.81 (−2.21, 9.84) Q3: 5.35 (−0.76, 11.47) Q4: 4.26 (−2.28, 10.81)	Q2: −5.71 (−23.71, 12.28) Q3: 3.05 (−15.20, 21.31) Q4: 5.86 (−13.67, 25.40)
			Cross-sectional OR (95% CI) for high levels	1.11 (0.96, 1.29)	NR	1.09 (0.94, 1.26)
			Prospective HR (95%) for high levels	**1.09 (1.00, 1.19)***	NR	**1.17 (1.02, 1.33)***
Cakmak et al. (2022)	Canadian Health Measures Survey (2007–2017) cross-sectional, Canada; 6,045 adults	0.6 (GM)	% change for increase equivalent to GM	1.9 (−0.3, 4.1)	**7.6 (3.2, 12.2)***	−3.3 (−9.3, 3.1)
Dunder et al. (2022)	Cohort study (2001–2004), Sweden; 864 older adults (70 yr at baseline)	0.7 (0.5–1.0)	β (95% CI) for ln-unit increase (for lipids over 10 years)	**0.15 (0.07, 0.23)***	0.05 (−0.02, 0.12)	**0.08 (0.04, 0.11)***

Reference confidence	Population	Median exposure (IQR) or as specified (µg/mL)	Effect estimate	Total cholesterol	LDL	Triglycerides
Dong et al. (2019)	NHANES cross-sectional (2003–2014 pooled), U.S.; 8,950 adults	1.0 (SD 1.5)	β (95% CI) for 1 unit increase	**3.96 (0.05, 7.86)***	**5.12 (0.14, 10.11)***	NR
Liu et al. (2020a)	Cross-sectional analysis from randomized clinical trial of weight loss; 326 overweight adults	1.5 (1.0–2.3)	Means ± SE for tertiles	T1: 185.6±7.7 T2: 186.1±7.8 T3: 190.8±7.5 p-trend: 0.4	NR	T1: 132.9±11.1 T2: 128.2±11.2 T3: 123.6±10.8 *p*-trend: 0.4
Pregnant women						
Starling et al. (2014b)	Cross-sectional analysis (2003–2004), Norway; 891 women	0.4 (0.3–0.5)	β (95% CI) for ln-unit increase	0.01 (−5.98,6.00)	−2.15 (−7.31, 3.02)	−0.02 (−0.07, 0.03)
			quartiles vs. Q1	Q2: −5.28 (−12.75, 2.19) Q3: −3.84 (−11.55, 3.86) Q4: 2.22 (−6.47, 10.90)	Q2: −5.04 (−11.78, 1.70) Q3: −3.82 (−10.71, 3.07) Q4: −0.81 (-8.30, 6.69)	Q2: −0.03 (−0.10, 0.04) Q3: −0.02 (−0.09, 0.05) Q4: −0.02 (−0.09, 0.06)
Matilla-Santander et al. (2017)	Cross-sectional analysis (2003–2008), Spain; 1,240 women	0.7 (0.5–0.9)	% change for log-unit increase (95% CI)	0.46 (−0.76,1.70)	NR	−4.75 (-8.16, −0.61)
			quartiles vs. Q1	Q2: 0.90 (−1.25,3.19) Q3: 1.11 (−1.13,3.47) Q4: 1.81 (−0.57,4.24)	NR	Q2: −5.82 (−12.2, 1.01) Q3: **−7.69 (−13.9, −1.00)*** Q4: −5.82 (−13.9, 2.02)
Gardener et al. (2021)	Cross-sectional analysis (2009), U.S., 433 women	0.7 (0.5–1.1)		Monotonic increase (quantitative	NR	Monotonic increase (quantitative estimates not provided)

Reference confidence	Population	Median exposure (IQR) or as specified (µg/mL)	Effect estimate	Total cholesterol	LDL	Triglycerides
				estimates not provided)		
Yang et al. (2020b)	Cohort (2013–2014), China, 436 women	1.3 (0.9–2.0)	β (95% CI) for ln-unit increase	0.09 (−0.07, 0.25)	−0.001 (−0.11, 0.11)	0.13 (−0.06, 0.32)
Cord blood						
Spratlen et al. (2020b)	Cohort (2001–2001), U.S. (WTC), 222 infants	0.4 (0.3–0.7)	Mean ratios for quartiles vs. Q1	Q2: 0.99 (0.91, 1.08) Q3: 1.00 (0.91, 1.09) Q4: 1.00 (0.90, 1.11)	NR	Q2: 0.96 (0.81, 1.14) Q3: 0.91 (0.77, 1.09) Q4: 0.87 (0.70, 1.07)
Tian et al. (2021)	Cohort (2012), China, 306 infants	prenatal 1.8 (1.4, 2.5)	β (95% CI) for ln-unit increase	**−0.15 (−0.25, −0.05)***	−0.12 (−0.25, 0.01)	−0.12 (−0.23, 0.00)
Adolescents and children						
Canova et al. (2021)	Cross-sectional study in highly contaminated area (2017–2019), Italy; 6,669 adolescents (14–19 yr) and 2,693 children (8–11 yr)	Adolescents 0.4 (0.4–0.6)	β (95% CI) for low and medium vs. BLOD exposure	L: 3.43 (1.91, 4.95)* M: 3.58 (1.83, 5.33)*	L: 2.35 (1.05, 3.65)* M: 3.04 (1.54, 4.54)*	L: 0.00 (−0.02, 0.03) M: −0.03 (−0.06, 0.00)
		children 0.4 (0.4–0.5)		L: 2.95 (0.39, 5.50)* M: 7.53 (3.81, 11.25)*	L: 1.58 (−0.71, 3.87) M: 6.06 (2.73, 9.39)*	L: −0.01 (−0.05, 0.03) M: −0.02 (−0.07, 0.04)
Papadopoulou et al. (2021)	Six birth cohorts, Europe, 1,301 children (6–11 yr)	prenatal 0.7 (0.4–1.1)	β (95% CI) for doubling exposure	NR	−0.06 (−0.16, 0.03)	0.00 (−0.10, 0.09)
		children 0.5 (0.3–0.7)		NR	0.03 (−0.05, 0.11)	0.04 (−0.04, 0.13)
Averina et al. (2021)	Cross-sectional (2010–2011), Norway, 980 adolescents (16 yr)	0.6 (0.4) in girls	β (95% CI) for log-unit increase	**0.15 (0.04, 0.26)***	**0.14 (0.05, 0.24)***	−0.01 (−0.08, 0.07)

Reference confidence	Population	Median exposure (IQR) or as specified (µg/mL)	Effect estimate	Total cholesterol	LDL	Triglycerides
Jensen et al. (2020a)	Cohort (2010–2012), Denmark; 613 children (3 and 18 mo)	prenatal 0.7 (5th–95th: 0.3–1.5)	β (95% CI) for 1 unit increase	3 mo: −0.15 (−0.44, 0.13) 18 mo: 0.37 (−0.05, 0.79)	3 mo: −0.06 (−0.35, 0.23) 18 mo: 0.24 (−0.21, 0.70)	3 mo: −0.10 (−0.39, 0.20) 18 mo: 0.18 (−0.26, 0.63)
Manzano-Salgado et al. (2017b)	Cohort (2003–2008), Spain; 627 children (4 yr)	prenatal 0.7 (0.5–0.9) (GM (IQR))	β (95% CI) for doubling exposure	0.07(−0.11,0.12) Boys: −0.05 (−0.22,0.12) Girls: 0.05 (−0.11,0.21)	0.07 (−0.10, 0.12) Boys: −0.01 (−0.17, 0.16) Girls: 0.02 (−0.13, 0.18)	0.03 (−0.07, 0.14) Boys: 0.02 (−0.12, 0.16) Girls: 0.05 (−0.11, 0.20)
Jain and Ducatman (2018)	Cross-sectional (NHANES 2013–2014), U.S.; 458 children (6–11 yr)	0.8	Means (95% CI)	Q1: 153 (148–158) Q2: 155 (150–160) Q3: 157 (152–163) Q4: 157 (151–164) *p*=0.3	NR	NR
Li et al. (2021)	Cohort (2003–2006), U.S.; 221 children (12 yr)	prenatal 0.9 (0.7–1.2)	β (95% CI) for IQR increase	NR	NR	0.0 (−0.2, 0.1)
		12 yr 0.3 (0.2–0.5)		NR	NR	0.1 (−0.1, 0.3)
Zeng et al. (2015)	Cross-sectional analysis (2009–2010), Taiwan; 225 adolescents (12–15 yr)	0.9 (range 0.3–2.5)	β (95% CI) for 1 unit increase	**12.92 (0.73, 25.10)***	**9.63 (0.20, 19.06)***	**23.01 (6.49, 39.52)***
Kang et al. (2018)	Cross-sectional analysis (2012–2014), Korea, 150 children (3–18 yr)	0.9 (0.7–1.3)	β (95% CI) for ln-unit increase	−1.62 (−10.22, 6.97)	2.30 (−6.56, 11.17)	0.07 (−0.09, 0.22)
Blomberg et al. (2021)	Birth cohort (2007–2009), Faroe	18 mo 1.0 (0.6–1.5)	β (95% CI) for doubling	−0.01 (−0.08, 0.07)	0.00 (−0.04, 0.05)	2.8 (−3.1, 9.1)

Reference confidence	Population	Median exposure (IQR) or as specified (µg/mL)	Effect estimate	Total cholesterol	LDL	Triglycerides
(additional results with different timing of exposure and outcome measurement are available in the publication)	Islands, 459 children (followed to 9 yr)		PFAS and lipids at birth			
			PFAS at birth and lipids at 18 mo	−0.02 (−0.17, 0.13)	−0.01 (−0.13, 0.11)	−2 (−9.5, 6.2)
			PFAS at 5 yr and lipids at 9 yr	0.11 (0.01, 0.21)*	0.08 (−0.0, 0.16)	0.49 (−6.1, 7.6)
			PFAS and lipids at 9 yr	0.16 (0.03, 0.29)*	0.12 (0.02, 0.22)*	−1.4 (−9, 6.9)
Zeng et al. (2022)	NHANES cross-sectional (2003–2004), U.S.; 491 boys	1.1 (GM)	β (95% CI) for ln-unit increase	4.82 (0.69, 8.94)*	3.15 (−2.20, 8.50)	5.15 (−3.92, 14.22)
Mora et al. (2018)	Cohort (1999–2002), U.S.; 682 children (7–8 yr)	prenatal 0.6 (0.5–0.9)	β (95% CI) for IQR increase	0.2 (−2.4,2.8) Boys: 2.0 (−2.3,6.2) Girls: −1.4 (−4.8,1.9)	0.5 (−1.8,2.8) Boys: 2.3 (−1.3,6.0) Girls: −1.2 (−4.0,1.7)	−2.5 (−5.8,0.8) Boys: −1.5 (−4.9,1.9) Girls: −2.9 (-8.1,2.4)
		child 1.5 (1.1–2.3)		0.6 (−0.7,1.9) similar for boys and girls	0.2 (−0.8,1.3) similar for boys and girls	0.9 (−0.2,2.0) similar for boys and girls

*$p < 0.05$; NR: not reported.

Not all results (e.g., subgroup analyses, different exposure classification) were extracted from each study if additional results did not change the interpretation.

Other risk factors for cardiovascular disease

Twenty-five studies reported on the association between PFNA exposure and other risk factors for cardiovascular disease, including blood pressure (16 studies), hypertensive disorders during pregnancy (4 studies), atherosclerosis (4 studies), and ventricular geometry (1 study). The study evaluations for these outcomes are summarized in Figure 3-93.

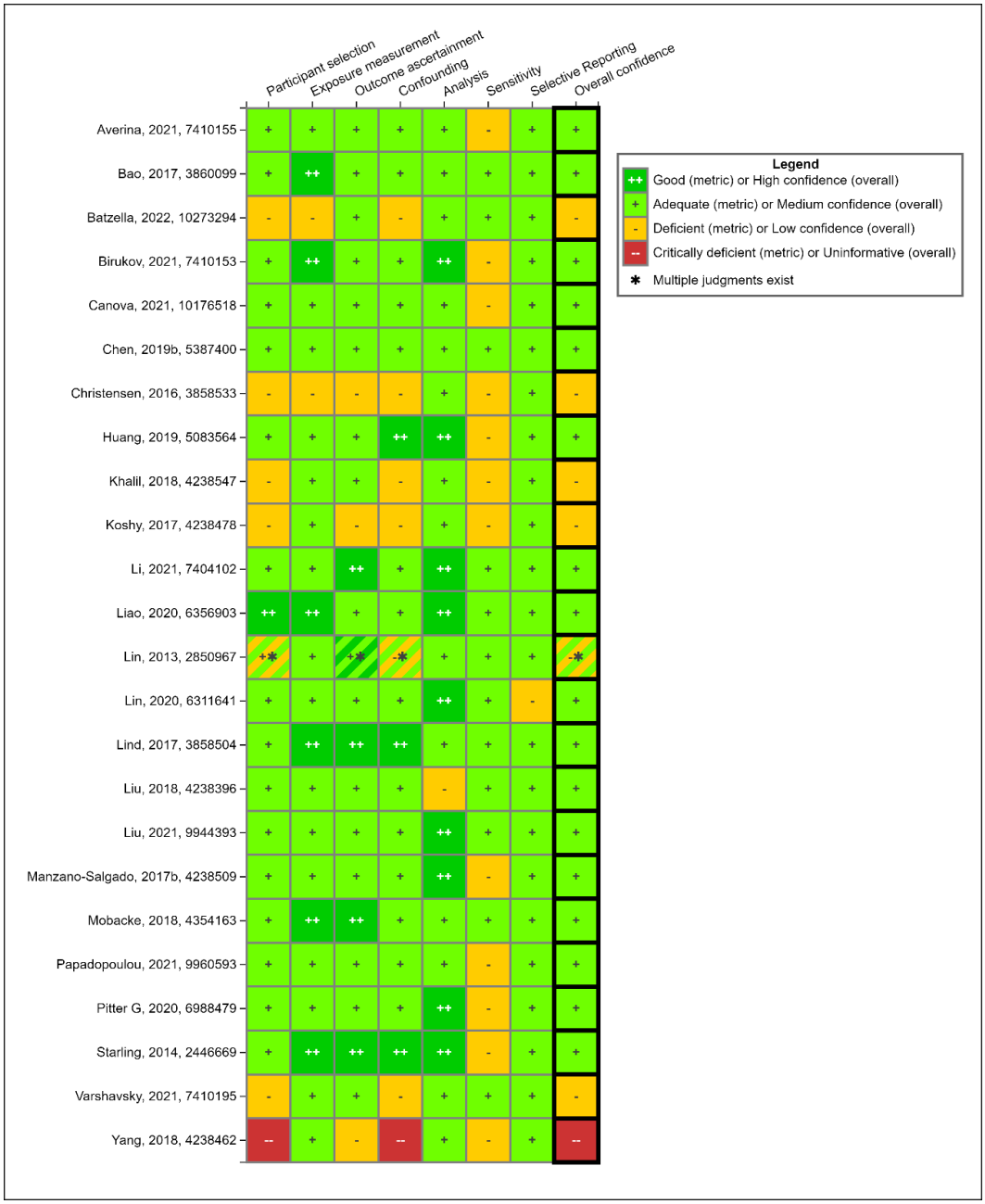


Figure 3-93. Study evaluation results for epidemiology studies of PFNA and cardiovascular disease risk factors. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500435/.

For blood pressure and hypertension endpoints, one study (Yang et al., 2018) was excluded from further analysis due to serious concerns for selection bias and lack of consideration of potential confounding. The remaining studies included a variety of populations and designs, which make direct comparison of blood pressure findings challenging given the occasionally large differences across demographics. Nine studies (13 publications) in adults included analyses of NHANES data (Jain, 2020a, b; Liao et al., 2020; Christensen et al., 2019), a cross-sectional study of government employees in China (Bao et al., 2017), a cross-sectional study of adults in Croatia (Chen et al., 2019), a prospective analysis of participants from a randomized trial of weight-loss diets (Liu et al., 2018), a prospective and cross-sectional analysis of participants from a randomized trial of a diabetes prevention program (Lin et al., 2020b), a cohort of adult male anglers in the United States (Christensen et al., 2016), a cross-sectional study of young adults in a highly contaminated region in Italy (Zare Jeddi et al., 2021; Pitter et al., 2020), a cross-sectional study of male workers with occupational exposure in the same region (Batzella et al., 2022), and a cross-sectional analysis of a cohort of adolescents and young adults in Taiwan (Lin et al., 2013a). There were also four studies in children, two birth cohorts in Spain (Manzano-Salgado et al., 2017b) and the United States (Li et al., 2021), a cross-sectional study of high school students in Norway (Averina et al., 2021), and a cross-sectional study of obese children in the United States (Khalil et al., 2018).

In adults (see Table 3-40), five *medium* and one *low* confidence studies examined hypertension as a dichotomous outcome. Three *medium* confidence studies reported higher odds of hypertension (measured using study protocols) with PFNA exposure (Liao et al., 2020; Pitter et al., 2020; Bao et al., 2017). In these studies, there was a 10%–20% increase in odds, with statistical significance in two (the third was statistically significant only in men). One *medium* confidence study (Chen et al., 2019) reported an inverse association (not statistically significant) and the last *medium* confidence study examining hypertension reported no association (Lin et al., 2020b). The single *low* confidence study of hypertension in adults reported an inverse association (Christensen et al., 2016). Examining potential effect modification, in Bao et al. (2017) and Liao et al. (2020), there is an indication that the association is stronger in women than men, but in Pitter et al. (2020), the opposite was observed. The NHANES population described in Liao et al. (2020) was examined in further analysis in Jain (2020a), which reported that the positive association was primarily among nonobese participants; results among obese participants were null.

Seven *medium* and one *low* confidence studies examined systolic and/or diastolic blood pressure as continuous outcomes in adults. Five *medium* confidence studies reported positive associations, two of which were statistically significant. Positive associations with both systolic and diastolic blood pressure were reported in Bao et al. (2017) ($p < 0.05$, β (95% CI) for ln-unit increase in exposure: systolic 3.01 (1.79, 4.23); diastolic: 2.48 (1.80, 3.16)), Lin et al. (2020b) (β (95% CI) for doubling of exposure: systolic 0.76 (−0.06, 1.59); diastolic: 0.38 (−0.16, 0.93)), and Pitter et al. (2020) (β (95% CI) for above vs. below median: systolic 0.99 (0.47, 1.51); diastolic 0.62 (0.24, 1.0)), and Batzella et al. (2022) (β (95% CI) for ln-unit increase in exposure: systolic 2.20 (−0.31, 4.71)).

In Liao et al. (2020), a nonlinear association was reported, also with both systolic and diastolic blood pressure, with a positive association observed at higher PFNA concentrations, but an inverse association at lower concentrations (β (95% CI) for log-unit increase in exposure: systolic, PFNA >1.72 ng/mL 4.45 (−0.31, 9.21); PFNA ≤1.72 ng/mL −2.32 (−4.35, −0.29); diastolic, PFNA >1.02 ng/mL 1.35 (−0.84, 3.55); PFNA ≤1.02–3.11 ng/mL (−5.29, −0.93). In contrast, the medium confidence study by Chen et al. (2019) reported an inverse association (not statistically significant), and the other by Liu et al. (2018) reported no association between PFNA exposure and change in diastolic blood pressure during the weight loss trial. The *low* confidence study (Lin et al., 2013a) in young adults and adolescents also observed no association with systolic blood pressure.

In children, Li et al. (2021), a *medium* confidence study reported an inverse association between PFNA exposure measured during gestation and mean blood pressure at 12 years but a positive association with cross-sectional measurement (neither statistically significant); results were null with exposure measured at 3 and 8 years and, even given the fairly long half-life of PFNA, this reduced confidence in the results for blood pressure measured at 12 years. Two other *medium* confidence studies reported no association with PFNA exposure (Canova et al., 2021; Papadopoulou et al., 2021). One *low* confidence study (Khalil et al., 2018) reported higher blood pressure; there was an increase in systolic (β = 32.4 (95% CI: 9.04,55.7), units not provided) and diastolic (β = 15.8 (95% CI: −3.59,35.3) blood pressure. The remaining two *medium* confidence studies (Averina et al., 2021; Manzano-Salgado et al., 2017b) reported no association with blood pressure.

Three *medium* confidence and two *low* confidence studies examined hypertensive disorders of pregnancy. One *medium* confidence study reported a statistically significant positive association (OR [95% CI] for quartiles vs. Q1: Q2 2.23 [1.31, 3.80]; Q3 2.11 [1.24, 3.57]) (Liu et al., 2021a). One *low* confidence study also reported positive associations between PFNA exposure and systolic and diastolic blood pressure measured continuously, (β (95% CI) for unit increase in exposure: systolic 5.4 (1.3, 9.5); diastolic 3.6 (0.4, 6.7) (Varshavsky et al., 2021). The other three studies did not report higher odds of preeclampsia (Birukov et al., 2021; Huang et al., 2019b; Starling et al., 2014a) or gestational hypertension (Birukov et al., 2021; Huang et al., 2019b). Non-statistically significant and non-monotonic inverse associations were observed with preeclampsia in Starling et al. (2014a) and gestational hypertension in Huang et al. (2019b).

In summary, there is some evidence of a positive association between PFNA exposure and blood pressure in nonpregnant adults. Among *medium* confidence studies, three of five studies of dichotomous hypertension evaluated in three different continents and five of six studies of continuous blood pressure reported positive associations, although there is some remaining uncertainty, including some concern for imprecision and inconsistency (particularly for blood pressure), and oftentimes unclear patterns of responses across sexes and exposure levels. Results in pregnant women and children do not indicate a consistent association.

For other cardiovascular risk factors, four studies were available of atherosclerosis. Three were *medium* confidence (Koskela et al., 2022; Lind et al., 2017b; Lin et al., 2013a) and one was *low*

confidence (Koshy et al., 2017) due to the potential for residual confounding. One *medium* confidence study examining abdominal aortic calcification, a marker of subclinical atherosclerotic disease, in adults aged 40 years and older reported a positive, although not statistically significant, association (OR (95% CI) for quartiles vs. Q1: Q2 1.04 (0.32, 3.39); Q3 1.20 (0.50, 2.87); Q4 1.47 (0.52, 4.14) (Koskela et al., 2022). One *medium* confidence study of seniors (Lind et al., 2017b) reported a non-statistically 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) in the study population overall (β = 1.9, 95% CI: −1.0,4.8), and a significant increase in women (β = 5.3, 95% CI: 1.0,9.5). A nonsignificant positive association was also observed with number of carotid arteries with atherosclerotic plaques (OR = 1.09, 95% CI: 0.88, 1.34). The other *medium* and *low* confidence studies reported no increase in atherosclerosis with increased PFNA exposure in adolescents and young adults (Lin et al., 2013a) or children (Koshy et al., 2017), respectively.

One *medium* confidence study reported on the association between PFNA exposure and ventricular geometry (Mobacke et al., 2018). There was a statistically significant decrease in relative wall thickness (RWT) (β = −0.02, 95% CI: −0.03, −0.01) and an increase in left ventricular end-diastolic volume (β = 1.01, 95% CI: 0.43,1.58). 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 some evidence of an association between PFNA exposure and blood pressure and atherosclerosis in adults, based on multiple *medium* confidence studies of blood pressure (and hypertension) and two studies of subclinical atherosclerosis. However, there is unexplained inconsistency and imprecision in the findings that results in substantial uncertainty. Associations with these outcomes were inconsistent in pregnant women (blood pressure), children/adolescents (blood pressure and atherosclerosis), and young adults (atherosclerosis).

Table 3-40. Associations between PFNA and hypertension in adults in *medium* confidence epidemiology studies

Reference confidence	Population	Median exposure (IQR) or as specified (µg/mL)	Effect estimate	Hypertension
Pitter et al. (2020), *Medium*	Cross-sectional in highly PFAS exposed region, Italy; 15,786 adults (20–39 yr)	0.4 (0.4–0.6)	OR (95% CI) for above vs. below median	1.10 (0.96, 1.26) Women 0.94 (0.71, 1.25) Men **1.19 (1.02, 1.40)***

Reference confidence	Population	Median exposure (IQR) or as specified (µg/mL)	Effect estimate	Hypertension
Lin et al. (2019), *Medium*	Participants from randomized trial of diabetes prevention, U.S.; 888 overweight and pre-diabetic adults	0.6 (0.4–0.8)	RR (95% CI) per twofold increase (cross-sectional)	1.04 (0.95, 1.14)
			HR (95% CI) per twofold increase (prospective)	0.99 (0.91, 1.08)
Liao et al. (2020), *Medium*	Cross-sectional (NHANES 2003–2012), U.S.; 6,967 adults	1.1 (0.7–1.6)	OR (95% CI) for tertiles vs. T1	T2: 1.07 (0.92, 1.25) T3: **1.18 (1.01, 1.37)*** Women T2: 0.97 (0.78, 1.21) T3: **1.29 (1.02, 1.61)*** Men T2: 1.10 (0.89, 1.36) T3: 1.04 (0.84, 1.28)
Chen et al. (2019), *Medium*	Cross-sectional of general population, Croatia; 123 adults	1.3 (range 0.5–3.5)	OR (95% CI) for ln-unit increase	0.89 (0.39, 2.04)
Bao et al. (2017), *Medium*	Cross-sectional of Chinese government employees, China; 1,228 adults	2.0 (1.1–3.1)	OR (95%) for ln-unit increase	**1.19 (1.04, 1.36)*** Women **1.49 (1.16, 1.92)*** Men 1.08 (0.92, 1.26)

*$p < 0.05$.
Note: Not all results (e.g., subgroup analyses, different exposure classification) were extracted from each study if additional results did not change the interpretation.

Cardiovascular disease

Five studies reported on the association between PFNA and cardiovascular disease. The study evaluations are summarized in Figure 3-94. Two cross-sectional studies, an analysis of NHANES data for 1999–2014 (Huang et al., 2018), data from the C8 Health Project (Honda-Kohmo et al., 2019), and a prospective cohort of farmers and other rural residents (Mattsson et al., 2015) were *medium* confidence. The other two were *low* confidence cross-sectional studies (Graber et al., 2019; Christensen et al., 2016). These had small sample sizes and were focused on participants in litigation over PFAS exposure (Graber et al., 2019) or anglers (Christensen et al., 2016). Concerns regarding confounding and sensitivity were identified in both these studies. Additionally, all the studies except (Mattsson et al., 2015) classified cardiovascular disease based on self-report, which is likely to result in misclassification. This could be differential in the study where exposure status was known to participants due to litigation (Graber et al., 2019) but is otherwise expected to be non-differential.

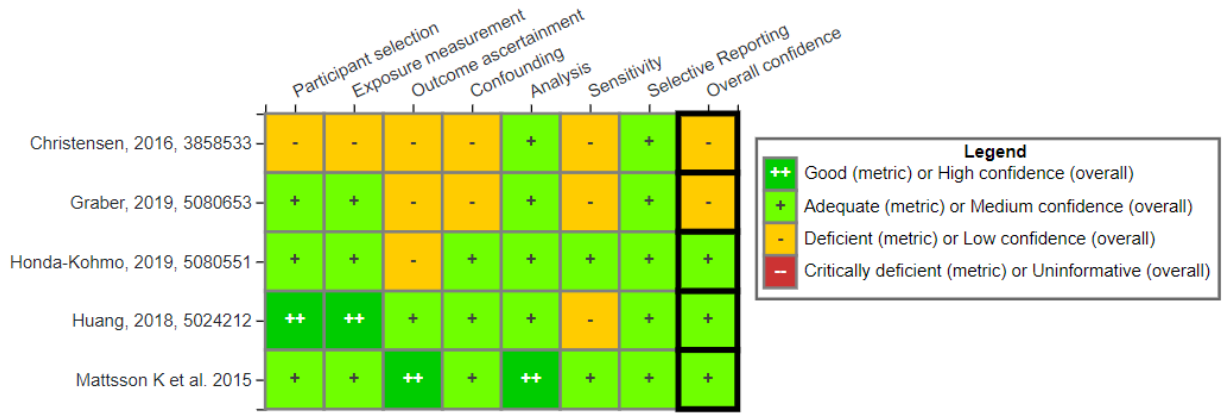


Figure 3-94. Heat map of study evaluations for PFNA and cardiovascular disease. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500452/.

Huang et al. (2018) reported higher odds of total cardiovascular disease, coronary heart disease, heart attack, and stroke with higher PFNA exposure (coronary heart disease and heart attack were statistically significant), with ORs in quartile 4 versus quartile 1 ranging from 1.2 to 1.9. No association was observed with congestive heart failure. In the other two *medium* confidence studies, there was no increase in coronary heart disease (Honda-Kohmo et al., 2019; Mattsson et al., 2015). The two *low* confidence studies also observed no association (Graber et al., 2019; Christensen et al., 2016). Overall, there is a lack of consistency across the studies of cardiovascular disease that cannot be explained by study design, risk of bias, or sensitivity.

Metabolic effects

Diabetes

Nine studies (12 publications) reported on the relationship between PFNA exposure and type 2 diabetes. Five out of the nine studies were cross-sectional and were considered *low* confidence due to concerns about timing of measurement exposure levels in relation to outcome ascertainment and other deficiencies noted in HAWC. Four studies (Charles et al., 2020; Donat-Vargas et al., 2019; Sun et al., 2018; Cardenas et al., 2017) measured PFNA at baseline and were able to determine incidence of diabetes since Cardenas et al. (2017) used a multicenter randomized clinical trial of intervention for prevention of type 2 diabetes among high-risk individuals and the other three used a prospective nested case-control design. Thus, these four studies were evaluated as *medium* confidence and given more weight in the synthesis because of measurement of exposure prior to diagnosis, reducing concern for reverse causation. A summary of study evaluations for PFNA and diabetes is presented in Figure 3-95, and additional details of the studies can be obtained from HAWC.

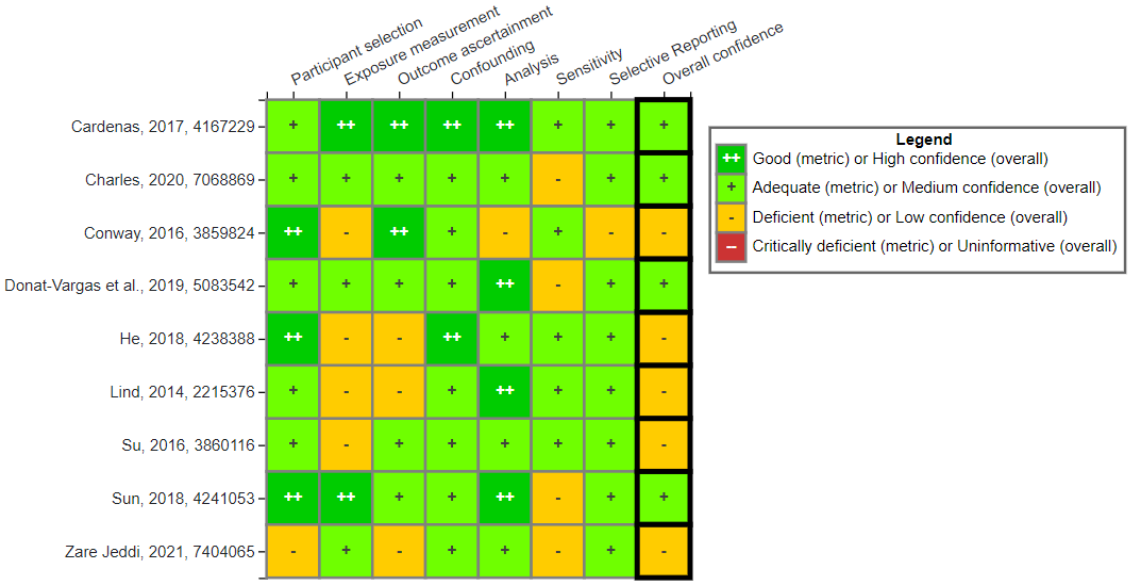


Figure 3-95. Heat map of study evaluations for PFNA and diabetes. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500196/.

Results of associations between PFNA exposures and diabetes are presented in Table 3-41. All studies evaluated exposure and outcome associations in adults; and in Conway et al. (2016), both adults and children were included in the study population. In the four studies of *medium* confidence (Charles et al., 2020; Donat-Vargas et al., 2019; Sun et al., 2018; Cardenas et al., 2017), only Charles et al. (2020) reported higher odds of type 2 diabetes with higher PFNA exposure, and this difference was not statistically significant. Donat-Vargas et al. (2019) reported a nonsignificant inverse association while the other two studies (Sun et al., 2018; Cardenas et al., 2017) reported null associations. Although some *low* confidence studies reported small positive associations (He et al., 2018; Lind et al., 2014), the association in He et al. (2018) was not monotonic and other studies reported inverse associations (Su et al., 2016) or null associations (Zare Jeddi et al., 2021; Conway et al., 2016). Across all the available studies, there is no clear pattern in the associations by exposure levels or contrast, as the studies reporting positive associations tended to have slightly lower exposure levels, and overall, the findings are inconclusive.

Table 3-41. Associations between PFNA and diabetes in epidemiology studies

Reference, confidence	Population	Median exposure (IQR) in ng/mL or as specified	Exposure change	Diabetes OR (95% CI)*
***Medium* confidence**				
Charles et al. (2020), *Medium*	Prospective nested case-control study (2001–2006), Norway; 133 women	Median (5th–95th) 0.4 (0.2–1.1)	IQR increase	1.37 (0.69, 2.75)

Reference, confidence	Population	Median exposure (IQR) in ng/mL or as specified	Exposure change	Diabetes OR (95% CI)*
Cardenas et al. (2017), *Medium*	Diabetes Prevention Program (1996–1999), U.S.; 957 adults (25+ yr)	Geometric mean (IQR) 0.5 (0.4)	log2-unit change	Incident type 2 0.99 (0.87, 1.12)[a]
Donat-Vargas et al. (2019), *Medium*	Prospective nested case-control study (1990–2013), Sweden; 248 adults	0.6 (0.4–0.8)	log-unit change	0.82 (0.57, 1.18)
Sun et al. (2018), *Medium*	Prospective nested case-control study of Nurses' Health Study II (1995–2000), U.S.; 793 adults (32–52 yr)	0.6 (0.4– –.9) controls	tertiles vs. T1	Incident type 2 T2: 1.08 (0.75, 1.56) T3: 0.99 (0.67, 1.48)
***Low* confidence**				
Zare Jeddi et al. (2021), *Low*	Cross-sectional in high contamination region (2017–2019), Italy; 15,876 young adults (20–39 yr)	0.4 (0.4–0.6)	log-unit change	0.95 (0.58, 1.53)
Lind et al. (2014), *Low*	Cross-sectional (2001–2004), Sweden; 1,016 adults (70 yr)[b]	0.7 (0.5–1.0)	ln-unit change	1.30 (0.85, 1.97)
He et al. (2018), *Low*	NHANES cross-sectional (2003, 2004, 2005–2006, 2007–2008, 2009–2010, 2011–2012), U.S.; 7,904 adults (20+ yr)	Mean ± SE Men 1.5 ± 0.02 Women 1.3 ± 0.03	quartiles vs. Q1	Men Q2: 1.25 (0.77, 2.04) Q3: 1.17 (0.74, 1.87) Q4: 1.19 (0.73, 1.95) Women Q2: 0.98 (0.14, 1.10) Q3: 1.50 (0.88, 2.57) Q4: 1.01 (0.62, 1.65)
Conway et al. (2016), *Low*	C8 Health Project cross-sectional (2005–2006), U.S.; 66,889 children and adults (<20 yr and 20+ yr)[c]	Mean (SD) 1.6 (0.9) no diabetes	unit change	0.94 (0.88–1.00)
Su et al. (2016), *Low*	Case-control study conducted at cardiology clinics (2009–2011), Taiwan; 571 adults (20–60 yr)	3.8 (2.6–5.1)	quartiles vs. Q1	Q2: 0.39 (0.14, 1.10) Q3: 0.72 (0.29, 1.10) Q4: 0.31 (0.11, 0.88)*

Prevalent diabetes unless indicated otherwise; *p-value or p-trend ≤ 0.05;
[a]HR (95% CI) reported in this study.
[b]Some studies listed as cross-sectional were cross-sectional analyses within a cohort study.
[c]Population includes children and adults.
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.

Gestational diabetes

Six studies reported on the relationship between PFNA exposure and gestational diabetes. The quality of gestational diabetes ascertainment was based on whether screening of gestational

diabetes mellitus (GDM) was defined by a study protocol or standards of practice at individual clinics. Another important consideration is that GDM associations with exposure are not interpretable in the presence of diabetes. Thus, for participant selection, it was important for studies to account for the diabetic status and/or the use of diabetic medications. Studies that did not consider these factors by exclusion or stratification were considered deficient for the participant selection domain and *low* confidence overall. Four studies that examined the association between PFNA exposure and gestational diabetes were of *medium* confidence (Yu et al., 2021; Rahman et al., 2019; Wang et al., 2018c; Valvi et al., 2017), and two studies were of *low* confidence (Matilla-Santander et al., 2017; Zhang et al., 2015). A summary of the study evaluations for PFNA and gestational diabetes is presented in Figure 3-96, and additional details of the studies can be obtained from HAWC.

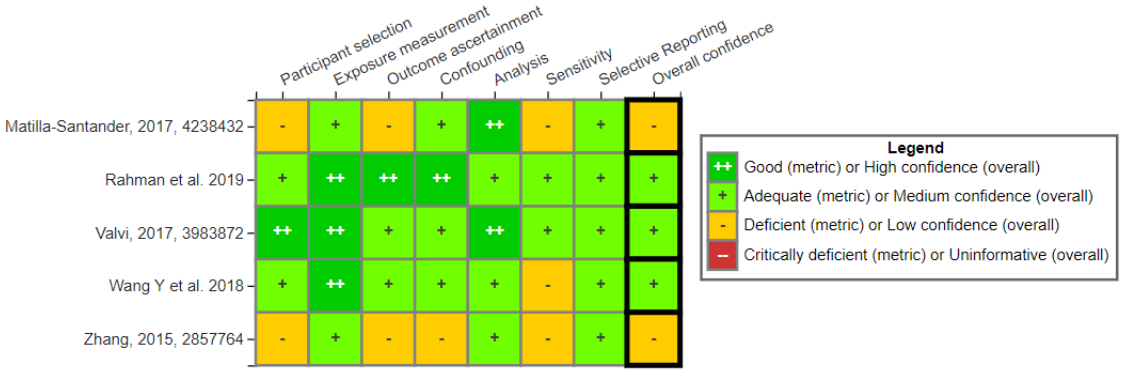


Figure 3-96. Summary of study evaluations for epidemiology studies of PFNA and gestational diabetes. See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500197/.

The results for the association between PFNA exposure and gestational diabetes for the six studies are presented in Table 3-96. Two *medium* confidence studies (Rahman et al., 2019; Wang et al., 2018c) reported slightly higher risk of GDM with higher exposure. These associations were not statistically significant, with the exception of a stratified analysis of participants with a family history of diabetes (the participants did not have a personal history of diabetes) in Rahman et al. (2019). The remaining studies reported ORs close to 1 (Yu et al., 2021; Zhang et al., 2015): or less than 1 but not statistically significant (Matilla-Santander et al., 2017; Valvi et al., 2017). Overall, there is not consistent evidence supporting an association.

Table 3-42. Associations between PFNA and gestational diabetes in epidemiology studies

Reference, confidence	Population	Median exposure (IQR) in ng/mL or as specified	Exposure contrast	Gestational diabetes mellitus (GDM) OR (95% CI)[a]
Wang et al. (2018c), *Medium*	Case-control study (2013), China; 252 pregnant women	0.4 (0.3–0.5)	Unit change	1.25 (0.37, 4.28)
Valvi et al. (2017), *Medium*	Cohort (1997–2000), Faroe Islands; 604 pregnant women	0.6 (0.5–0.8)	Per doubling of exposure	0.88 (0.53, 1.47)
Rahman et al. (2019), *Medium*	Cohort (2009–2013), U.S.; 2,334 pregnant women	GM (95% CI) 0.8 (0.8–0.2)	SD increment	Overall cohort[b] 1.05 (0.82, 1.35) With family history of type 2 diabetes[b] **1.43 (1.22, 1.68)**
Yu et al. (2021), *Medium*	Cohort (2013–2016), China; 2,747 pregnant women	1.7 (1.1)	Log-unit increase	1.03 (0.81, 1.30)
Matilla-Santander et al. (2017), *Low*	Cohort (2003–2008), Spain; 2,150 pregnant women	GM (GSD) 0.6 (1.8)	Log-unit increase	0.85
Zhang et al. (2015), *Low*	Cohort (2005–2009), U.S.; 272 pregnant women	GM (95% CI) Non-GDM 1.2 (1.1–1.3)	SD increment	1.06 (0.70–1.60)

[a]Results shown are from most-adjusted models unless noted.
[b]RR (95% CI) reported in this study and has other estimates by subgroups.
Note: Not all results (e.g., subgroup analyses, different exposure classification) were extracted from each study if additional results did not change the interpretation.

Blood glucose and insulin resistance

Homeostatic model assessment (HOMA) is a method for assessing insulin resistance and β-cell function, based on 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 of diabetes and was considered a primary outcome for this review along with fasting blood glucose. It is important to consider that insulin and HOMA-IR associations with exposure are not interpretable in the presence of diabetes, especially if diabetes is treated with hypoglycemic medication since the treatment will affect insulin production and secretion. Thus, for participant selection, it was important to account for the diabetic status and/or the use of diabetic medications. Studies that did not consider these factors by exclusion or stratification were considered deficient for the participant selection domain and *low* confidence overall. For the timing of the exposure

measurement, unlike for diabetes, exposure and outcome can be assessed concurrently as the outcome can be a short-term response, and establishing temporality was not deemed a major concern.

Twenty-nine studies (30 publications) reported on the relationship between PFNA exposure and blood glucose and/or insulin resistance. Of these, 17 were of *medium* confidence (Cakmak et al., 2022; Gardener et al., 2021; Goodrich et al., 2021; Li et al., 2021; Valvi et al., 2021; Yu et al., 2021; Duan et al., 2020; Ren et al., 2020; Christensen et al., 2019; Heffernan et al., 2018; Jensen et al., 2018; Kang et al., 2018; Wang et al., 2018c; Cardenas et al., 2017; Fleisch et al., 2017; Starling et al., 2017; Lin et al., 2011; Lin et al., 2009) and 8 were *low* confidence. Many of these *low* confidence studies did not account for diabetic status of the adult participants and were thus deficient for participant selection. In addition, three studies were *uninformative* due to lack of consideration of potential confounding (Zhang et al., 2019; Yang et al., 2018; Jiang et al., 2014). Study evaluation results are summarized in Figure 3-97 and additional details are available in HAWC. Of the included studies, 12 reported on insulin resistance using HOMA-IR and 23 studies reported on fasting blood glucose. Thirteen studies reported on general population adults, one study reported on occupationally exposed adults, six studies reported on pregnant women, and seven studies reported on children and adolescents.

Figure 3-97. Summary of study evaluations epidemiology studies of PFNA and insulin resistance and blood glucose.[a] See interactive HAWC link: https://hawcprd.epa.gov/summary/visual/100500198/.

[a]Two publications Lin et al. (2009) and Nelson et al. (2010) reported NHANES data from overlapping years (2003–2004), and these were considered one study. However, the results from all publications were reviewed.

The results for the association between PFNA exposure and these outcomes for all studies are presented in Table 3-43. The majority of studies of both insulin resistance and blood glucose indicated no association with PFNA exposure. The most consistent findings of an association were in pregnant women, with two of five studies reporting positive associations with blood glucose (Yu et al., 2021; Ren et al., 2020). In particular, results in these studies were statistically significant in when examining results for one hour post oral glucose tolerance test, which the other studies did not include. Looking at HOMA-IR scores in pregnant women, one of two studies reported a positive association (Jensen et al., 2018). Results in general population adults, adolescents, and children were inconsistent. Overall, there is not a clear association between PFNA exposure and insulin resistance or blood glucose, but there is an indication of an association in pregnant women.

Table 3-43. Associations between PFNA and insulin resistance or blood glucose in epidemiology studies

Reference and confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate	Insulin resistance (HOMA-IR)	Blood glucose
General population, adults					
Heffernan et al. (2018), *Medium*	Cohort of women with and without polycystic ovarian syndrome (PCOS), United Kingdom; 59 adults (20–45 yr)	GM (95% CI) Control 0.5 (0.4, 0.6)	β(SE) for ln-unit change	Controls 0.03 (0.11) PCOS −0.10 (0.17)	Controls 0.16 (0.09) PCOS 0.00 (0.10)
Cardenas et al. (2017), *Low*	Diabetes Prevention Program trial, non-medication arms (1996–1999), U.S.; 957 adults (25+ yr)	GM (IQR) 0.5 (0.40)	β(95% CI) for twofold change	0.20 (−0.02, 0.42)	0.45 (0.03, 0.87)
Cakmak et al. (2022), *Medium*	Canadian Health Measures Survey (2007−2017), Canadia; 6,768 all ages	GM 0.6	% change for GM increase	1.2 (−5.6, 8.5)	0.2 (−1.0, 1.5)
Valvi et al. (2021), *Medium*	Faroe Islands cohort (1986–1987 enrollment), Denmark; 699 adults (28 yr)	0.7 (0.5–0.9) at age 7 yr	β(95% CI) for twofold change	0.01 (−0.06, 0.08)	0.00 (−0.02, 0.02)

Reference and confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate	Insulin resistance (HOMA-IR)	Blood glucose
Lind et al. (2014), *Low*	Cross-sectional (2001–2004), Sweden; 1,016 adults (70 yr)	0.7 (0.5–1.0)	β(95% CI) for ln-unit change	0.004 (−0.059, 0.066)	NR
Lin et al. (2009), *Low*	Cross-sectional (NHANES 1999–2000, 2003–2004), U.S.; 969 adults (>20 yr)	NR	Mean ± SEM for log-unit change	Adults −0.04 ± 0.04	Adults 0.00 ± 0.04
Khalil et al. (2020), *Low*	Cross-sectional of firefighters (2009), U.S.; 87 men	GM 0.9	β (95% CI) for log-unit change	NR	No association
Christensen et al. (2019), *Medium*	Cross-sectional (NHANES 2007–2008, 2009–2010, 2011–2012, 2013–2014), U.S.; 2,975 adults (≥20 yr)	2007–2008 1.2 (0.8, 1.7) 2009–2010 1.2 (0.79, 1.8) 2011–2012 0.8 (0.6, 1.2) 2013–2014 0.6 (0.4, 1.0)	OR (95% CI) for quartiles vs. Q1	NR	Q2: 1.36 (0.89, 2.07) Q3: 1.37 (0.83, 2.25) Q4: 1.62 (0.90, 2.92)
Chen et al. (2019), *Low*	Cross-sectional of general population, Croatia; 123 adults	1.3 (range 0.5–3.5)	β (95% CI) for ln-unit change	NR	−0.11 (−0.40, 0.18)
Liu et al. (2018), *Low*	POUNDS clinical trial (2003–2007), U.S.; 621 adults (30–70 yr)	Men 1.6 (1.1–2.4) Women 1.5 (1.0–2.4)	Spearman correlation	0.15	Change in glucose 0–6 mo in trial: −0.06 6–24 mo: 0.03
Lin et al. (2011), *Medium*	Cross-sectional (1992–2000); Taiwan; adolescents and adults (12–30 yr)	1.7 (25.03)	Mean ± SE	<50th: −0.66 (0.11) 50th–74th: −0.77 (0.13) 75th–89th: −0.90 (0.15) ≥90th: −0.76 (0.17)	<50th: 84.77 (0.58) 50th–74th: 85.33 (0.75) 75th–89th: 85.51 (0.98) ≥90th: 85.18 (1.15)
Duan et al. (2020), *Medium*	Cross-sectional (2017), China; 294 adults	3.2 (2.0, 5.2)	% change (95% CI) for 1% change	NR	**0.02 (0.01, 0.04)***
Lin et al. (2020c), *Low*	Cross-sectional (2016–2017), Taiwan; 938 older adults (55–75 yr)	3.2 (range 0.8–9.9)	β (95%) for quartiles vs. Q1	NR	Q2: 0.06 (−7.27, 7.4) Q3: −1.6 (−8.95, 5.76) Q4: 4.25 (−3.18, 11.69) Women:

Reference and confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate	Insulin resistance (HOMA-IR)	Blood glucose
					Q2: −2.58 (−10.99, 5.84) Q3: −5.33 (−13.73, 3.06) Q4: 5.99 (−2.49, 14.47) Men: Q2: **15.64 (1.43, 29.85)*** Q3: 7.65 (−6.45, 21.75) Q4: 5.05 (−9.15, 19.24)
Su et al. (2016); *Low*	Case-control study(2009–2011), Taiwan; 571 adults (20–60 yr)	3.8 (2.6–5.1)	GM (95% CI) for quartiles vs. Q1	NR	Q2: 0.97 (0.94, 1.00) Q3: 0.99 (0.96, 1.02) Q4: 0.97 (0.94, 1.00)
Pregnant women					
Wang et al. (2018c), *Medium*	Case-control study (2013); 252 women	GDM 0.4 (0.3–0.5) Non-GDM 0.4 (0.3–0.5)	OR (95% CI) for medium and high exposure vs. low	NR	Medium vs. Low 1.37 (0.74, 2.52) Highest vs. Low 1.08 (0.58, 2.00)[a]
Starling et al. (2017), *Medium*	Cohort (2009–2014); U.S.; 1,410 women	0.4 (0.6, 1.1)	% Change (95% CI) for high vs. low exposure	NR	0.5–6.0 ng/mL −0.025 (−0.042, −0.009)
Gardener et al. (2021), *Medium*	Cross-sectional analysis (2009), U.S.; 184 women	0.7 (0.5–1.1)	Mean (95% CI) for quartiles	Insulin No association	NR
Jensen et al. (2018), *Medium*	Cohort (2010–2012), Denmark; 649 women	0.7 (0.4–1.6)	% Change (95% CI) for twofold change	12.2 (−0.5, 26.4)	0.03 (−2.1, 2.2)
Yu et al. (2021), *Medium*	Cohort (2013–2016), China; 2,747 pregnant women	1.7 (1.1)	β (95% CI) for log-unit change	NR	0.03 (−0.01, 0.07) 1 hr OGGT **0.18 (0.06, 0.30)*** 2 hr OGGT **0.15 (0.04, 0.25)***

Reference and confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate	Insulin resistance (HOMA-IR)	Blood glucose
Ren et al. (2020), *Medium*	Cohort (2012), China; 856 women	1.8 (1.3–2.5)	OR (95% CI) high glucose for ln-unit change	NR	Fasting: 1.55 (0.98, 2.46) 1 hr post oral glucose tolerance test: **2.15 (1.24, 3.74)***
			β (95% CI) for ln-unit change	NR	Fasting: **0.09 (0.02, 0.16)*** 1 hr post oral glucose tolerance test: **0.044 (0.22, 0.66)***
Children and adolescents					
Khalil et al. (2018), *Low*	Cross-sectional of obese children (2016); U.S.; children (8–12 yr)	0.2 (0.2)	β(95% CI) for unit change	−2.16 (−10.2, 5.86)	−1.92 (−21.2, 17.4)
Goodrich et al. (2021), *Medium*	Participants from cohort of obese children (SOLAR 2001–2012) and a cross-sectional analysis in a second cohort of adolescents to early adults (CHS 2011–2012), U.S.; 328 and 137 participants	GM SOLAR 0.6 CHS 0.4	Difference (95% CI) for high vs. low levels	NR	Results stratified by sex, oral glucose tolerance test timepoint, puberty stage. No clear pattern in direction of association.
Koshy et al. (2017), *Low*	Cohort from World Trade Center Health Registry (WTCHR), U.S.; 402 adolescents	Control 0.5 (0.33) WTCHR 0.6 (0.36)	β(95% CI) for ln-unit change	0.01 (−0.13, 0.14)	NR
Fleisch et al. (2017), *Medium*	Cohort (1992–2002), U.S.; 665 children (7 yr)	GM (IQR) Prenatal 0.6 (0.5, 0.9) Mid-childhood 1.7 (1.1, 2.3)	% Change (95% CI) for quartiles vs. Q1	Prenatal Q2: 7.8 (−11.8, 31.9) Q3: 13.1 (−8.6, 39.8) Q4: 2.9 (−19.2, 31.1) Mid-childhood Q2: −25.0 (−37.0, −10.7) Q3: −27.1 (−39.4, −12.1) Q4: −25.6 (−38.0, −10.7)	NR

Reference and confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate	Insulin resistance (HOMA-IR)	Blood glucose
Li et al. (2021), ***Medium***	Cohort (2003–2006), U.S.; 221 children (12 yr)	prenatal 0.9 (0.7–1.2)	β (95% CI) for IQR increase	NR	−1.7 (−3.4, 0.1)
		12 yr 0.3 (0.2–0.5)		NR	0.6 (−1.0, 2.2)
Kang et al. (2018), *Medium*	Cross-sectional (2012–2014), South Korea; children (3–18 yr)	GM (SD) 0.9 (1.8)	β (95% CI) for ln-unit change	NR	0.43 (−1.78, 2.16)
Lin et al. (2009), *Medium*	Cross-sectional (NHANES 1999–2000, 2003–2004), U.S.; 474 adolescents (12–20 yr)	NR	Mean ± SEM for log-unit change	Adolescents −0.08 ± 0.04	Adolescents 0.07 ± 0.04

GM = geometric mean; OR = odds ratio; NR = not reported.
*p-value or p-trend ≤ 0.05.

Metabolic syndrome

The criteria for clinical diagnosis of metabolic syndrome include the following: elevated waist circumference; elevated triglycerides ≥150 mg/dL (1.7 mmol/L); reduced high-density lipoprotein (HDL) cholesterol <40 mg/dL (1.0 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women; elevated blood pressure: systolic ≥130 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 diagnosis of metabolic syndrome and that country- or population-specific cut points for waist circumference should be used (Alberti et al., 2009). Medication use for any of these criteria can also be used for classification; without this consideration, the participant selection domain is rated as deficient, similar to the individual outcomes above.

Five studies reported on the association between PFNA exposure and metabolic syndrome. One study was considered *uninformative* due to critical deficiencies in participant selection and confounding (Yang et al., 2018). Two studies were *low* confidence (Zare Jeddi et al., 2021; Lin et al., 2009) due to lack of consideration of medication use and other limitations. The remaining two studies were *medium* confidence (Christensen et al., 2019; Lin et al., 2009). A summary of study evaluations for PFNA and metabolic syndrome is presented in Figure 3-98, and additional details of the studies can be obtained from HAWC.

Christensen et al. (2019) found a dose-dependent, positive association between PFNA exposure and metabolic syndrome in adults greater than 20 years of age (OR: 2.25 (1.58, 3.20) with

ln (PFNA); quartile 2 vs. quartile1: 1.60 (1.07, 2.42), quartile 3: 2.45 (1.50, 4.00), and quartile 4: 2.83 (1.54, 5.20)). Lin et al. (2020c) also reported a positive association in older adults (55–75 years), although not statistically significant (OR (95% CI) for quartiles vs. Q1: Q2: 0.94 (0.51, 1.71); Q3: 1.04 (0.57, 1.89); Q4: 1.48 (0.82, 2.67)). The *low* confidence studies in adolescents and adults (Lin et al., 2009) and young adults (Zare Jeddi et al., 2021) did not report higher odds of metabolic syndrome with higher exposure. In addition to the studies of metabolic syndrome, one *medium* confidence study calculated a cardiometabolic risk summary score more appropriate for children; no association was observed with PFNA exposure (Li et al., 2021). In summary, there is some evidence of an association between metabolic syndrome and PFNA exposure in adults, based on the two available *medium* confidence studies, although there are uncertainties.

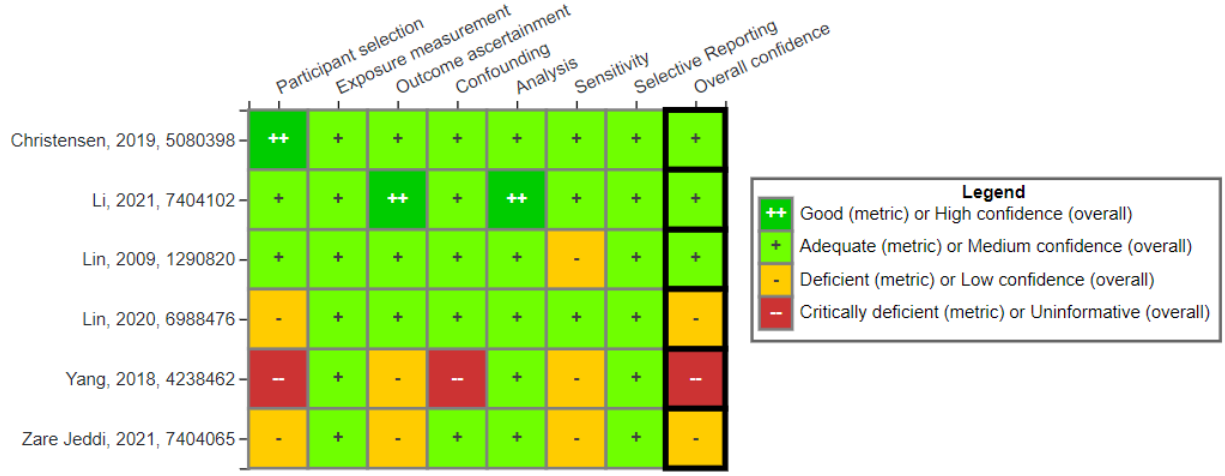


Figure 3-98. Heat map of study evaluations for PFNA and metabolic syndrome.
See interactive HAWC link:
https://hawcprd.epa.gov/summary/visual/100500199/.

Adiposity

Twenty-six studies (30 publications) reported on the association between PFNA exposure and obesity, body mass index (BMI), and/or other measures of adiposity. Three studies were considered *uninformative* because of lack of consideration of confounding as well as other deficiencies (Zhang et al., 2019; Yang et al., 2018; Halldorsson et al., 2012). Of the remaining studies, seven were cross-sectional (Lind et al., 2022; Wise et al., 2022; Yu et al., 2022; Canova et al., 2021; Thomsen et al., 2021; Zare Jeddi et al., 2021; Domazet et al., 2020; Scinicariello et al., 2020a; Chen et al., 2019; Christensen et al., 2019; Khalil et al., 2018; Nelson et al., 2010) and were classified as *low* confidence because of concerns related to the timing of exposure measurement. Eight studies were prospective, including six that examined the association between prenatal or early-life exposure measurements and adiposity during childhood (Bloom et al., 2022; Chen et al., 2019; Mora et al., 2018; Hartman et al., 2017; Karlsen et al., 2017; Manzano-Salgado et al., 2017b; Braun et

al., 2016), one cohort of people living near a uranium processing plant (Blake et al., 2018), one cohort of pregnant women (Romano et al., 2020), and one clinical trial of weight-loss diets that examined weight change (Liu et al., 2018). All of the prospective studies were classified as *medium* confidence. The evaluations for these studies are summarized in Figure 3-99.

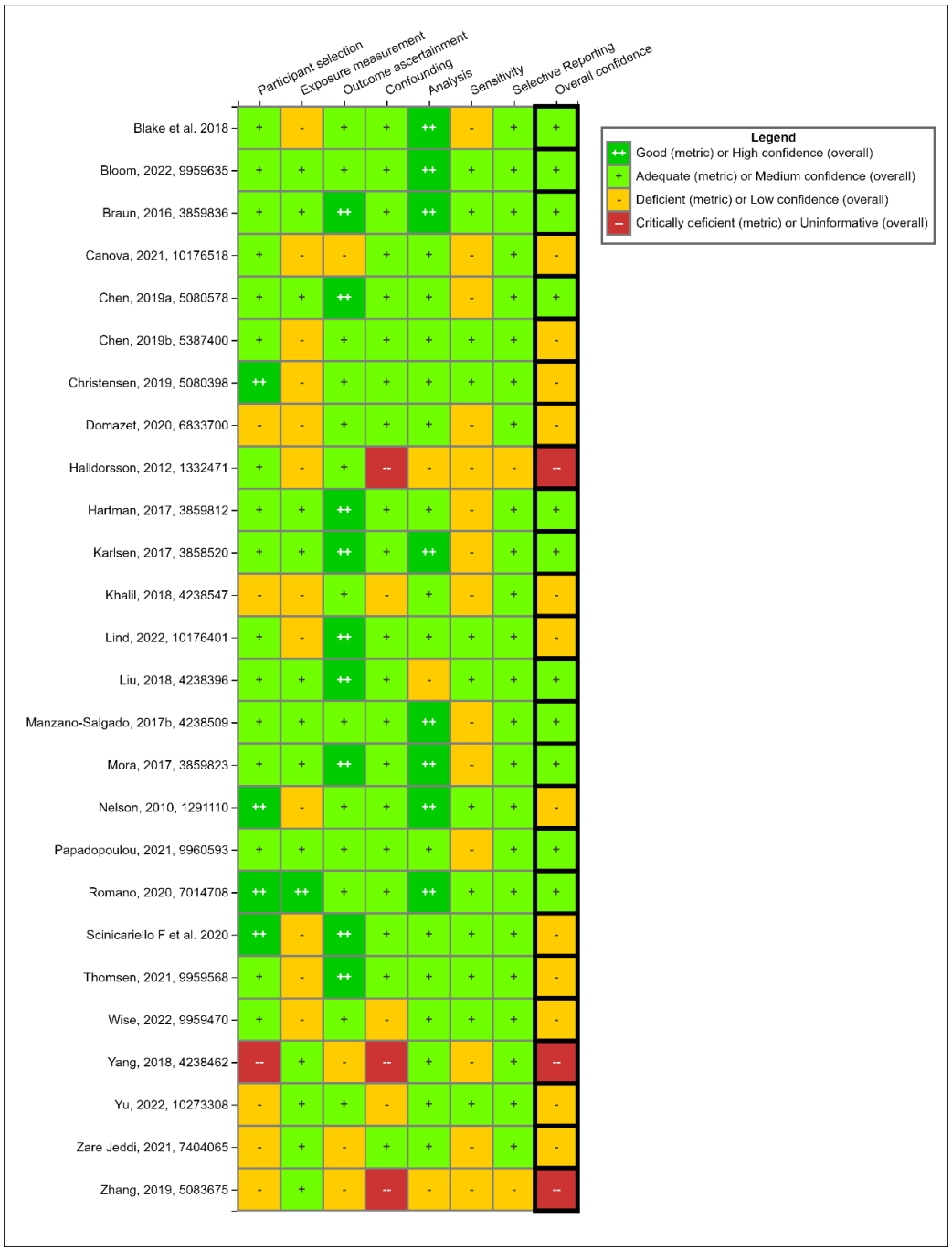


Figure 3-99. Summary of study evaluations for epidemiology studies of adiposity[a]. See interactive HAWC link:

https://hawcprd.epa.gov/summary/visual/assessment/100500071/pfna-evaluation-adiposity/.

[a]Multiple publications of the same study: Braun et al. (2016) also includes Braun et al. (2020); Liu et al. (2020c), and Li et al. (2021). Mora et al. (2017) also includes Janis et al. (2021).

The results from the eight prospective studies of adiposity in children are summarized in Tables 3-44 and 3-45, which contain the continuous outcome measures (BMI, waist circumference, and body fat) and dichotomous outcome (overweight), respectively. One study reported a statistically significant positive association with body fat percent in boys only (Chen et al., 2019). Positive, although not statistically significant associations with adiposity were also observed in Hartman et al. (2017), Mora et al. (2018), and Braun et al. (2016). The direction of association was not consistent across all outcomes (BMI, waist circumferences, and other measures of body fat) in three of these four studies reporting positive associations (Chen et al., 2019; Hartman et al., 2017; Braun et al., 2016). The follow-up analyses from Mora et al. (2018) and Braun et al. (2016) did not report a positive association (Janis et al., 2021; Braun et al., 2020; Liu et al., 2020a). Three studies in children reported no association with continuous measures of adiposity (Papadopoulou et al., 2021; Karlsen et al., 2017; Manzano-Salgado et al., 2017b) and one study reported a statistically significant inverse association in participants with obesity but not in those without obesity (Bloom et al., 2022). Exposure levels and contrast do not appear to explain this inconsistency, as they were similar across the available studies, and the strongest associations were observed in the studies with the lowest exposure levels. However, all four studies examining dichotomous outcomes of overweight and obese observed some positive associations, including an exposure-response gradient across tertiles in Braun et al. (2016), although none were statistically significant. In addition, five *low* confidence studies in children and adolescents examined cross-sectional associations with adiposity; one study reported a statistically significant inverse association with fat mass (Domazet et al., 2020), while the other study reported no association (Yu et al., 2022; Canova et al., 2021; Scinicariello et al., 2020a), including with MRI measures of body fat (Thomsen et al., 2021).

In adults, one prospective study (Liu et al., 2018) reported no difference in weight loss associated with PFNA exposure but found a statistically significant increase in weight gain associated with PFNA exposure in women following weight loss (changes in body weight: tertile 1: 2.5 ± 0.9, tertile 2: 2.9 ± 0.9, tertile 3: 4.7 ± 0.9, *p*-trend: 0.006). In men, there was less weight gain with higher exposure. A second prospective study (Blake et al., 2018) reported higher BMI with higher exposure (% change 0.65, 95% CI: −0.73, 2.04). One *low* confidence cross-sectional study of NHANES data for 2007–2014 (Christensen et al., 2019) also reported higher odds of increased waist circumference with higher PFNA exposure (OR (95% CI) for Q2 vs. Q1: 1.67 (1.05, 2.65), Q3: 2.12 (1.14, 3.93), Q4: 2.20 (1.08, 4.51), which was statistically significant and indicated an exposure-response gradient across quartiles. The other NHANES analysis (for 2003–2004) reported no "meaningful associations" but quantitative estimates were not available (Nelson et al., 2010). Chen et al. (2019) reported positive, although not statistically significant, associations with

BMI and hip circumference (β (95% CI) for ln-unit increases: 0.48 (−0.95, 1.91) and 0.81 (−2.50, 4.12), respectively). In contrast, in young adults (20–39 years old), Zare Jeddi et al. (2021) reported a statistically significant inverse association with BMI of 30 or greater (OR (95% CI) for log-unit increases in exposure: 0.85 (0.72, 1.00)). In other cross-sectional studies (*low* confidence), no association was observed in Wise et al. (2022), while in Lind et al. (2022), inverse associations were observed with some indices of body composition in women but not men. Lastly, in a single *medium* confidence pregnancy cohort, overweight and obese women had higher gestational weight gain with higher PFNA exposure, but this difference was not statistically significant and was not observed in underweight and normal weight women.

In summary, there is inconsistent evidence of an association between PFNA exposure and adiposity in both adults and children.

Table 3-44. Associations between maternal exposure to PFNA and adiposity in *medium* confidence epidemiology studies in children

Reference	Population	Median exposure (IQR) (µg/mL)	Effect estimate	BMI	Waist circumference	Body fat
Hartman et al. (2017)	ALSPAC birth cohort (1991–1992), United Kingdom; 359 children with follow-up at 9 yr)	0.5 (0.4–0.7)	β (95% CI) for 1 unit increase	0.22 (−0.83,1.27)	−0.11 (−2.91,2.70)	DXA total body fat 1.71 (−1.29,4.71) DXA trunk fat −0.03 (−1.83,1.77)
Mora et al. (2018) Janis et al. (2021)	Cohort (1999–2002), U.S.; 1,006 children with follow-up at median 3 yr	0.6 (0.5–0.9)	β (95% CI) for IQR increase	0.02 (−0.05,0.08)	−0.01 (−0.23,0.22)	Sum of subscapular and triceps skinfold thickness −0.01 (−0.28,0.25)
	876 children with follow-up at median 7 yr			0.04 (−0.02,0.11)	0.31 (−0.19,0.82)	Sum of subscapular and triceps skinfold thickness 0.62 (0.01,1.22) DXA total fat mass index 0.08 (−0.02,0.18) DXA trunk fat mass index 0.04 (−0.03,0.11)
	537 children with follow-up at 11–16 yr		Longitudinal change for twofold increase	BMI z-score −0.01 ($p > 0.05$)	NR	Total fat mass index 0.1 ($p > 0.05$) Truncal fat mass index 0.05 ($p > 0.05$)

Reference	Population	Median exposure (IQR) (µg/mL)	Effect estimate	BMI	Waist circumference	Body fat
Papadopoulou et al. (2021)	Six birth cohorts, Europe, 1,301 children at 6–11 yr	prenatal	β (95% CI) for Quartiles vs. Q1	NR	Q2: −0.03 (−0.22, 0.16) Q3: 0.11 (−0.12, 0.35) Q4: 0.11 (−0.18, 0.40)	NR
		children 0.5 (0.3–0.7)			Q2: 0.00 (−0.18, 0.18) Q3: −0.12 (−0.33, 0.08) Q4: −0.09 (−0.32, 0.15)	
Manzano-Salgado et al. (2017b)	Cohort (2003–2008), Spain; 1,230 children with follow-up at 4 yr	0.7 (GM) (0.5–0.9)	β (95% CI) for doubling exposure	0.05 (−0.03,0.13)	0.02 (−0.07,0.10)	NR
	1,086 children with follow-up at 7 yr			0.06 (−0.04,0.16)	0.02 (−0.07,0.10)	NR
Karlsen et al. (2017)	Cohort (2007–2009), Faroe Islands; 444 children with follow-up at 18 mo	0.7 (0.5–0.9)	β (95% CI) for log- unit increase; T2 and T3 vs. T1	0.01 (−0.19,0.21) T2: −0.13 (−0.33,0.06) T3: 0.01 (−0.19,0.20)	NR	NR
	371 children with follow-up at 5 yr			0.0 (−0.21,0.20) T2: −0.15 (−0.35,0.05) T3: −0.06 (−0.26,0.14)	NR	NR
Bloom et al. (2022)	ECHO cohort (2017–2019), U.S. 803 children at 4–8 yr	0.8 (0.6–1.2)	β (95% CI) for log-unit increase	BMI z-score Without obesity −0.02 (−0.12, 0.07) With obesity −0.15 (−0.36, 0.05)	Without obesity 0.06 (−0.02, 0.14) With obesity −0.04 (−0.26, 0.18)	Fat mass Without obesity 0.20 (−0.08, 0.49) With obesity **−1.19 (−2.19, −0.18)*** Percent body fat Without obesity 0.004 (−0.004, 0.01) With obesity **−0.02 (−0.04, −0.004)***

Reference	Population	Median exposure (IQR) (µg/mL)	Effect estimate	BMI	Waist circumference	Body fat
Braun et al. (2016) Liu et al. (2020a)	Cohort (2003–2006), U.S.; 204 children with follow-up at 8 yr	0.9 (0.7–1.2)	Adjusted difference (95% CI) T2 and T3 vs. T1	T2: −0.12 (−0.43,0.19) T3: −0.05 (−0.36,0.26)	T2: −0.2 (−2.8,2.5) T3: 1.1 (−1.6,3.8)	Body fat percent T2: −0.4 (−2.3,1.5) T3: 0.2 (−1.7,2.2)
	212 children with follow-up at 12 yr		β (95% CI) for IQR increase	BMI z-score Gestation exposure −0.08 (−0.36, 0.20) 3 yr exposure −0.11 (−0.31, 0.09) 8 yr exposure 0.05 (−0.17, 0.27)	NR	Fat mass index Gestation exposure 0.03 (−0.19, 0.25) 3 yr exposure −0.09 (−0.22, 0.03) 8 yr exposure 0.00 (−0.17, 0.17)

T = tertile, GM = geometric mean, DXA = dual-energy X-ray absorptiometry, NR = not reported.
*p-value or p-trend ≤ 0.05

Table 3-45. Associations between maternal exposure to PFNA and overweight in children in *medium* confidence epidemiology studies

Reference	Population	Median exposure (IQR) (µg/mL)	Effect estimate	Overweight
Mora et al. (2018)	Cohort (1999–2002), U.S.; 1,006 children with follow-up at median 3 yr	0.6 (0.5–0.9)	RR (95% CI) for IQR increase	Overweight 1.12 (0.96, 1.30) Obese 0.97 (0.75,1.27)
	876 children with follow-up at median 7 yr			Overweight 1.06 (0.85,1.32) Obese 1.21 (0.99,1.47)
Manzano-Salgado et al. (2017b)	Cohort (2003–2008), Spain; 1,230 children with follow-up at 4 yr	0.7 (GM) (0.5–0.9)	RR (95% CI) for doubling exposure	1.08 (0.96,1.21)
	1,086 children with follow-up at 7 yr			1.06 (0.93,1.20)
Karlsen et al. (2017)	Cohort (2007–2009), Faroe Islands; 444 children with follow-up at 18 mo	0.7 (0.5–0.9)	OR (95% CI) for log-unit increase; T2	1.02 (0.79,1.31) T2: 0.80 (0.62,1.02) T3: 1.02 (0.81,1.28)

Reference	Population	Median exposure (IQR) (µg/mL)	Effect estimate	Overweight
	371 children with follow-up at 5 yr		and T3 vs. T1	1.15 (0.6,1.98) T2: 0.61 (0.35,1.06) T3: 1.04 (0.64,1.66)
Braun et al. (2016) Liu et al. (2020a)	Cohort (2003–2006), U.S.; 204 children with follow-up at 8 yr	0.9 (0.7–1.2)	RR (95% CI) for tertiles vs. T1	T2: 1.18 (0.63,2.22) T3: 1.26 (0.64,2.48)
	212 children with follow-up at 12 yr		RR (95% CI) for IQR increase	By BMI 1.14 (0.74, 1.75) By fat mass index 1.19 (0.67, 2.13)

Animal Studies

The evidence base for PFNA cardiometabolic effects in animals was limited to short-term testing that included one *high* confidence 28-day study in adult male and female rats (NTP, 2018) and three *medium* confidence 14-day studies in adult male rats and mice (Wang et al., 2015a; Fang et al., 2012a; Fang et al., 2012c), as well as a *medium* confidence gestational exposure in SD rats (Rogers et al., 2014) (see Table 3-46). Endpoints evaluated in these studies were heart weight, heart histopathology, and blood pressure, the latter in male and female rat offspring only. In adult rodents, serum lipids and glucose were also measured, and are considered markers indicative of potential cardiotoxicity (Zhao et al., 2020; Daugherty et al., 2017).

Due to mortality reported at the two high doses tested in the NTP 28-day study, this synthesis considers PFNA treatments at 2.5 mg/kg-day in males and 6.25 mg/kg-day in females to be the highest dose with reportable results for all cardiometabolic endpoints except the histopathology where tissue analyses were performed at the time of death. Another 7-day oral exposure study with PFNA in diabetically induced rats was judged to be *uninformative* and is not discussed further due to a lack of reporting on blood glucose levels across treatment groups that could introduce variability that influences results, as well as a lack of appropriate negative controls (Fang et al., 2015).

Table 3-46. Overall evaluation results of toxicity studies examining effects of PFNA exposures on cardiometabolic endpoints in rodents[a]

Reference	Species, strain (sex)	Exposure design	Exposure route and dose range	Serum lipids	Serum glucose	Heart weight	Histopathology	Blood pressure
Fang et al. (2012a)	Rat, Sprague-Dawley (adult male)	Short-term 14 d	Gavage 0, 0.2, 1, 5 mg/kg-d	+	+	NM	NM	NM
Fang et al. (2012c)	Rat, Sprague-Dawley (adult male)	Short-term 14 d	Gavage 0, 0.2, 1, 5 mg/kg-d	+	NM	NM	NM	NM
Fang et al. (2015)	Rat, Sprague-Dawley (adult male, diabetically induced)	Short-term 7 d	Gavage 0, 0.2, 1, 5 mg/kg-d	--	NM	NM	NM	NM
NTP (2018)	Rat, Sprague-Dawley Harlan (adult male/female)	Short-term 28 d	Gavage; 0, 0.625, 1.25, 2.5, 5, 10 mg/kg-d (m); 0, 1.56, 3.12, 6.25, 12.5, 25 mg/kg-d (f)	++	++	-	-	NM
Rogers et al. (2014)	Rat, Crl:CD, Sprague-Dawley, Charles River (male/female)	Developmental GD 1–20	Gavage, maternal 0, 5 mg/kg-d	NM	NM	NM	NM	+
Wang et al. (2015a)	Mouse, BALB/c (adult male)	Short-term 14 d	Gavage 0, 0.2, 1, 5 mg/kg-d	+	NM	NM	NM	NM

Dark green (++) = *high* confidence; light green (+) = *medium* confidence; yellow (-) = *low* confidence; red (--) = *uninformative;* NM = not measured. Study evaluation details for all outcomes are available in HAWC.

Serum lipids and glucose

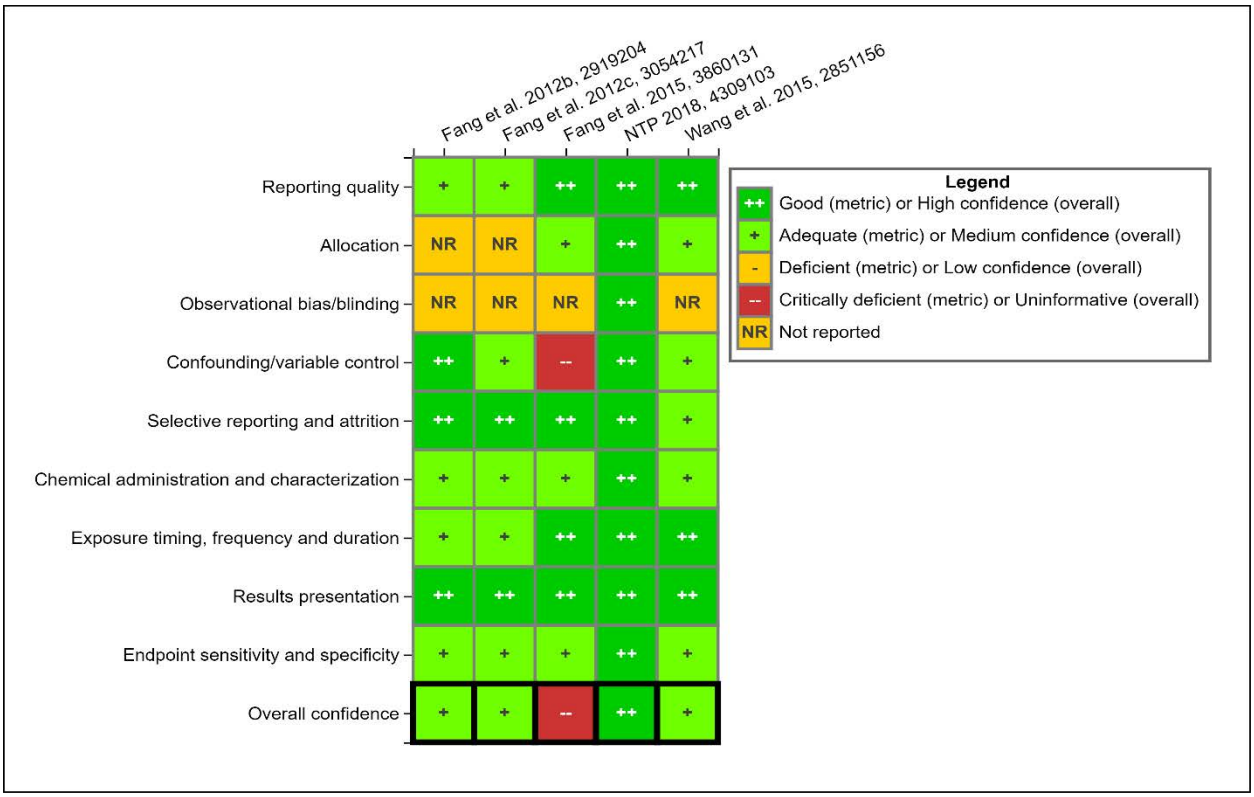


Figure 3-100. Study evaluation heatmap of animal studies that examined PFNA effects on serum lipids and glucose. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Cardiometabolic-serum-lipids-and-glucose/.

Four *high* and *medium* confidence studies evaluated effects of short-term PFNA exposures on serum levels of lipids (triglyceride and cholesterol) and glucose (NTP, 2018; Wang et al., 2015a; Fang et al., 2012a; Fang et al., 2012c) (see Figure 3-100).

A generally consistent decrease in serum triglyceride and cholesterol was observed in male rats and mice across most of the short-term experiments (see Figure 3-101), which is consistent with observations for several other PFAS. The biological significance of these decreases is currently unknown, and it was unclear from the study reporting if animals were fasted in three of the four *high*/*medium* confidence studies which could have introduced variability in results if animals were not fasted (fasting generally increases serum lipids). EPA test guidelines recommend overnight fasting in rodents for clinical chemistry measurements to reduce variability and minimize the potential masking of more subtle effects (EPA, 2000, TG 870.3050; Repeat Dose 28-day Oral Toxicity Study in Rodents). While there is a preference for fasting rodents, the clinical chemistry results may have increased variability but did not raise concerns about the overall direction of the findings.

Additionally, the NTP 28-day study, effects in male rodents at dose levels of 2.5 mg/kg-day were considered inconclusive due to severe body weight loss (44%) (NTP, 2018; Wang et al., 2015a; Fang et al., 2012c), as dietary restriction studies show serum lipids to decline in rats with weight losses ranging from ~20% to 40% (Hubert et al., 2000). However, non-dose-dependent but statistically significant reductions in serum triglycerides were reported in male rats by 51% and 32% at 0.625 and 1.25 mg/kg-day PFNA, where there was no or modest weight loss. The NTP 28-day study also reported statistically significant reductions in serum total cholesterol at 0.625 mg/kg-day (27%) and 1.25 mg/kg-day (23%). The 14-day experiments in rats and mice showed significant reductions in serum triglycerides at 5 mg/kg-day PFNA where body weight loss also may have influenced the results (body weight was reduced by >20% in adult male rats and mice at this dose level) (Wang et al., 2015a; Fang et al., 2012c). Serum total cholesterol was also significantly reduced in the 14-day study in adult male SD rats with reductions of 22% and 32% at 0.2 mg/kg-day and 1 mg/kg-day, respectively (Fang et al., 2012c). A similar range of statistically significant reductions in serum HDL cholesterol was reported in another 14-day study by the same coauthors at 0.2 mg/kg-day (21%) and 1 mg/kg-day (44%), with lower HDL/LDL ratios at 1 mg/kg-day PFNA (Fang et al., 2012a). Wang et al. (2015a) also reported reduced serum concentrations of total cholesterol (31%) in mice exposed for 14 days at higher dose levels (5 mg/kg-day PFNA) where there was significant body weight loss. No significant changes were observed at lower doses (0.2 and 1 mg/kg-day).

The evidence base for PFNA effects on serum glucose was small and inconsistent, with both decreases and increases reported in adult male SD rats (see Figure 3-102). The NTP 28-day study reported a statistically significant decrease in serum glucose in adult male SD rats exposed to PFNA at 2.5 mg/kg-day (likely attributable to reduced body weight and decreases in food intake at this dose level). In contrast, a study in adult male SD rats by Fang et al. (2012a) reported a modest but significant increase in serum glucose at 1 and 5 mg/kg-day PFNA after a 14-day exposure.

PFNA had no significant effects on levels of serum triglycerides, cholesterol, or glucose of adult female rats in the 28-day study (see Figures 3-101 and 3-102) (NTP, 2018). Overall, the effects of declines in serum lipids in male rodents is unclear but may be related to body weight loss and potentially hepatoxicity (see Section 3.2.3), and there is some uncertainty regarding the inconsistencies in the directionality of changes in glucose and cholesterol.

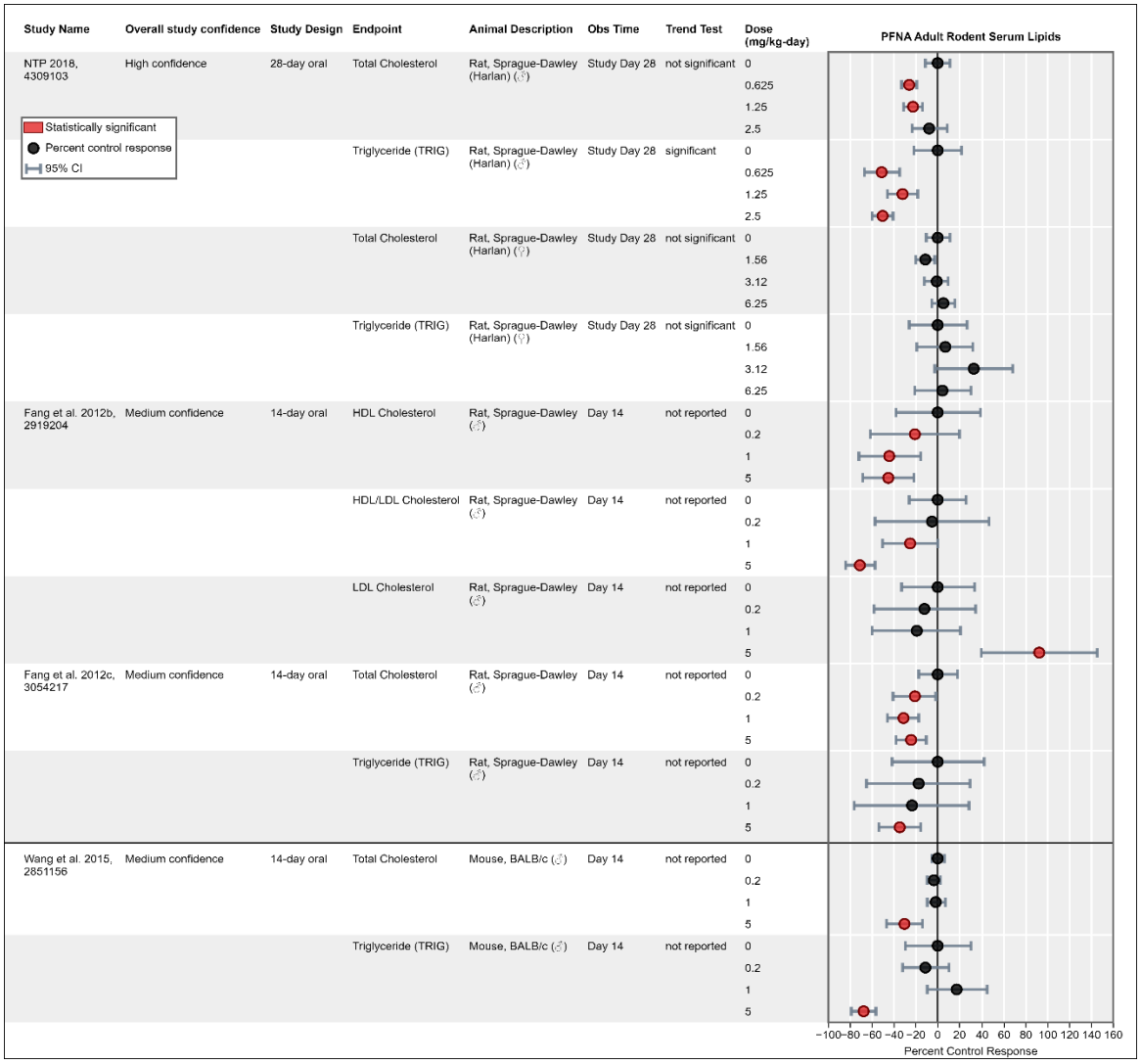


Figure 3-101. Total serum triglyceride and cholesterol in *high* and *medium* confidence studies in male rodents (top) and female rats (bottom). See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/pfna-serum-lipids/.

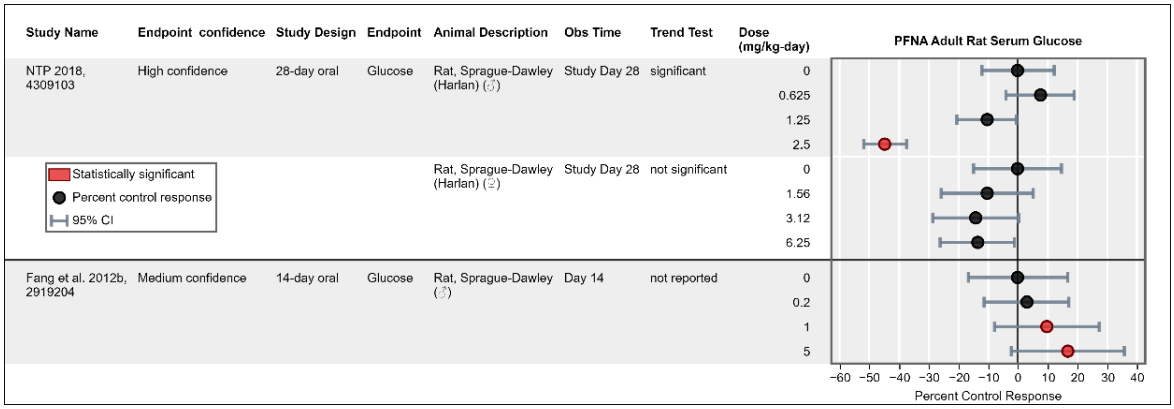


Figure 3-102. Total serum glucose in *high* and *medium* confidence studies in adult male rats (top) and adult female rats (bottom). See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/PFNA-serum-glucose/.

Heart weight and histopathology

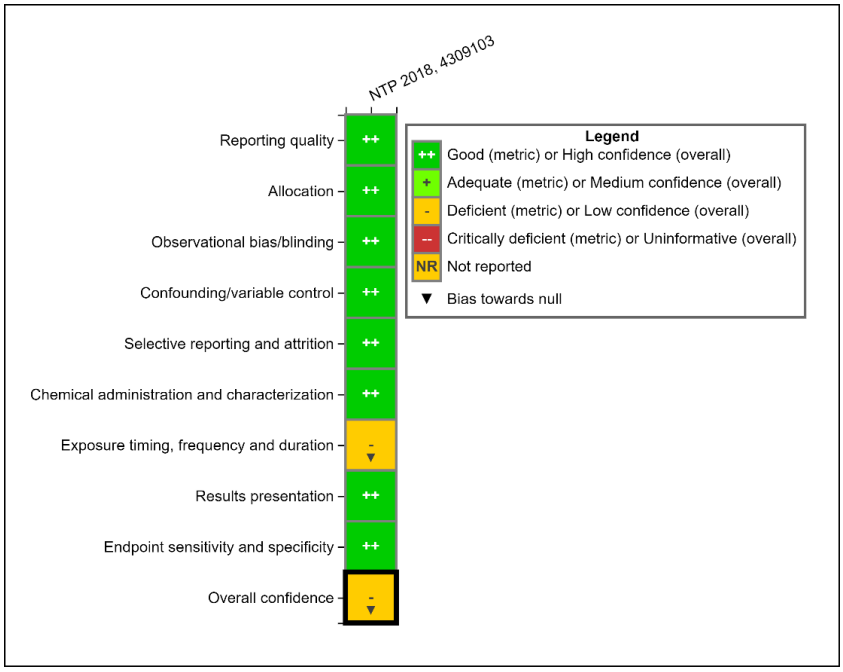


Figure 3-103. Study evaluation heatmap of animal studies that examined PFNA effects on heart weight and histopathology. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Cardiometabolic-heart-weight-and-histopathology/.

The only study that evaluated potential PFNA effects on heart weight and cardiac tissue histopathology is the 28-day toxicity study in rats (NTP, 2018) (see Figure 3-103). As noted earlier, this study was judged to be overall *low* confidence for these endpoints because the exposure duration was insufficient to evaluate the potential for direct cardiac abnormalities; this was the

only limitation identified, resulting in a predicted bias toward the null. Nonetheless, dose-dependent reductions in absolute, but not relative, heart weights were observed in both adult male and female rats (see Figure 3-104). Although some studies show heart weight to be non-proportional to body weight (Bailey et al., 2004), other studies reported heart and body weight to be strongly correlated, with better correlation in males (Nirogi et al., 2014). Thus, both absolute and relative heart weights can be inferred as potentially biologically relevant metrics. Absolute heart weight was significantly decreased in male rats by 14% and 43% at 1.25 mg/kg-day and 2.5 mg/kg-day PFNA, respectively (both of which appear to be directly influenced by body weight reductions, which were 17% and 44%, respectively). In female rats, there was also a decrease in absolute heart weight after the short-term exposure, which was statistically significant at 3.12 mg/kg-day (8%) and 6.25 mg/kg-day (11%), noting that body weight in females was decreased 6% at 3.12 mg/kg-day and 10% at 6.25 mg/kg-day. The biological significance of these changes, which closely mirror the observed body weight loss, is notably uncertain. No significant differences were reported in relative heart weights or histopathological abnormalities in the heart or blood vessels of either male or female rats, however 28 days is not expected to be a sufficient exposure time to develop lesions.

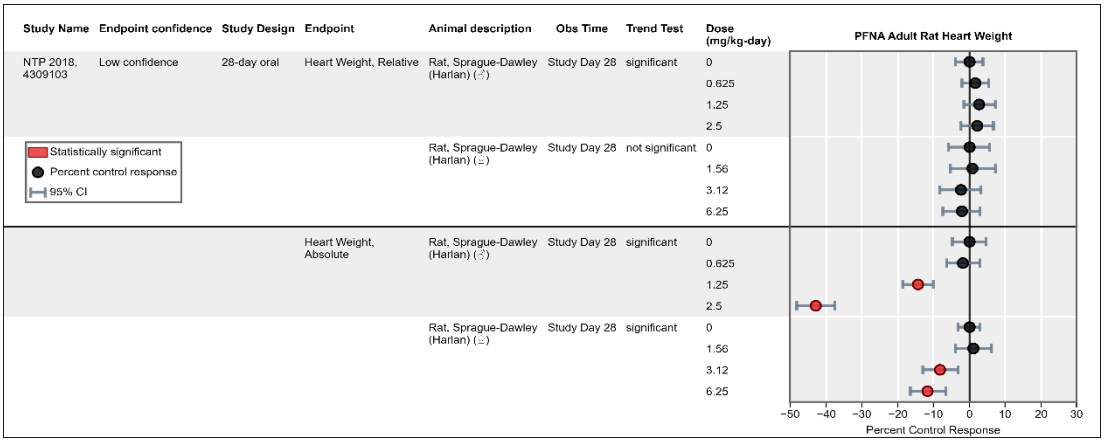


Figure 3-104. PFNA effects on relative heart weight (top) and absolute heart weight (bottom) of adult male and female rats in a 28-day study. See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/PFNA-Heart-Weight/.

Blood pressure

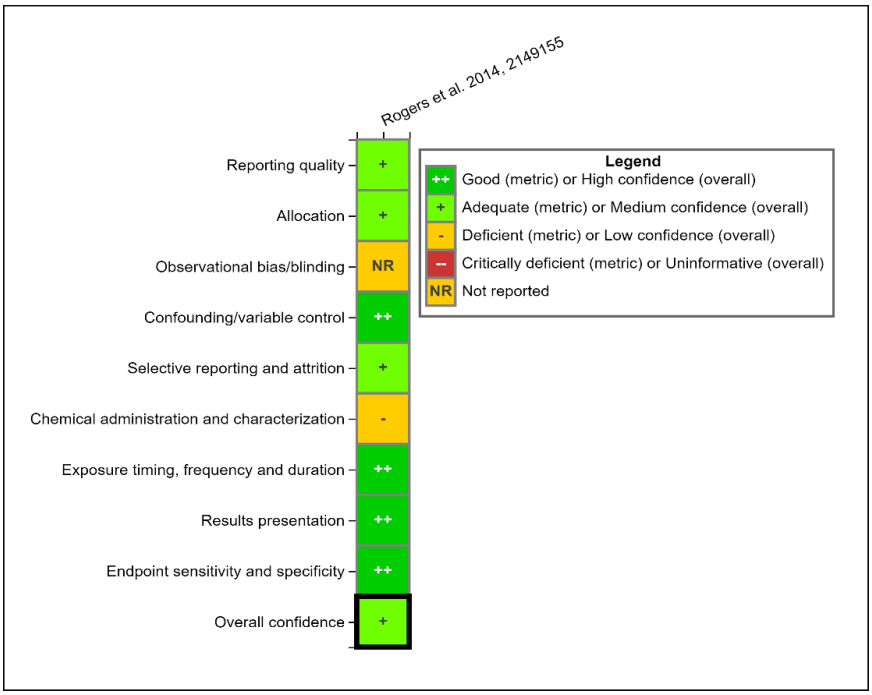


Figure 3-105. Study evaluation heatmap of animal studies that examined PFNA effects on gestationally exposed offspring blood pressure). See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Cardiometabolic-blood-pressure/.

One *medium* confidence developmental toxicity study by Rogers et al. (2014) examined effects of PFNA gestational exposures on offspring blood pressure, as well as other developmental endpoints (see Figure 3-105 and Section 3.2.2, "Animal Studies"). In this study, statistically significantly elevated systolic blood pressure was reported in 10-week-old male and female offspring exposed to 5 mg/kg-day PFNA from GD 1 to 20, although the biological significance of the effect magnitude is unclear. Specifically, a 9% increase in systolic pressure was observed in females and a 7% increase was seen in males; these changes were not statistically different from controls in either sex by 26 or 56 weeks of age. The elevated blood pressure in 10-week males was reported to be correlated with significant reductions in nephron counts at PND 22 (see 3.2.10, "Organ weight and histopathology"). While the exposure period encompassed a critical window of cardiovascular development, it was not possible to interpret the relevance of the exposure period to latent cardiovascular effects. The single-dose design also prevented evaluation of dose gradients. Additionally, reduced pregnancy weight gain was observed in dams from GD 4 to 19, as well as reduced birth weights. Maternal feed restriction studies in rats show dams may produce smaller offspring with elevated blood pressure and lower nephron counts (Ellis-Hutchings et al., 2010;

Abdel-Hakeem et al., 2008; Woods et al., 2004). Therefore, maternal stress may have played a role in the elevated blood pressure in offspring. Overall, this finding is considered inconclusive.

Mechanistic and Supplemental Information

There was limited evidence to elucidate mechanisms potentially involved in mediating PFNA effects on cardiometabolic outcomes. A study in a rat hepatoma cell line showed PFNA exposure significant upregulation in the lipoprotein metabolism genes *ApoA4* and *Mito-3-Keto-β* and a twofold increase in *Mito-3-Keto-α* (100 μM) after 72 hours of exposure (Naile et al., 2012). There is also some evidence in humans and animals showing PFNA interference with hepatic lipid metabolism and glucose metabolism pathways that may perturb serum lipid and glucose homeostasis (see Sections 3.2.3 and 3.2.10). Experiments in larval zebrafish showed PFNA significantly increased the expression of cardiotoxic genes (Gong et al., 2022). Specifically, 20 μM at 120 hpf upregulated *amhc*, which is associated with recovery of cardiac dysfunction, *nkx2.5* which is a cardiac transcription factor, *end1* which regulates vasoconstriction, and *tgfb2* which plays a role in cardiomyocyte proliferation (Gong et al., 2022). PFNA was also associated with cardiac malformations in these experiments.

Considerations for Interpreting the Human Relevance of the Animal Cardiometabolic Evidence

Although the results from the available animal studies on PFNA and potential cardiometabolic effects are overall inconclusive, this section highlights what is known regarding cardiometabolic differences between humans and laboratory animal models commonly used in toxicological studies to inform potential future studies. The pathophysiology of cardiovascular disease in humans is a complex process driven by multiple risk factors (e.g., diabetes, hyperlipidemia, hypertension, and aging), which lead to metabolic and pro-inflammatory alterations. Unfortunately, there is no single animal model that completely recapitulates all the features of human disease (Oppi et al., 2019). Furthermore, there are significant differences between rodent and human cardiovascular systems. Murine plasma cholesterol is approximately threefold lower, the major lipoprotein in mice is HDL, not LDL (Getz and Reardon, 2012), and differences in bile acid composition contribute to lower intestinal absorption of cholesterol and higher cholesterol excretion (Oppi et al., 2019). These differences contribute to significantly lower cholesterol levels in mice when compared with humans, and having lower cholesterol levels in turn confers protection from cardiovascular injuries such as atherosclerosis (Oppi et al., 2019).

Future studies using experimental models and designs that better emulate human disease would allow for a more informed characterization of potential cardiometabolic responses to PFNA. Future studies should focus on the use of genetically manipulated or experimentally induced rodent models that can better emulate human metabolic and pathological conditions (Kodavanti et al., 2015). For example, studies aimed at evaluating vascular injuries such as atherosclerosis should focus on the use of animal models that can generate non-HDL-based hypercholesterolemia such as LDL Receptor or apolipoprotein E (ApoE) null mice (Getz and Reardon, 2012) and expose animals

for sufficient time to develop of arterial injuries (Daugherty et al., 2017). Furthermore, future studies focused on potential effects to the cardiovascular system should include analysis of physiological and biochemical parameters (e.g., heart rate, blood gases, oxygen consumption, and additional studies on blood pressure,), which are considered indicative of adverse responses in the cardiovascular system (Gad, 2015).

Evidence Integration

The human epidemiological evidence of cardiometabolic toxicity is considered *slight*. The majority of studies on serum lipids in general population adults and adolescents, including those of *medium* confidence, indicate a positive association between PFNA exposure and cholesterol levels, however findings were less consistent for related measures of LDL cholesterol and triglycerides and among pregnant women and children. There is further uncertainty regarding the biological significance of the small positive associations observed. There is some coherent evidence of an association with other cardiovascular risk factors such as blood pressure, atherosclerosis, metabolic syndrome, and adiposity, but there is uncertainty in these findings due to unexplained inconsistencies within and across studies and concerns for imprecision. Additionally, while one *medium* confidence study reported higher odds of total cardiovascular disease, coronary heart disease, heart attack, and stroke with higher PFNA exposure, two other *medium* confidence studies and two low confidence studies showed no increase in coronary heart disease. There was little evidence of an association between PFNA exposure and diabetes and insulin resistance.

The animal evidence of cardiometabolic toxicity is considered *indeterminate* based on the narrow scope and unclear biological significance of the findings observed in a small number of studies. Although some perturbations in serum lipids and glucose were reported, the changes in serum lipid levels in animals do not follow the same pattern observed in human studies (generally, decreases in serum lipids, as compared with increases in the epidemiological studies), which may be due to biological differences between species and the short duration of the study, and changes in serum glucose levels were inconsistent. These and other endpoints related to cardiovascular effects were sparsely studied using largely insensitive study designs to allow for full consideration of the potential for effects.

Overall, the currently available ***evidence suggests*** but is not sufficient to infer that PFNA may cause cardiometabolic impairments in humans given sufficient exposure conditions (see Table 3-47).[20] This judgment is based primarily on studies in humans that assessed median exposure levels of 0.6–1.5 ng/mL, and showed generally increased serum lipids, and some potentially supportive but mixed results for other increased risk factors for cardiovascular disease; however, important uncertainties remain.

[20]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).

Table 3-47. Evidence profile table for cardiometabolic effects

Summary of human and animal evidence[a]					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.9. Cardiometabolic Effects, Human Studies)					⊕⊙⊙ ***Evidence suggests but is not sufficient to infer*** *Primary basis:* Human studies report generally positive associations with serum lipids and some limited but coherent evidence of other cardiovascular risk factors *Human relevance:* Judgment based on epidemiological studies *Cross-stream coherence:* N/A, animal evidence *indeterminate* *Susceptible populations and lifestages:*
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Serum lipids</u> Seventeen *medium* confidence, 13 *low* confidence studies	• 5 of 5 *medium* confidence studies in general population adults (3 statistically significant), 2 of 4 studies in pregnant women, 3 of 3 studies in adolescents, and 2 of 5 studies in children/cord blood reported positive associations between PFNA exposure and total cholesterol. • Results in children and infants were inconsistent	• Positive association observed in most studies of general population adults • *Medium* confidence studies reporting an effect	• Unexplained inconsistency across studies and interrelated lipid measures	⊕⊙⊙ *Slight* Most studies report positive associations between PFNA exposure and serum lipids; Limited evidence of associations with other CV risk factors provide some coherence, although there was no increased risk of CV disease in most studies; Evidence in children is inconsistent	
<u>Other cardiovascular risk factors</u> Eighteen *medium* confidence, 6 *low* confidence studies	• Positive associations were observed with hypertension (3/5 studies) and continuous blood pressure (5/7 studies) in general population of adults. Two of 4 studies reported positive associations with atherosclerosis	• Positive associations observed in the majority of studies of blood pressure in nonpregnant adults	• Unexplained inconsistency across studies • Imprecision for blood pressure		
<u>Cardiovascular disease</u> Three *medium* confidence, 2 *low* confidence studies	• One study reported higher odds of cardiovascular disease including coronary heart disease, heart attack, and stroke. The other 5 studies reported no increase	• No factors noted	• Unexplained inconsistency across studies		

Summary of human and animal evidence[a]					Evidence integration summary judgment
Evidence from in vivo animal studies (**see Section 3.2.9. Cardiometabolic Effects, Animal Studies**)					The evidence base is overall insufficient to determine potential susceptibility, noting that the evidence base for effects in infants and children is small and inconsistent
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Heart Weight and Histopathology</u> One *low* confidence study in adult rats: • 28 d	• Decrease in absolute heart weight at ≥1.25 mg/kg-d in males and ≥3.12 mg/kg-d in females • No change in histopathology or relative heart weights	• *Dose-responsive* decreases in absolute heart weight • *Magnitude of effect*, up to 43% in males, although this corresponded with a 44% decrease in body weight	• Concern for potential confounding of absolute heart weight by body weight decreases, making biological significance questionable	⊙⊙⊙ *Indeterminate* Although some consistent changes were observed for these sparsely studied endpoints, there were concerns regarding confounding and biological significance	
<u>Serum Lipids</u> Four *high/medium* confidence studies in adult rats and mice: • 28 d • 14 d (×3)	• Significant reductions in serum cholesterol and triglycerides in male rats and mice exposed short-term	• *Consistent* reductions in serum cholesterol and triglycerides in 2 species (males only)	• Unclear biological significance of decreases in serum lipids		
<u>Blood Pressure</u> One *medium* confidence developmental study in rats: • GD 1–20	• Significant increases in systolic blood pressure in gestationally exposed male and female rats (5 mg/kg-d) at 10 wk of age, but not 26 or 56 wk	• No factors noted	• Unclear biological significance of small transient changes in blood pressure • Concern for potential confounding		

[a]No mechanistic evidence directly applicable to human or animal cardiometabolic outcomes.

3.2.10. Urinary System Effects

This section describes and integrates the evidence informative to assessing the potential for effects on the urinary system following exposure to PFNA. Both the epidemiological and animal toxicological evidence bases are focused on renal effects. There is some overlap in this section with the animal evidence synthesis of cardiometabolic and developmental effects in rodent offspring (see Sections 3.2.9 and 3.2.2, respectively).

Human Studies

Fifteen studies (23 publications) investigated the relationship between PFNA exposure and renal function, specifically measures of GFR and uric acid (UA); impaired renal function typically manifests as decreased GFR and increased UA, although the latter is a more indirect and less specific measure of kidney function (and is thus given less weight). The study evaluations are summarized in Figure 3-106. Two studies (Zhang et al., 2019; Seo et al., 2018) were considered *uninformative* due to critical deficiencies in confounding. The remaining studies were primarily cross-sectional analyses and were classified as *low* confidence primarily due to concerns for reverse causality without other major methodological limitations. In essence, as described in Watkins et al. (2013), decreased renal function could plausibly lead to higher levels of PFAS, including PFNA, in the blood. This hypothesis is supported by data presented by Watkins et al. (2013), although there is some uncertainty in the conclusions due to the use of modeled exposure data as a negative control and the potential for a causal effect to occur in addition to reverse causality. The results least likely to be affected by reverse causality were analyses in four studies designed to assess reverse causality (e.g., stratification by glomerular filtration stage or modeling with PFHxS as the dependent variable: (Lin et al., 2021; Moon, 2021; Jain, 2019; Zeng et al., 2019c; Conway et al., 2018)) and two studies with prospective designs (Lin et al., 2021; Blake et al., 2018). Of these, Lin et al. (2021) had the benefit of both prospective data analysis and additional analyses to assess reverse causation and was thus rated as *medium* confidence. Across studies, because of the potential for reverse causation, there is considerable uncertainty in interpreting the results of the available studies. However, the informative studies were otherwise well conducted and had adequate or good ratings for domains other than exposure measurement.

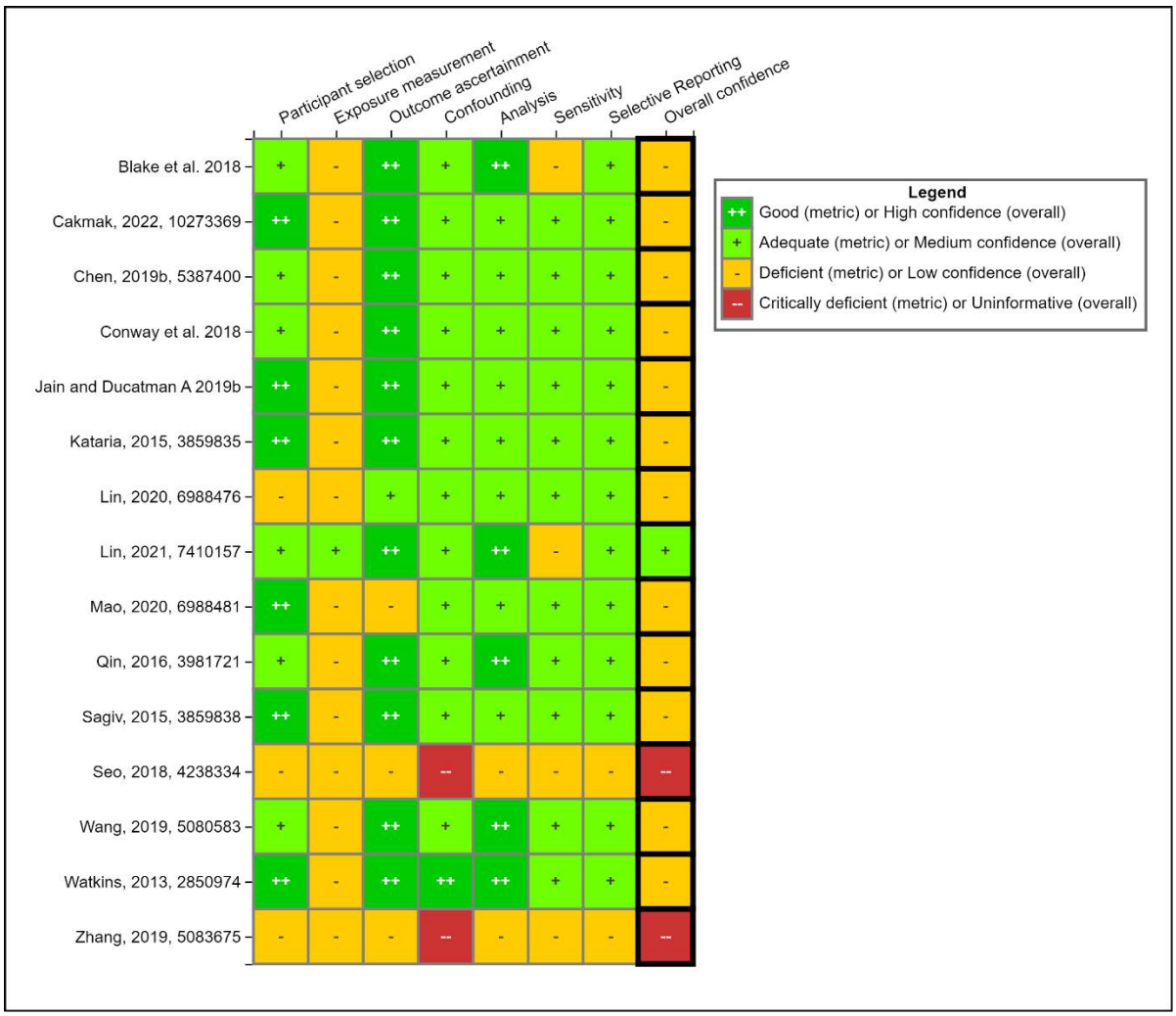


Figure 3-106. Summary of study evaluation for epidemiology studies of PFNA exposure and urinary effects.[a] See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/pfna-epidemiology-urinary-study-evaluations/.

[a]Multiple publications of the same study: Jain and Ducatman (2019b) also includes Jain and Ducatman (2019a), Jain (2019), Jain (2013), Jain (2021b), Jain (2020a), Jain (2021a), Moon (2021), and Scinicariello et al. (2020b). Sagiv et al. (2015) also includes evaluation for Sagiv et al. (2018). Results from each publication were reviewed and extracted if different from the primary publication.

Across the 13 included studies, there is an indication of impaired renal function (i.e., lower GFR, higher UA) in 8 (Lin et al., 2021; Lin et al., 2020c; Chen et al., 2019; Jain and Ducatman, 2019a; Blake et al., 2018; Qin et al., 2016; Sagiv et al., 2015; Watkins et al., 2013), but there are some inconsistencies (see Table 3-48). In adults, Blake et al. (2018), Sagiv et al. (2015), Scinicariello et al. (2020b), Lin et al. (2021) reported lower GFR with higher exposure, although the association in Lin et al. (2021), the single *medium* confidence study, was observed only in participants with hypertension (the direction of association was opposite for participants without hypertension. Jain

and Ducatman (2019a) and a related publication Scinicariello et al. (2020b) reported higher UA, with an exposure-response gradient observed across quartiles. However, in another analysis of NHANES data in adults, Jain and Ducatman (2019b) reported an inverted U-shape of exposure by GFR (higher exposure levels in the second and third GF stages than the first; i.e., normal function to mild renal damage) and fourth (moderate to severe renal damage). This was also observed in analyses stratified by sex and without a known biological explanation for this trend. Conway et al. (2018) reported a similar trend, with higher odds of GF stages 2 and 3 than 1 and 4/5 (which was the reference group) with higher exposure. The remaining studies of GFR, uric acid, and creatinine in adults did not indicate an association between PFNA exposure and renal function. In children and adolescents, Watkins et al. (2013) reported lower GFR with higher exposure and Qin et al. (2016) reported higher odds of high UA, while Kataria et al. (2015) also reported an inverted U-shape with GFR.

Considering dichotomous outcomes, Wang et al. (2019) reported lower odds of chronic kidney disease were associated with higher exposure (not statistically significant). A single study of kidney stones reported no association with PFNA exposure (Mao et al., 2020)

While multiple studies reported an association between PFNA exposure and changes consistent with impaired renal function, there is notable unexplained inconsistency in the direction of association. More importantly, because of the potential for reverse causation for this outcome, there is considerable uncertainty in interpreting a causal relationship.

Table 3-48. Associations between PFNA exposure and renal function in epidemiology studies (all *low* confidence)

Reference, study confidence	Study population	Median exposure level (IQR) in ng/mL	Form and units of effect estimate	Effect estimate
Glomerular filtration rate ***Decrease* indicates impaired renal function**				
Watkins et al. (2013), *Low*	Cross-sectional study of 9,660 children in U.S. exposed to high PFOA levels in water	IQR 0.5	Mean change (95% CI) per IQR increase exp	**−0.88 (−1.41,−0.36)***
Blake et al. (2018), *Low*	Prospective cohort of residents near a uranium processing site (1990–2008); U.S.; 210 adults	0.5 (0.4–0.7)	Percent change (95% CI) in eGFR per IQR change	**−1.61 (−3.00, −0.22)***
Lin et al. (2021), Medium	Cohort study within placebo and lifestyle intervention arms of a diabetes prevention randomized controlled	0.6 (0.4–0.9)	β (95 CI) for doubling of baseline exposure	0.18 (−0.90, 1.27) With hypertension −1.85 (−4.20, 0.49) Without hypertension 0.90 (−0.33, 2.14)

Reference, study confidence	Study population	Median exposure level (IQR) in ng/mL	Form and units of effect estimate	Effect estimate
	trial of 875 adults in the U.S.			
Sagiv et al. (2015), *Low*	Cross-sectional study of 1,645 pregnant women in U.S.	0.7 (0.5–0.9)	% change PFNA with increased GFR	**−4.2 (−4.8, −3.5)***
			Geometric means (IQR) of PFNA by quartile of GFR	Q1: 0.8 (0.6, 1.1) Q2: 0.7 (0.5, 0.9) Q3: 0.6 (0.5, 0.9) Q4: 0.5 (0.4, 0.8)
Jain and Ducatman (2019b), *Low*	Cross-sectional study (NHANES) (2007–2014); U.S.; 6,836 adults	1.0	Adjusted geometric means of PFNA (95% CI) by glomerular function (GF) stage	All participants GF-1: 0.97 (0.92, 1.02) GF-2: 1.09 (1.02, 1.17) GF-3: 1.12 (0.99, 1.26) GF-3B/4: 0.88 (0.71, 1.08)
Kataria et al. (2015), *Low*	Cross-sectional study of 1,960 adolescents in U.S.	1	β (95 CI) for quartiles vs. Q1	Q2: 1.2 (−4.3,6.7) Q3: 2.8 (−1.7,7.2) Q4: −1.1 (−5.5,3.4)
Conway et al. (2018), *Low*	Cross-sectional study of 53,650 adults in U.S. exposed to high PFOA	1.4 (1.1–1.8)	OR (95% CI) for 1-unit increase	GF-1: 2.64 (2.10, 3.48) GF-2: 3.16 (2.41, 4.15) GF-3A: 3.58 (2.71, 4.73) GF-3B: 4.04 (2.94, 5.54) GF-4/5: 1.0 (ref)
Wang et al. (2019), *Low*	Cross-sectional study (2015–2016); China; 1,612 adults	2.0 (1.1, 3.1)	Mean change (95% CI) in eGFR per ln-unit change	0.36 (−0.46, 1.18)
			OR (95% CI) for chronic kidney disease per ln-unit change	0.86 (0.7, 1.07)

Reference, study confidence	Study population	Median exposure level (IQR) in ng/mL	Form and units of effect estimate	Effect estimate
Uric acid (UA) ***Increase* in this marker might indirectly reflect impaired renal function**				
Qin et al. (2016), *Low*	Cross-sectional study of 225 children in Taiwan	0.8 (0.6–1.1)	β (95 CI) for ln-unit increase	−0.07 (−0.39,0.25) Girls: −0.19 (−0.58,0.20) Boys: 0.10 (−0.45,0.66)
			OR (95% CI) for quartile increase exp and high UA	1.3 (0.8,2.0) Girls: 1.4 (0.7,2.9) Boys: 1.2 (0.7,2.1)
Scinicariello et al. (2020b), Low	Cross-sectional study (NHANES) (2009–2014); U.S.; 4,917 adults	0.9	β (95% CI) in serum uric acid for quartiles vs. Q1	Q2: 0.06 (−0.05, 0.17) **Q3: 0.22 (0.11, 0.32)*** **Q4: 0.29 (0.15, 0.42)***
			OR (95% CI) in hyperuricemia for quartiles vs. Q1	Q2: 1.14 (0.83, 1.57) **Q3: 1.55 (1.20, 2.01)*** **Q4: 1.65 (1.23, 2.22)***
Jain and Ducatman (2019a), *Low*	Cross-sectional study (NHANES) (2007–2014); U.S.; 6,836 adults	1.0	β (*p*-value) for 1-unit increase	In GF-1 participants Women: 0.02 (0.09) Men: 0.03 (<0.01)
Kataria et al. (2015), *Low*	Cross-sectional study of 1,960 adolescents in U.S.	1	β (95% CI) for quartiles vs. Q1	Q2: 0.01 (−0.2,0.2) Q3: −0.04 (−0.2,0.1) Q4: 0.1 (−0.05,0.3)
Chen et al. (2019), *Low*	Cross-sectional study of 122 adults in China	GM 1.3, range 0.5–3.5	β (95% CI) for ln-unit increase	4.76 (−23.16, 32.67)
Creatinine ***Increase* in this marker might indirectly reflect impaired renal function**				
Cakmak et al. (2022), Low	Cross-sectional study (2007–2017); Canada; 6,045 adults	0.6 (GM)	% change per 1 mean increase in PFDA	−0.7 (−2.7, 1.4)
Chronic kidney disease ***Increase* (OR > 1) in this marker reflects impaired renal function**				
Wang et al. (2019), *Low*	Cross-sectional study (2015–2016); China; 1,612 adults	2.0 (1.1, 3.1)	OR (95% CI) for chronic kidney disease per ln-unit change	0.86 (0.7, 1.07)
Kidney stones ***Increase* (OR > 1) in this marker reflects impaired renal function**				

Reference, study confidence	Study population	Median exposure level (IQR) in ng/mL	Form and units of effect estimate	Effect estimate
Mao et al. (2020), Low	Cross-sectional study (NHANES) (2007–2016); U.S.; 8,453 adults	0.9 (0.6–1.5)	OR (95% CI) for kidney stone history for tertiles vs. T1	T2: 0.99 (0.82, 1.20) T3: 0.98 (0.80, 1.20)

*$p < 0.05$.

Animal Studies

The animal evidence base for renal effects included a *high* confidence 28-day exposure study in adult male and female rats (NTP, 2018) and a *medium* confidence developmental toxicity study in rats (Rogers et al., 2014). Both studies were found generally sound except for some deficiencies in methodological reporting and results presentation in the *medium* confidence study. Endpoints evaluated in these studies relevant to renal function included serum markers (there were no urine markers), kidney weights and histopathology in adult rats, and nephron counts in prenatally exposed rat offspring (Rogers et al., 2014). Due to mortality reported at the two high doses tested in the NTP (2018) study, this synthesis considers PFNA treatments at 2.5 mg/kg-day in males and 6.25 mg/kg-day in females to be the highest doses with reliable results for all outcomes except the histopathological results where tissue analyses were performed at the time of early death (see Section 3.2.1).

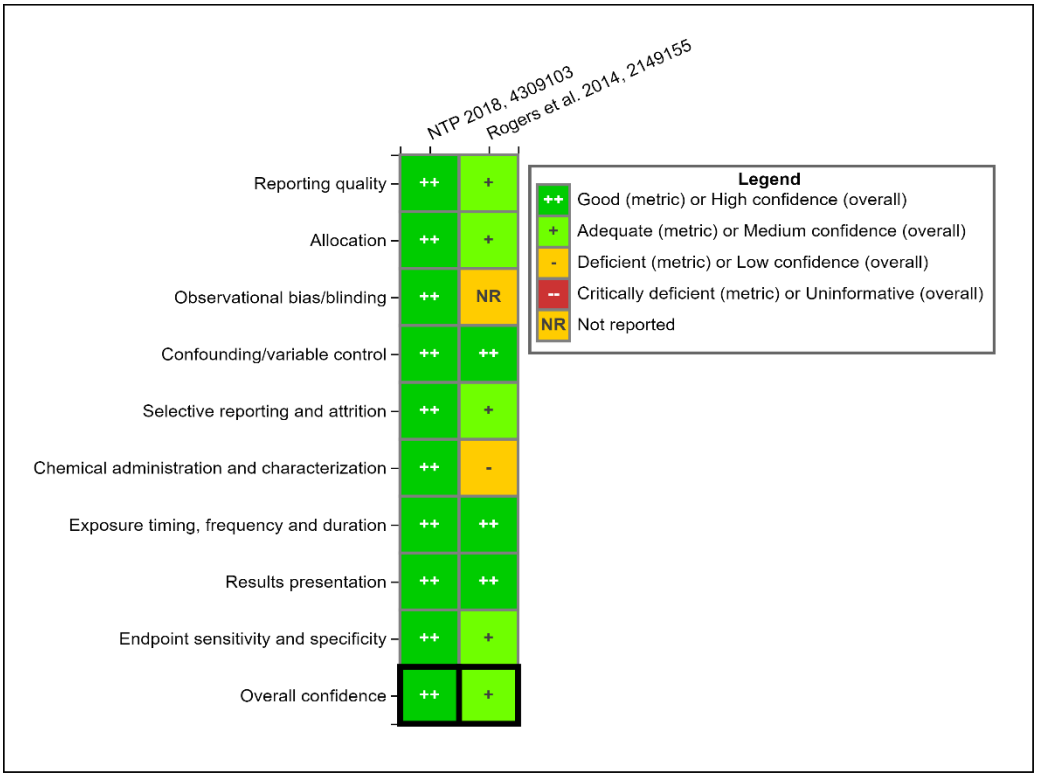


Figure 3-107. Heat map of studies that examined PFNA effects on renal endpoints (i.e., serum markers, organ weights, histopathology). See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/Renal-effects-animals/.

Serum markers

PFNA effects on blood urea nitrogen (BUN), creatinine, and total protein were evaluated in the *high* confidence 28-day study by NTP (2018) in adult male and female rats. Dose-dependent increases in BUN occurred in males (17%, 34%, and 49% at 0.625, 1.25, and 2.5 mg/kg-day, respectively) and in females at 3.12 and 6.25 mg/kg-day (24 and 28%, respectively) (see Figure 3-108). While the dose-responsive increases in BUN suggest potential kidney injury, body weight loss in males at 1.25 mg/kg-day (17%) and 2.5 mg/kg-day (44%) could have contributed to the elevated BUN. Dietary restriction testing shows that reduced food intake and body weight loss may increase BUN by accelerating protein catabolism (Moriyama et al., 2008). However, BUN levels were also elevated in males and females at doses where body weight loss did not occur or were modest, and so a direct effect cannot be ruled out. Dose-dependent declines in creatinine and total protein were also reported in males. Serum creatinine was significantly decreased by 15% at 2.5 mg/kg-day PFNA and total protein was significantly decreased by 9%, 17%, and 34% at 0.625, 1.25, and 2.5 mg/kg-day, respectively. Like the BUN values, body weight loss at 1.25 and 2.5 mg/kg-day complicated interpretation as dietary restriction studies show reduced total protein and creatinine with food intake reductions and body weight loss (Moriyama et al., 2008; Hubert et al., 2000). Accordingly, the change in creatinine at only 2.5 mg/kg-day PFNA is likely a secondary effect driven

by the weight reductions. However, it should be noted that decreases in total protein in males also occurred at doses where body weight loss <4% occurred in males. In females, the significant but slight increases (<7%) in total protein were not considered toxicologically relevant, with no significant changes in creatinine reported (NTP, 2018). This could be due to sex differences in adult rats in renal absorption of PFAS chemicals, with males having lower clearance rates that may increase renal toxicity (Hethey et al., 2019; Kim et al., 2019).

Organ weight and histopathology

Absolute and relative kidney weights and histopathology (kidney and bladder) were examined in the *high* confidence NTP 28-day study in adult male and female rats. Additionally, the *medium* confidence prenatal exposure study in rats (Rogers et al., 2014) examined PFNA effects at a single dose (5 mg/kg-day) on neonate kidney weight and nephron counts at PND 22, as well as latent effects on blood pressure (Rogers et al., 2014). Both absolute and relative kidney weights can be inferred as potentially biologically relevant metrics for this endpoint. Although some testing shows kidney weight to be correlated with body weight favoring relative measures (Nirogi et al., 2014), other studies show kidney and body weight to be nonproportional (Bailey et al., 2004) with increases in absolute kidney weights generally correlated with renal histopathology in rats (Craig et al., 2015).

In the NTP 28-day study, dose-dependent increases in relative kidney weight were observed in both adult male and female rats, whereas effects on absolute kidney weight were not consistent in direction between sexes (see Figure 3-108). In female rats, there was a dose-dependent increase in both absolute and relative kidney weights. Absolute weights increased by 8% at 1.56 mg/kg-day and 11% at both 3.12 and 6.25 mg/kg-day. Relative kidney weights increased by 7%, 17%, and 28%, respectively, at these doses. Consistent with females, relative kidney weights of males showed a dose-dependent increase of 15%, 23%, and 43% at 0.625, 1.25, and 2.5 mg/kg-day, respectively. However, a statistically significant 20% decrease in absolute kidney weight at 2.5 mg/kg-day PFNA was not observed at lower doses and is inconclusive due to the large body weight loss (>40%). Rogers et al. (2014) reported no statistically significant differences in kidney weight between the control group and male SD rats prenatally exposed to PFNA at a maternal dose of 5 mg/kg-day (data not shown in original article).

The kidney and urinary bladder were histopathologically evaluated for nonneoplastic lesions in the 28-day rat study (NTP, 2018). Beyond minimal chronic progressive nephropathy that was reported in all animals (treatments and control), which is a common age-related renal disease in rats (Hard et al., 2012; Melnick et al., 2012), no abnormalities were reported except for a focal cyst in a high dose (25 mg/kg-day) female on day 15. No lesions were reported in the urinary bladder of either sex. In the developmental study, nephron counts were significantly reduced by 16% in male offspring at PND 22 after gestational exposures to 5 mg/kg-day (Rogers et al., 2014). The reductions in nephron counts were correlated with statistically significant elevations in blood pressure in 10-week-olds, although the etiology of the latent response on blood pressure is

unknown. Female nephron counts and blood pressure were not evaluated in this study. Notably, maternal effects were reported with significantly reduced pregnancy weight gain from GD 4 to 19 in dams exposed to PFNA, as well as reduced fetal birth weights. Maternal dietary restriction in rats has been shown to produce smaller offspring with decreased nephron counts and elevated blood pressure (Ellis-Hutchings et al., 2010; Abdel-Hakeem et al., 2008; Woods et al., 2004). Thus, maternal stress may have played a role in the offspring nephron reductions. Interpretation of the findings was further limited by single-dose design preventing analysis by dose gradient.

No mechanistic studies were identified for renal effects in the PFNA evidence base.

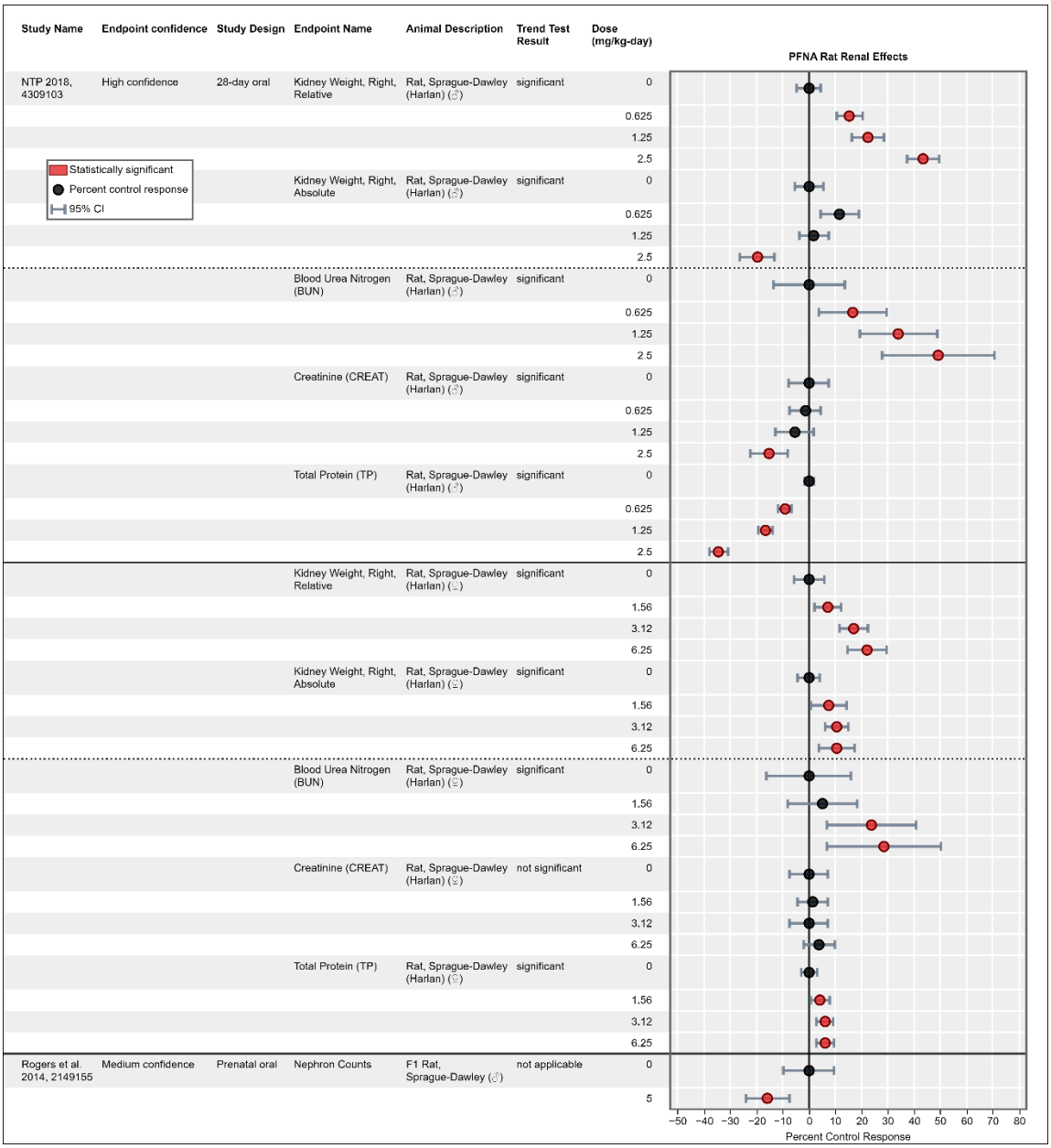


Figure 3-108. PFNA effects on renal serum markers and absolute and relative kidney weights of adult rats in a high confidence 28-day study (adult male and female) (NTP, 2018) and a medium confidence study in prenatally exposed rats at PND 22 (bottom row of figure) (Rogers et al., 2014).[a] See interactive HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500071/PFNA-rodent-kidney/.

[a]Note that narrative results in Rogers et al. (2014) indicated kidney weights were unchanged in prenatally exposed rat neonates at 5 mg/kg-day PFNA (data not shown).

Evidence Integration

The human epidemiological evidence base of urinary system toxicity is considered *indeterminate.* While associations between PFNA exposure and impaired renal functioning (i.e., lower GFR or higher serum UA) were reported in 8 of 13 epidemiological studies, considerable uncertainty remains due to the potential for reverse causation and unexplained inconsistencies in the direction of association that makes interpretation of these findings difficult.

The animal evidence base was judged to have *slight* evidence for renal toxicity but with a large amount of uncertainty. The evidence base includes one *high* confidence 28-day study in adult rats and a *medium* confidence single-dose developmental exposure study in rats (NTP, 2018; Rogers et al., 2014). The 28-day study showed consistent and coherent increases in relative kidney weights and serum markers of potential renal dysfunction (most notably, BUN) in adult female and male rats that were dose-dependent and occurred at PFNA doses that did not cause overt toxicity. Decreases in nephron counts also were reported in gestationally exposed male rats at 5 mg/kg-day. However, this finding did not influence the hazard judgment because considerable uncertainty due to co-occurring effects on pregnancy weight gain and possible associated maternal stress could have influenced this marker.

Taken together, the currently available ***evidence is inadequate*** that PFNA may cause renal injury in humans given sufficient exposure conditions (see Table 3-49). This judgment is based on a short-term animal bioassay showing increased relative kidney weights in adult male and female rats with elevations in a serum marker of potential renal injury. However, the adversity of the animal findings is unclear.

Table 3-49. Evidence profile table for renal effects

Summary of human and animal evidence[a]					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.10. Urinary System Effects, Human Studies)					⊙⊙⊙ ***Evidence is inadequate*** *Primary basis:* A *high* confidence short-term study in adult male and female rats provided some evidence of potential renal effects with unclear adversity and substantial uncertainties *Cross-stream coherence:* Potential coherence between nitrogenous wastes in humans (uric acid) and rats (BUN). *Human relevance, Susceptible populations and lifestages:* No factors noted
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
Impaired Renal Function One *medium* and 9 *low* confidence studies	• Association between PFNA exposure and impaired renal function (i.e., lower GFR or higher serum uric acid) in 8 of 13 studies	• No factors noted	• Almost all studies were *low* confidence • Potential for reverse causality • Unexplained inconsistencies in direction of effects	⊙⊙⊙ *Indeterminate*	
Evidence from in vivo animal studies (see Section 3.2.10. Urinary System Effects, Animal Studies)					
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Serum markers</u> One *high* confidence study in adult rats: • 28 d	• Increased BUN at doses ≥0.625 mg/kg-d in males and ≥3.12 mg/kg-d in females after short-term exposure	• *Dose-dependent* increase in BUN in males and females • *Magnitude of effect*, up to 49% in males and 28% in females	• Lack of expected *coherence* with histopathology (null) • Unclear biological significance in the absence of coherent evidence	⊕⊙⊙ *Slight* Consistent effects on relative kidney weights and BUN in a single study of adult female and male rats after short-term exposure; however, there was a lack of corresponding histopathology and unclear adversity	
<u>Organ weight</u> One *high* confidence study in adult rats: • 28 d	• Increased relative kidney weight at ≥1.56 and ≥0.625 mg/kg-d in adult females and males exposed short-term; • No effects in PND 22 offspring exposed prenatally	• *Dose-responsive* increase in absolute and relative kidney weights in adult females and in relative kidney weights in males	• Lack of expected *coherence* with histopathology (null) • Unclear biological significance in the absence of coherent evidence		

Summary of human and animal evidence[a]					Evidence integration summary judgment
One *medium* confidence gestational exposure study in rats: • GD 1–20		• *Magnitude of effect,* up to 43% in males and 28% in females (relative wt.)	• Some concern for potential confounding (magnitude of changes at doses not affecting body weight were of unclear biological significance)		
<u>Histopathology</u> One *high* confidence study in adult rats: • 28 d One *medium* confidence gestational exposure study in rats: • GD 1–20	• No abnormalities reported in adult rats exposed short-term except for a focal cyst in a high dose (25 mg/kg-d) female on d 15 • Nephron counts significantly reduced by 16% in male offspring (PND 22) after gestational exposures to 5 mg/kg-d; female nephron counts not evaluated	• No factors noted	• Maternal stress and reduced pregnancy weight gain potentially played role in decreased nephron counts in offspring		

[a]No mechanistic studies available.

3.2.11. Other Noncancer Health Effects

No epidemiological studies in the evidence base were identified to inform health effects other than those discussed in the previous sections.

Several other health effects were examined in experimental animals; however, the data were too limited to inform whether PFNA exposure has the potential to cause these effects (i.e., *indeterminate* evidence). Specifically, the *high* confidence, 28-day rat study conducted by NTP (2018) investigated the alimentary system (including the esophagus, small and large intestine, pancreas, salivary glands, and stomach), musculoskeletal system, skin, eye, harderian gland, adult nervous system, and respiratory system. For each of these systems, no PFNA exposure-related effects were reported in male or female rats, with the exception of the respiratory and hematological observations.

For the respiratory system, there was a significant increase in relative lung weight in male rats at all doses with an upward trend (up to 2.5 mg/kg-day). In females there was also an upward trend in relative lung weight that was statistically significant at 3.12 but not 6.25 mg/kg-day (NTP, 2018). Absolute weight was not affected in either sex, and no treatment-related histopathological lesions of the lung were reported for any of these doses in either sex (some minimal to mild olfactory lesions were reported at the highest tested doses causing substantial systemic toxicity; 10 and 25 mg/kg-day in males and females, respectively) (NTP, 2018).

Hematological observations in male rats showed significant reductions in reticulocytes (immature red blood cells) at all reportable doses with reductions of 19%, 49%, and 95% at 0.625, 1.25, and 2.5 mg/kg-day, respectively. Potentially coherent with this, an observed decrease in mean cell volume at 2.5 mg/kg-day in male rats was likely due, at least in part, to the severe depletion of reticulocytes since they are larger in size. There were no significant changes in red blood cell (RBC) counts; however, this could be due to the short study duration, since rat erythrocytes have a life span of approximately 60 days (NTP, 2018). The 19% decrease in reticulocytes at the low dose in male rats, which was not associated with overt toxicity (i.e., significant body weight loss) is of unknown biological significance due to the short duration of the study and the lack of other effects associated with suppression of erythropoiesis such as bone marrow hypocellularity at this dose (see Section 3.2.6, Table 3-31). No significant hematological changes were reported in female animals.

Overall, the currently available ***evidence is inadequate*** to determine whether PFNA exposure has the potential to cause other health effects, including those related to alimentary, musculoskeletal, hematological, respiratory, and adult nervous systems. In general, the data available for these health outcomes were sparse (limited to one short-term study) and were either largely null or had little support for biological significance or coherence.

3.3. CARCINOGENICITY

3.3.1. Cancer

Human Studies

Ten studies were identified that evaluated the risks of cancer associated with exposures to PFNA (see Figure 3-109). However, five of these studies were identified as *uninformative* due to critical deficiencies in exposure measurement (Wielsøe et al., 2017; Christensen et al., 2016), outcome ascertainment (e.g., outcomes were only self-reported) (Christensen et al., 2016), confounding (Lin et al., 2020a), participant selection (Velarde et al., 2022; Wielsøe et al., 2017), or analysis (Omoike et al., 2021), and are not discussed further. Of the remaining studies, Hardell et al. (2014) was considered *low* confidence due to directional bias toward the null and potential for confounding. Bonefeld-Jørgensen et al. (2014) examined the effect of PFNA on breast cancer risk in Danish women using a case-control study, and initially found a nonsignificant decreased risk of developing breast cancer with increases in continuously measured PFNA, and no effect in the categorical analysis. However, 72 breast cancer cases (29% of the cases) were later withdrawn from the National Patient Registry calling into question the validity of the outcome ascertainment for this analysis. Additional analyses excluding these 72 cases were not conducted for PFNA. Bonefeld-Jørgensen et al. (2014) was deemed *low* confidence primarily because there was no explanation of why 29% of the cases were withdrawn and the study did not include comparisons of the subjects' details between the withdrawn cases and the originally selected cases. Liu et al. (2021b) and Tsai et al. (2020) were case-control studies and were also *low* confidence due to concerns for confounding as well as participant selection (Tsai et al., 2020) and exposure misclassification (Liu et al., 2021b). Hurley et al. (2018) was *medium* confidence.

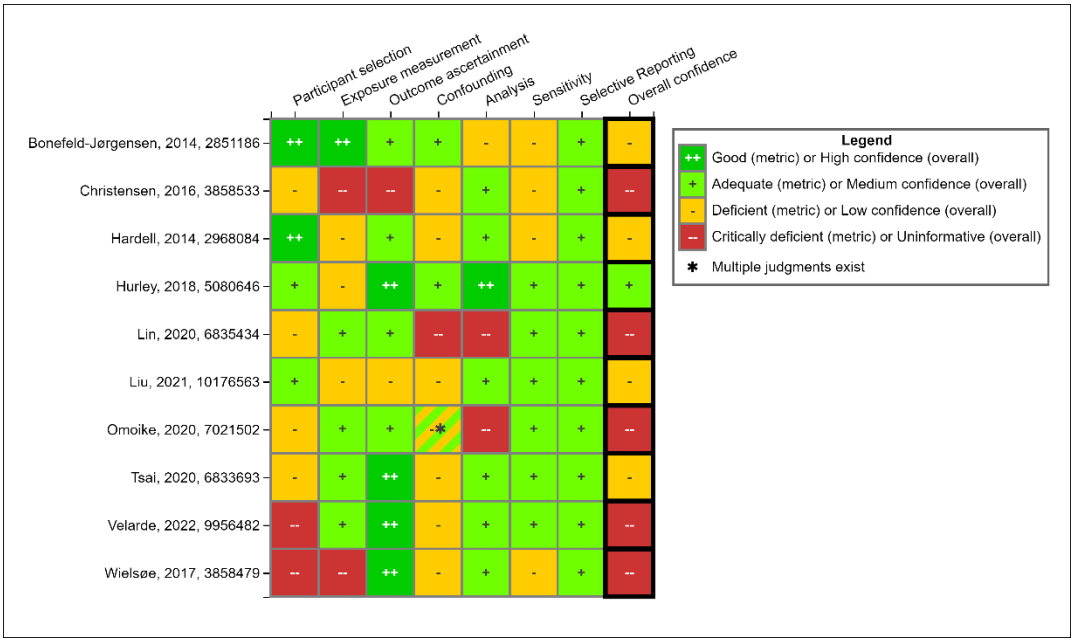


Figure 3-109. Summary of human study evaluations that examined PFNA effects on carcinogenicity. See interactive HAWC link: https://hawc.epa.gov/summary/visual/assessment/100500071/PFNA-and-carcinogenicity-epidemiology/.

Among the informative studies, three (one *medium* and two *low* confidence) examined the risk of breast cancer (Tsai et al., 2020; Hurley et al., 2018; Bonefeld-Jørgensen et al., 2014). None found a clear positive association between PFNA exposure and breast cancer. The *medium* confidence study (Hurley et al., 2018) found no association in the full study population. When stratified by menopausal status, there was an indication of a positive association in pre- or perimenopausal women only, but this association was nonmonotonic across quartiles. One *low* confidence study did not present unstratified results but found differences in direction of association based on age and ER status (none were statistically significant). A positive association was observed in women 50 years of age and younger, but an inverse association was observed in women greater than 50 years of age (Tsai et al., 2020). The other *low* confidence study found no association (Bonefeld-Jørgensen et al., 2014) overall. A nonmonotonic and nonsignificant positive association was observed in women over 40 years of age but not in women 40 years of age and under, opposite to what was observed in the other studies (although the cutoffs differed, which complicates interpretation).

One *low* confidence study by Hardell et al. (2014) reported nonsignificant increases in prostate cancer incidence associated with PFNA above the median compared with below the median (OR = 1.8; 95% CI: 0.8, 4.1) and somewhat higher nonsignificant increases associated with PFNA among men with a first-degree relative with prostate cancer (OR = 2.1; 95% CI: 0.9, 4.8). This study had deficiencies in assessment of confounding and exposure measurement, which would generally be expected to bias results toward the null.

One *low* confidence study examined risk of thyroid cancer associated with PFNA exposure (Liu et al., 2021b) and found a strong inverse association (OR [95% CI] versus Q1: Q2 0.69 [0.36, 1.32], Q3 0.33 [0.16, 0.67], Q4 0.22 [0.10, 0.50]).

In summary, the available epidemiological evidence on PFNA and the risks of any type of cancer is limited. The studies are generally subject to serious sources of bias and results are inconsistent.

Animal Studies

No chronic/carcinogenicity studies are available for PFNA. The only animal testing was the NTP 28-day study that evaluated incidences of neoplastic lesions in male and female rats exposed orally to PFNA at doses ranging from 0 to 10 and 0 to 25 mg/kg-day, respectively (NTP, 2018). No neoplasms were reported. However, due to the short exposure duration, this study was considered *low* confidence for evaluation of carcinogenicity potential.

Mechanistic Studies and Supplemental Information

This mechanistic evidence synthesis focuses on genotoxicity studies. A limited evidence base of studies evaluating the genotoxic and mutagenic potential of PFNA is available from in vivo assays in rats (of short duration, which would bias results toward the null), and in vitro assays with prokaryotic organisms, mammalian cells, and human sperm (see Table 3-50).

In vivo experiments in rats showed no increase in the frequency of micronucleated polychromatic or normochromatic erythrocytes in peripheral blood in males or females after 28 days of oral PFNA exposure (NTP, 2012). Similarly, results from an in vitro test system with hamster V79 fibroblast cells were also negative for micronuclei (Buhrke et al., 2013). A 14-day oral immunotoxicity study in male rats indicated genotoxic potential via DNA damage in the spleen at 3 and 5 mg/kg-day (Fang et al., 2010). This effect was associated with oxidative stress.

Three in vitro studies in mammalian cell test systems (Yahia et al., 2016; Wielsøe et al., 2015; Eriksen et al., 2010) and one study in human sperm (Emerce and Çetin, 2018) examined PFNA-induced DNA damage using single cell gel electrophoresis (Comet assay), which detects the presence of DNA strand breaks. Two of these studies reported positive results for DNA damage in human hepatoma HepG2 cells; one associated DNA damage with ROS production at noncytotoxic concentrations (Wielsøe et al., 2015), while the other reported damage without ROS production. This was concurrent with cytotoxicity at the high dose (400 µM) where LDH release was measured at 66% in the cell medium, but not at the lower dose of 100 µM where LDH release was below 5% (Eriksen et al., 2010). Comet assay results were also positive in human lymphoblastoid (TK6) cells, which also showed an increase in 8-hydroxy-2'-deoxyguanosine (8-OHdG) adducts, a common marker of oxidative DNA damage (Yahia et al., 2016). In contrast, human sperm treated with noncytotoxic concentrations of PFNA up to 1 mM for 1 hour showed no DNA damage (Emerce and Çetin, 2018).

Mutagenicity test results for PFNA in *Salmonella typhimurium* (TA98, TAl00) and in *Escherichia coli* strain (WP2 uvrA pKM101) conducted by NTP were consistently negative in the presence or absence of rat liver S9 cellular fractions (cytosolic) (NTP, 2005). These findings were consistent with a separate study using five salmonella strains with or without S9 (Buhrke et al., 2013). A prokaryotic gene profiling assay designed to evaluate potential toxicological MOAs showed a positive dose-response relationship with some DNA damage-associated genes after PFNA exposure, but the significance of these results to human biology is difficult to interpret due to the type of test system (Nobels et al., 2010).

In summary, there are mixed genotoxicity results for PFNA, and some of these results are difficult to interpret due to cytotoxicity and questionable human relevance. Studies by NTP that evaluated both in vivo micronucleus formation after short-term PFNA exposure and in vitro mutations via the Ames assay exhibited null results (NTP, 2012, 2005). One in vivo and several in vitro studies indicated oxidative stress may be involved in DNA damage responses although these results were not consistent (Emerce and Çetin, 2018; Yahia et al., 2016; Wielsøe et al., 2015; Eriksen et al., 2010; Fang et al., 2010). While some ***evidence suggests*** that PFNA may induce weak genotoxic activity, possibly involving oxidative stress, additional data would be needed to support this hypothesis.

Table 3-50. Tests evaluating genotoxicity and mutagenicity

Test	Materials and methods	Results	Conclusions	Reference
Genotoxicity studies in mammalian species – in vivo				
DNA damage (double strand breaks)	Male Sprague-Dawley rats were exposed to 1, 3, and 5 mg/kg-d by oral gavage for 14 d.	PFNA induced double strand DNA breaks, detected by TUNEL apoptosis assay, in rat spleen at 3 and 5 mg/kg-d.	PFNA exposure is associated with DNA damage associated with apoptosis and oxidative stress in the rat spleen after 14 d of repeat dose oral exposure.	Fang et al. (2010)
Micronucleus assay	Male Sprague-Dawley rats were exposed daily to PFNA by oral gavage at doses of 0, 0.625, 1.25 (n = 5), and 2.5 (n = 1) mg/kg for 28 d. Female Sprague-Dawley rats were exposed daily to PFNA by oral gavage at doses of 0, 1.56, 3.12, and 6.25 (n = 5) 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 PFNA genotoxicity or mutagenicity in the erythrocyte micronucleus assay after 28 d of repeat dose oral exposure.	NTP (2012)
Genotoxicity studies in mammalian cells – in vitro				
Micronucleus assay	Chinese hamster lung fibroblast V79 cells exposed to highest non-cytotoxic concentration of 10 µM PFNA for 3 hr and cultured for another 21 hr without test article (OECD TG 487). S9 fraction added in parallel experiment.	Test results were negative with and without S9.	There is no evidence of PFNA mutagenicity/clastogenicity in an in vitro mammalian cell test system.	Buhrke et al. (2013)
Oxidative DNA damage (8OHdG)	Human lymphoblastoid (TK6) cells were exposed to PFNA at non-cytotoxic concentrations of 125 and 250 ppm for 2 hr.	Comet assay indicated PFNA induced DNA damage and a dose-dependent increase in 8-OHdG adducts was measured by HPLC-MS/MS.	PFNA induced oxidative DNA damage, evidenced by 8-OHdG adducts, in a human lymphoblastoid cell line.	Yahia et al. (2016)
DNA single and double strand breaks	Human hepatoma HepG2 cells exposed to PFNA at non-cytotoxic concentrations between 200 nM and 20 µM for 24 hr.	Comet assay indicated PFNA induced DNA damage and concomitant with an increase in intracellular ROS generation.	PFNA induced DNA damage corresponding with increased ROS production.	Wielsøe et al. (2015)

Test	Materials and methods	Results	Conclusions	Reference
DNA single- and double-strand breaks	Human hepatoma HepG2 cells exposed to PFNA at 100 and 400 µM for 24 hr.	Comet assay indicated PFNA increased DNA strand breaks at both concentrations and was associated with cytotoxicity at the high dose (measured by LDH release) but not and the lower dose. DNA damage at both doses was observed without significant increases in ROS production.	Non-cytotoxic and cytotoxic concentrations of PFNA-induced DNA damage without significant changes in ROS production (measured by DCFH-DA fluorescence assay).	Eriksen et al. (2010)
DNA single- and double-strand breaks	Human sperm were treated with non-cytotoxic concentrations of 0.1–1 mM PFNA for 1 hr.	Comet assay indicated PFNA did not induce DNA damage in human sperm at any concentration tested.	PFNA did not cause DNA damage in human sperm.	Emerce and Çetin (2018)
Genotoxicity studies in prokaryotic organisms				
Prokaryotic gene profiling assay	14 *E. coli* strains (including DNA damage response reporter genes Nfo, RecA, UmuDC, Ada, SfiA, and DinD) were incubated with 15.6 µM–1 mM PFNA for 90 m. The reduction of ONPG (O-nitrophenyl-b-D-galactopyranoside) to ONP (O-nitrophenol) by β-galactosidase was measured to quantify activity of the promoters.	PFNA caused significant dose-dependent increases in expression of Ada, SfiA, and DinD, as well as genes involved in oxidative stress, membrane damage, and osmotic stress.	PFNA induces expression of genes associated with DNA damage.	Nobels et al. (2010)
Ames assay	*S. typhimurium* strains (TA98 and TA100) and *E. coli* strain (WP2 uvrA pKM101) in the presence or absence of S9. Concentrations of PFNA were between 0−10,000 µg/plate.	Test results were negative in all bacterial strains irrespective of the presence of S9.	There is no evidence of PFNA mutagenicity in S. typhimurium and *E. coli* strains.	NTP (2005)
Ames assay	*S. typhimurium* strains (TA98, TA100, TA1535, TA1537, TA1538) in the presence or absence of S9. Concentrations of PFNA were between 5 µmol/plate (highest dose that did not induce cytotoxicity).	Test results were negative in all bacterial strains irrespective of the presence of S9.	There is no evidence of PFNA mutagenicity in five strains of S. typhimurium.	Buhrke et al. (2013)

Evidence Integration

The available evidence to evaluate a potential association between PFNA exposure and cancer is weak, consisting of primarily *low* confidence studies in humans and a single, short-term *low* confidence oral exposure study, with some mechanistic information on genotoxicity. The low confidence human and animal studies were largely null, and the available mechanistic evidence was inconclusive. While there is some evidence of carcinogenicity for PFOA and PFOS, the ability to relate the findings for these other longer chain PFAS to PFNA is currently too uncertain to influence the carcinogenicity judgment for PFNA.

Taken together, based on the EPA cancer guidelines (U.S. EPA, 2005), there is ***inadequate information to assess carcinogenic potential*** for PFNA.

4. SUMMARY OF HAZARD IDENTIFICATION CONCLUSIONS

4.1. SUMMARY OF CONCLUSIONS FOR NONCANCER HEALTH EFFECTS

The currently available ***evidence demonstrates*** that perfluorononanoic acid (PFNA) causes developmental growth impairments in humans given sufficient exposure conditions, and the ***evidence indicates*** that hazards likely exist with respect to hepatic and male reproductive effects in humans given sufficient exposure conditions.[21] Additionally, the currently available ***evidence suggests*** but is not sufficient to infer that PFNA may cause immune, neurodevelopmental, cardiometabolic, and thyroid effects given sufficient exposure conditions.[22] These judgments were derived primarily from epidemiological and experimental animal studies, the latter of which exposed test organisms to PFNA during short-term (7–28 days) and developmental (GD 1–18) exposure periods. There is currently ***inadequate evidence*** to determine the potential for health hazards in humans for female reproductive, urinary, adrenal, and other health effects not indicated above. A summary of justifications for the evidence integration judgments for each of the main hazard sections is provided below.

The hazard identification judgment that the ***evidence demonstrates*** that PFNA exposure causes developmental effects in humans is based on *robust* epidemiological evidence of decreased birth weight in *high* and *medium* confidence studies in exposed individuals, with some residual uncertainties from potential sources of bias related to sample timing and the potential impact of PFAS co-exposures. The median PFNA serum values during pregnancy ranged from 0.2 to 2.3 ng/mL with maximum values of 0.81 to 22 ng/mL (median of the maximums = 4.5 ng/mL). There was a wide range of mean birth weight differences in the 18 studies of the overall population (β range: −15 to −133 g per ln-unit PFNA increase) showing inverse associations with 12 studies ranging from –40 to –62 g and 9 studies ranging from –40 to –47 g. The range of values in this cluster of studies was slightly larger than the overall pooled estimate based on EPA's meta-analysis (β = –33 g). The birth weight evidence is further bolstered by coherent support from consistent observations of inverse associations of PFNA exposures with related epidemiological endpoints, including birth length and postnatal weight and height. Additional cross-stream coherence is provided by *moderate* evidence of PFNA-induced developmental effects in *high* and *medium*

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

[22]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).

confidence studies in rodents that observed a reduced number of live pups born, reduced preweaning survival and body weight gain (with generally no catch-up in growth by weaning at higher doses and that extended post-weaning), and delays in attainment of developmental landmarks (eye opening, vaginal opening, preputial separation) in gestationally exposed rodent offspring generally at PFNA doses ≥2 mg/kg-day.

The hazard identification judgment that the ***evidence indicates*** PFNA exposures are likely to cause hepatoxicity in humans given sufficient exposure conditions is based on *moderate* evidence of PFNA associations with increased ALT, AST, and GGT in epidemiological studies and are considered indicators of potentially impaired liver functioning. The positive associations provide some cross-stream coherence with the animal evidence base (considered *robust*) that included a coherent pattern of effects in *high* and *medium* confidence short-term studies (≤28 days) in adult rats and mice and developmental toxicity studies in gestationally exposed mouse offspring. These effects include liver enlargement in multiple species and lifestages, large increases in liver enzymes in mice (ALT/AST), large increases in hepatobiliary components in rats (total bile acids, bilirubin, liver accumulation of triglycerides), and evidence of histopathology (hepatocellular hypertrophy, cytoplasmic alterations, and mild necrosis in rats). Together, the constellation of effects provided support for the adversity of PFNA. However, the PFNA evidence base in animals and adversity finding for liver effects in animals is uncertain due to the lack of longer duration exposure studies. Mechanistic evidence from in vivo and in vitro rodent studies and in human in vitro models also provided support for the adversity finding in rodents and informed the biological plausibility and human relevance of effects observed in animals—including providing strong support for PPARα dependent and independent modes of action (MOAs) and support for liver triglyceride accumulations and steatosis in PFNA-induced liver toxicity.

The hazard identification judgment that the ***evidence indicates*** PFNA exposures are likely to cause male reproductive effects in humans given sufficient exposure conditions is based on a consistent, dose-dependent, and coherent pattern of effects in studies in adult and prepubertal rodents. These effects included decreased organ weights and testosterone, impaired spermatogenesis, and corresponding structural changes to reproductive tissues at ≥1.25 mg/kg-day PFNA. There were some concerns for bias toward the null due to the short-term exposure durations that did not capture the entire spermatogenic cycle. The animal evidence base is considered *moderate*. The human evidence base is considered *slight*, with one *medium* confidence study reporting an inverse association between PFNA exposure and testosterone. While the MOA for PFNA-induced male reproductive toxicity is unclear, there is some mechanistic data from in vivo and in vitro studies in rodents that suggest reductions in testosterone and impaired spermatogenesis may involve functional damage to Sertoli cells and atrophy in Leydig cells.

The hazard identification judgment that the ***evidence suggests*** but is not sufficient to infer that PFNA exposures may cause immunosuppression in humans given sufficient exposure conditions is based on *slight* evidence of reduced antibody responses following vaccination in

children. These observations were generally consistent in the direction of association across vaccine type, timing of vaccination, and age at antibody response measurement, with reductions generally being fairly large despite poor sensitivity (the majority were greater than a 10% decrease per doubling of exposure, ranging up to 35%). However, there is considerable uncertainty in the evidence base that is due to potential confounding across PFAS and weaker effect patterns for PFNA than for other correlated PFAS, including PFDA, PFHxS, PFOA, and PFOS. Specifically, EPA's benchmark dose modeling did not show statistically significant effects of PFNA on antibody concentrations in children aged 5 and 7 years in both the single-PFAS model and in the multi-PFAS model of PFNA controlling for PFOS and PFOA. The animal evidence was considered *indeterminate,* due in large part to the fact that there are currently no functional studies of PFNA-induced immunomodulatory activity to aid in interpreting the immunosuppression observed in epidemiological studies.

The hazard identification judgment that the ***evidence suggests*** but is not sufficient to infer that PFNA exposures may cause endocrine effects, specifically thyroid toxicity, in humans given sufficient exposure conditions are based on *moderate* evidence from the *high* confidence NTP 28-day study that observed consistent and dose-dependent reductions in serum free and total T4 in female rats and free T4 in male rats. PFNA effects on serum total T4 in males lacked a clear dose gradient. Results in males from the 28-day study were difficult to decipher because of body weight loss at the higher end of the dose range tested and uncertainty as to the biological basis for observed declines in serum TSH. Nonetheless, the reductions in serum T4 in both sexes was of a concerning magnitude (75% reduction in free T4 and 91% reduction in total T4 in males at 0.625 mg/kg-day, a dose level unaffected by weight loss, and 36%–53% in females at ≥3.12 mg/kg-day). While there are uncertainties related to the lack of longer duration exposures and evaluation in younger animals, effects in females are especially concerning given the importance of thyroid hormone in pregnancy for fetal brain development. The epidemiological evidence was found to be *indeterminate* with the strongest evidence from *medium* confidence studies indicating positive associations between PFNA and T4 in children/adolescents, and evidence of inverse associations with T4 in infant boys. However, there was considerable uncertainty in the results because of inconsistencies in the direction of associations across age groups that were difficult to interpret biologically, as well as concern for study sensitivity in some studies. The MOA for PFNA-induced effects on the thyroid are not well characterized, and the available evidence from in vitro testing in human and rodent cell lines is equivocal.

The hazard identification judgment that the ***evidence suggests*** but is not sufficient to infer that PFNA exposures may cause neurodevelopmental toxicity given sufficient exposure conditions is based on *slight* evidence from *medium* confidence epidemiological studies that indicate positive associations between PFNA exposures and diagnosis of attention-deficit/hyperactivity disorder (ADHD) and related behavioral disorders. However, there is substantial uncertainty because of imprecision in neurobehavioral diagnoses, as well as inconsistent results of associations for other

outcomes (e.g., cognition, social behavior). The animal evidence is *indeterminate* as there are no studies currently available in mammalian models that have evaluated effects or mechanisms. The hazard identification judgment that the ***evidence suggests*** but is not sufficient to infer that PFNA exposures may cause cardiometabolic effects given sufficient exposure conditions is based on *slight* epidemiological evidence with considerable uncertainty. *Medium* confidence studies in adults, including pregnant women, suggest that higher PFNA exposures may be associated with elevated serum cholesterol. There is also some coherent evidence from *medium* confidence studies of associations with cardiovascular risk factors (blood pressure, atherosclerosis, metabolic syndrome, and adiposity) but that are uncertain because of unexplained inconsistencies, and the available animal evidence is *indeterminate*.

Finally, there is ***inadequate evidence*** to evaluate the potential for effects on female reproductive, urinary, adrenal, and other noncancer health outcomes. The available epidemiological and animal evidence bases for these health outcomes are limited in number and/or lacked consistency and coherence. Table 4-1 summarizes hazard judgments for PFNA in comparison with other completed Agency PFAS assessments. Additional EPA assessments of PFAS have been completed for perfluoropropanoic acid (PFPrA) (U.S. EPA, 2023d) and lithium bis[(trifluoromethyl)sulfonyl]azanide (HQ-115) (U.S. EPA, 2023c). For these assessments, candidate chronic oral reference doses (RfDs) based on liver effects and developmental effects from animal evidence have been derived for PFPrA and HQ-115, respectively.

Table 4-1. Hazard conclusions across published EPA PFAS human health assessments

Health outcome	PFAS Assessments[a,b,c]						
	PFNA (this assessment)	**PFHxA** U.S. EPA (2023h)	**PFBA** U.S. EPA (2022b)	**PFBS** U.S. EPA (2018b)	**Gen X chemicals** U.S. EPA (2021a)	**PFOA[d]** U.S. EPA (2016b)	**PFOS[d]** U.S. EPA (2016a)
Thyroid	+/-	+	+	+	ND	Human: + Animal: +/-	Human: +/- Animal: +/-
Liver	+	+	+	-	+	Human: + Animal: +	Human: - Animal: +
Developmental	+	+	+	+	+/-	Human: + Animal: +	Human: + Animal: +
Reproductive	Male: + Female: -	-	-	-	+/-	Human: - Animal: +/-	ND
Immunotoxicity	+/-	-	-	-	+/-	Human: + Animal: +	Human: +/- Animal: +
Renal	-	-	-	+	+/-	Human: +/- Animal: +/-	ND
Hematological	-	+	-	ND	+/-	ND	ND
Ocular	ND	ND	-	ND	ND	ND	ND
Serum lipids	+/-	ND	ND	-	ND	Human: + Animal: +	Human: +
Hyperglycemia	ND	ND	ND	ND	ND	Human: - Animal: -	Animal: +/-
Nervous system	+/-	-	ND	ND	ND	Human: - Animal: -	Animal: +/-
Cardiovascular	+/-	ND	ND	-	ND	ND	ND

Health outcome	PFAS Assessments[a,b,c]						
	PFNA (this assessment)	**PFHxA** U.S. EPA (2023h)	**PFBA** U.S. EPA (2022b)	**PFBS** U.S. EPA (2018b)	**Gen X chemicals** U.S. EPA (2021a)	**PFOA[d]** U.S. EPA (2016b)	**PFOS[d]** U.S. EPA (2016a)
Cancer	-	-	-	-	+/-	+/-	+/-

[a]Assessments used multiple approaches for summarizing their noncancer hazard conclusion scales; for comparison purposes, the conclusions are presented as follows: + = ***evidence demonstrates*** or ***evidence indicates***, or ***evidence supports*** (e.g., PFBS); +/- = ***evidence suggests***, - = ***evidence inadequate*** or ***equivocal evidence*** (e.g., PFBS); -/- = sufficient evidence to conclude no hazard (no assessment drew this conclusion); ND = no data available for this outcome for this PFAS.

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

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

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

4.2. SUMMARY OF CONCLUSIONS FOR CARCINOGENICITY

The currently available evidence is insufficient to make a judgment on whether PFNA exposure might affect the development of any specific cancers. Consistent with the EPA cancer guidelines (U.S. EPA, 2005), a weight of evidence hazard descriptor of ***inadequate information to assess carcinogenic potential*** is applied for PFNA for all routes of exposure.

4.3. CONCLUSIONS REGARDING SUSCEPTIBLE POPULATIONS AND LIFESTAGES

The available human and animal studies indicate that early lifestages represent a susceptible window for health effects related to PFNA exposure. The epidemiological ***evidence indicates*** the developing fetus and children are vulnerable to PFNA, which is supported by toxicological studies in animals. Specifically, most human developmental studies (16 of 21) reported birth weight deficits with higher PFNA exposure (see Section 3.2.2) in either the overall population (including 11 of 15 *high* and *medium* studies) or at least one of the sexes. Although statistically significant results were detected along with some exposure-response evident in different sexes, the lack of consistent patterns and often insufficiently powered studies preclude any definitive sex-specific conclusion. Results from *high/medium* animal studies support the human findings, reporting reduced survival, birth weight, postnatal body weight, and delays in attainment of developmental milestones (Das et al., 2015; Rogers et al., 2014; Wolf et al., 2010). Susceptibility to immunosuppressive effects in children is indicated by generally consistent but imprecise evidence for immunosuppression with PFNA exposure based on lower antibody responses (see Section 3.2.6). Evidence in animals suggests that other health outcomes might also be affected by PFNA exposure during development. Specifically, gestationally exposed rodents have shown liver enlargement that appears to be partially independent of PPARα (see Section 3.2.3) and effects on the developing male and female reproductive system (see Sections 3.2.4 and 3.2.5). Additionally, reductions in serum T4 in adult rats (NTP, 2018) suggest potential susceptibilities to early lifestages with added concerns due to the delays in eye opening observed in prenatally exposed mice (see Section 3.2.2. "Postnatal developmental landmarks") that are a well-characterized effect in rodents to chemically induced T4 reductions (Gilbert, 2011; Dong et al., 2009; Goldey et al., 1995). While no animal studies have evaluated PFNA effects on the thyroid during pregnancy and development (and the thyroid epidemiology evidence base was *indeterminate*), young individuals exposed during gestation, early childhood, and puberty may be susceptible given that T3 and T4 play a critical role in brain development and somatic growth (O'Shaughnessy et al., 2019; Bernal, 2015; Zoeller and Rovet, 2004). There was also suggestive evidence of an association between PFNA exposures during gestation/childhood and diagnoses of ADHD and related neurological outcomes but with substantial inconsistencies in the evidence base (see Section 3.2.8).

5. DERIVATION OF TOXICITY VALUES

5.1. NONCANCER AND CANCER HEALTH EFFECT CATEGORIES CONSIDERED

For noncancer health effects, the currently available ***evidence demonstrates*** that exposure to perfluorononanoic acid (PFNA) causes developmental effects given sufficient exposure conditions, and the ***evidence indicates*** that PFNA exposure likely causes hepatic and male reproductive effects in humans given sufficient exposure conditions. These conclusions are based on results from epidemiological and experimental animal studies. The dose levels associated with these hazards are considered for derivation of oral reference doses (RfDs), as presented below. The available ***evidence suggests*** but is not sufficient to infer that PFNA exposure may cause immune, thyroid, developmental neurotoxicity, and cardiometabolic effects in humans given sufficient exposure conditions. Given the uncertainty of these latter conclusions, no toxicity values were derived for these potential effects. However, while a dose-response assessment is typically not conducted for health effect judgments of "***evidence suggests***," when the evidence base includes at least one well-conducted study, quantitative analysis may still be useful for some purposes, such as providing a sense of the magnitude and uncertainty of estimates for health effects of concern, informing responses in potentially susceptible populations, or setting research priorities (U.S. EPA, 2020, 2005). For this assessment, the ***suggestive evidence*** of immunosuppression in children was modeled by EPA to compare with other PFNA points of departure (PODs) and to inform the uncertainty factor (UF) given that this effect is observed with other PFAS (e.g., PFDA, PFOA, PFOS, PFHxS). Consistent with the weaker pattern of effects on antibody levels for PFNA than has been observed for other PFAS (see Section 3.2.3), the benchmark dose (BMD) modeling results were null (see Appendix D.1.2). Thus, the immune effects data did not inform any other dose-response decisions, including UF selection. Reductions in serum T4 in adult female rats were also advanced for dose-response modeling for comparison with other PFNA PODs and to inform UF selection. Reduced T4 has been identified as a human health concern for other PFAS (e.g., PFBS, PFBA, PFHxA), and this outcome is of particular interest to PFNA given the very strong evidence for developmental effects observed for this PFAS and given the critical importance of thyroid hormone in fetal and offspring development. The ***evidence is inadequate*** to assess whether PFNA exposure may cause other potential health effects in humans for which studies were available (i.e., female reproductive, adrenal, hematological, and urinary), and these endpoints were not considered for dose-response analysis. One acute, single-dose inhalation exposure study was identified but was considered *low* confidence and inadequate for reference value derivation, so an inhalation reference concentration (RfC) was not estimated. Similarly, the evidence base related to potential

carcinogenicity was determined to contain ***inadequate information to assess carcinogenic potential***, and thus no cancer toxicity values were estimated for any route of exposure.

5.2. NONCANCER TOXICITY VALUES

The noncancer reference 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 lifestages) that are likely to be without appreciable risk of adverse health effects over a lifetime (U.S. EPA, 2002c, 1993). The lifetime RfD derived in Section 5.2.1 corresponds to chronic, lifetime exposure. In addition, a less-than-lifetime, subchronic toxicity value (referred to as a "subchronic RfD"), which corresponds to exposure durations ranging from a month to 10% of the life span in humans, is derived in Section 5.2.2. Subchronic toxicity values may be useful for certain decision purposes (e.g., site-specific risk assessments with less-than-lifetime exposures). Both the lifetime and subchronic RfDs include organ/system-specific reference doses (osRfDs) associated with each health effect considered for POD derivation, as 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 indicates the ***evidence is inadequate*** to inform the potential toxicity of inhaled PFNA.

5.2.1. Oral Reference Dose (RfD) Derivation

Study/Endpoint Selection

Data sufficient to support dose-response analyses for oral chronic and/or subchronic PFNA exposures were available for the following human health hazards: developmental, hepatic, and male reproductive effects. Additionally, for some health outcomes with suggestive evidence of an effect (immune, thyroid), PODs were derived to compare with other PFNA PODs. Rationales for study selection and the specifics of POD and candidate toxicity value calculations, as well as the determination of confidence in the derived toxicity values, are detailed in this section.

Using the methods described in Section 1.2.5 and Appendix A, the following general considerations were used to prioritize studies for estimating PODs for potential use in toxicity value derivation. Well-conducted (i.e., *high* or *medium* confidence) human studies that were deemed most influential to the hazard conclusions were prioritized for POD derivation and compared with PODs derived from *high* or *medium* confidence animal data. *High* and *medium* confidence human studies were available for developmental, hepatic, and immunotoxic effects and were advanced, with multiple *high* confidence studies in humans serving as candidates for RfD derivation of developmental effects. While there are also *high* and *medium* confidence studies in experimental animals that evaluated developmental endpoints, these were not advanced as candidates for RfD derivation because the *high/medium* confidence human data were preferred and were more sensitive (see "Developmental effects," below). Additionally, the *high* and *medium* confidence studies on potential immune effects were considered for POD-comparative purposes as discussed

above. However, PODs of immune effects observed in epidemiological studies were not advanced for candidate toxicity value derivation because of the limited evidence for hazard (i.e., *slight* in humans and *indeterminate* in animals) and results of EPA's BMD modeling (see the *Immune effects* discussion below). For subchronic health effects (i.e., hepatic and male reproductive), evidence from short-term animal studies was considered most influential for hazard characterization, and these data were therefore also advanced for dose-response assessment and potential use in subchronic toxicity value derivation. The short duration PFNA exposures available in these studies were not deemed sufficient to support derivation of candidate values for the lifetime RfD. *High* and *medium* 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 (including doses that were not associated with overt toxicity) across the evidence base were preferred for subchronic toxicity values, along with *medium* or *high* confidence animal studies evaluating exposure periods relevant to developmental outcomes (the latter were also considered for use in deriving the lifetime RfD). These types of studies increase the confidence in the resultant RfD because they represent data with low risk of bias and reduce the need for low-dose and exposure-duration extrapolation (see Appendix A, Section 11.1 of the protocol).

A summary of endpoints and rationales considered for toxicity value derivation is presented below.

Developmental effects

The epidemiological evidence provides *robust* evidence for effects of PFNA exposure on fetal growth restriction, specifically decreased birth weight, making it the focus for dose-response analysis (see Section 3.2.2). Twenty-six *medium* and *high* confidence studies that examined mean (n = 20 *high/medium*) or standardized (two additional *high* studies) birth weight associations in the overall population or either sex (four additional *high*/*medium* studies) were considered for dose-response analysis. Two of the *high* confidence and one *medium* confidence study were not considered further because they did not present results amenable to modeling (Hall et al., 2022; Gardener et al., 2021; Xiao et al., 2019). In addition, three *high* (Eick et al., 2020; Buck Louis et al., 2018; Shoaff et al., 2018) and one *medium* (Chen et al., 2012) confidence studies that did not observe decrements in birth weight were not modeled individually, although the results of three of these studies are included in the meta-analysis (note: Eick et al. (2020) only presented categorical results and thus was not included in the meta-analysis). Overall, 20 *high* (n = 10) and *medium* (n = 10) confidence studies showing some inverse birth weight associations in either the overall population or individual sexes were amenable to modeling. Given this large number of studies and the potential concern over an impact of pregnancy hemodynamics, the studies that had exposure sampling during early pregnancy were prioritized for individual study dose-response modeling, as

were the meta-analytical results for the pooled early pregnancy sampling studies (see Appendix C.1.5 and (Wright et al., 2023)).[23]

Thus, for individual study dose-response modeling, five of the 10 *high* (Wikström et al., 2020; Sagiv et al., 2018; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016) and six of the 10 *medium* (Chang et al., 2022; Chen et al., 2021; Hjermitslev et al., 2020; Meng et al., 2018b; Wang et al., 2016; Robledo et al., 2015) confidence studies with early pregnancy sampling were further considered. One *high* (Lind et al., 2017a) and two medium (Wang et al., 2016; Robledo et al., 2015) confidence studies were not carried forward because of mixed results across sexes which complicated interpretation. Likewise, the *high* confidence study by Bach et al. (2016) was not carried forward as it did not show an inverse association based on continuous PFNA measurements. Further considering the remaining seven studies, EPA's meta-analysis of mean birth weight stratified by confidence level showed comparable pooled estimates (i.e., betas) for *medium* and *high* confidence studies, and the individual study estimates for the four remaining medium confidence studies with sampling during early pregnancy were also similar to the individual study estimates for the three remaining *high* confidence studies being considered. Therefore, only the three remaining *high* confidence studies with early pregnancy sampling were advanced to dose-response modeling (Wikström et al., 2020; Sagiv et al., 2018; Manzano-Salgado et al., 2017a).

The EPA meta-analysis of mean birth weight changes in relation to continuous PFNA exposures from 27 studies reported an overall birth weight deficit (β =−32.9 g; 95% CI: −47.0, −18.7 per ln-unit change). Although the meta-analysis showed some differences by sample timing, all strata examined by sample timing and confidence level showed birth weight deficits of 22 grams or larger per ln-unit increase which provide additional supportive evidence of an adverse effect on birth weight from maternal exposure to PFNA. Within these analyses, EPA considered a subset of 10 studies with predominately trimester one (or early trimester two) studies classified as "early pregnancy" to be the most relevant and homogenous meta-analytical result to model. The BMDL of 1.81 ng/mL based on this subset was higher than the PODs based on the individual studies (range: 0.84 to 1.25 ng/mL) (see Tables D-11 and D-12 in Appendix D.1.4).

The human evidence is supported by coherent results from rodent bioassays with two *high/medium* confidence developmental toxicity studies in CD-1 and 129S1/SvlmJ wild type mice by Das et al. (2015) and Wolf et al. (2010), respectively, found to be suitable for modeling and derivation of candidate RfDs. A single-dose, *medium* confidence study by Rogers et al. (2014) for most endpoints was not prioritized for modeling as animal studies with multiple dose groups are preferred for quantitation. The studies in mice indicated consistent and mostly dose-dependent effects on postnatal survival and growth metrics, including reduced body weight (pre- and post-weaning) and delayed attainment of developmental landmarks (eye opening, vaginal opening, preputial separation), all of which were advanced for modeling (Das et al., 2015; Wolf et al., 2010).

[23]Results for two studies with later exposure sampling are included for comparison purposes in Table 5-9; these results are discussed more fully in Appendix D (e.g., Appendix D.1.3).

The reduced survival in Wolf et al. (2010) did not follow a monotonically decreasing response gradient, but the strong downward trend for this endpoint was taken into consideration (results of the BMD modeling are presented in Appendix D.2.3). While the data could not be successfully modeled, the consistent negative association of all doses with mortality supports the effects observed in another strain of mouse in Das et al. (2015) that reported dose-dependent reductions in offspring survival. The largest percent reduction in body weight in both mouse studies occurred at PND 7. Thus, the reduced survival at PND 21 and deficits in body weight at PND 7 in Das et al. (2015) and Wolf et al. (2010) were prioritized for modeling. Additionally, reduced offspring body weight from PND 21 was advanced to allow consideration of the full lactational term (see Table 3-10). The post-weaning body weight decrements from Das et al. (2015) at PND 24 (largest post-weaning deficits in both sexes), PND 42 (females), and PND 287 (males) also were selected for modeling. Both Das et al. (2015) and Wolf et al. (2010) observed endpoints of delayed eye opening. The findings from Das et al. (2015) were prioritized for modeling as time to attainment, which was the endpoint evaluated in this study, is the preferred output metric for quantitative analysis. Delayed eye opening, also found following exposures to PFOA (Lau et al., 2006) and PFBA (Das et al., 2008), is identified as a "simple but reliable" indicator of impaired postnatal development in rodents (Lau et al., 2006), and eye opening delays are a form of visual deprivation that prevents ocular visual signals from reaching the brain during a critical period of development (Espinosa and Stryker, 2012; Wiesel, 1982). Evidence in humans further supports the adversity of this endpoint, given that infants born with congenital cataracts that interfere with the processing of visual signals have permanent visual defects if the cataracts are removed after the critical window for visual development (Wiesel, 1982). Therefore, any delay in the development of sight or development of the visual neurological system results in permanent functional decrements and is relevant to human health. PFNA effects on the developing reproductive system included delays in vaginal opening and preputial separation (Das et al., 2015). EPA's Reproductive Toxicity Guidelines (U.S. EPA, 1996) state that significant effects in the development of the male and female reproductive systems "either early or delayed, should be considered adverse…" and thus supports considering these endpoints for reference value derivation.

The human and experimental animal studies and endpoints selected for dose-response modeling are shown below in Tables 5-1 and 5-2.

Table 5-1. Endpoints considered for dose-response modeling and derivation of points of departure for selected developmental effects studies in humans and meta-analysis subsets

Endpoint	Study reference	Overall population, sex-specific and all births vs. term births only	PFAS sample timing	POD derivation	Notes
Decreased birth weight	Manzano-Salgado et al. (2017a)	Overall population; all births	Trimesters 1–3	Yes	Results based on continuous exposure and birth weight deficits were moderately small in magnitude (−10.3 (95% CI: −38.1, 17.6) g/log2(ng/mL)); study showed some coherence across some fetal growth endpoints. Maternal samples were collected primarily during trimester one which should minimize the pregnancy hemodynamic impact.
Decreased birth weight	Sagiv et al. (2018)	Overall population; Term Births	Trimesters 1–2	Yes	Birth weight deficits were relatively large in magnitude (−56.4 (95% CI: −104.0, −8.8) g/ng/mL) and consistent with larger birth weight deficits based on categorical data. Maternal samples were collected primarily during trimester one which should minimize the pregnancy hemodynamic impact.
Decreased birth weight	Wikström et al. (2020)	Overall population; all births	Trimesters 1–2	Yes	Birth weight deficits were relatively large in magnitude (−46.0 (95% CI: -89.0, −4.0) g/ln(ng/mL)) and statistically significant. Maternal samples were collected primarily during trimester one which should minimize the pregnancy hemodynamic impact.
Decreased birth weight	All 27 studies; meta-analysis[a]	Multiple	Trimesters 1–3	Yes	Meta-analysis of 27 birth weight epidemiologic studies.
Decreased birth weight	22 *high* and *medium* confidence studies, meta-analysis[a]	Multiple	Trimesters 1–3	Yes	Meta-analysis of 12 *high* and *medium* confidence birth weight epidemiologic studies.
Decreased birth weight	11 early pregnancy studies; meta-analysis[a]	Multiple	Trimesters 1–3[b]	Yes	Meta-analysis of 11 early pregnancy birth weight epidemiologic studies.

Endpoint	Study reference	Overall population, sex-specific and all births vs. term births only	PFAS sample timing	POD derivation	Notes
Decreased birth weight	10 early pregnancy studies; meta-analysis[a,b]	Multiple	Trimesters 1–3[b]	Yes	Meta-analysis of 10 early pregnancy birth weight epidemiologic studies with maternal sampling during pregnancy (excluding Robledo et al. (2015)).[c]

[a]Meta-analyses results and methodology provided in Section C.1.5 and in Wright et al. (2023).
[b]Early pregnancy studies predominately collected maternal PFNA biomarkers during trimester 1 although samples for some participants were measured later in pregnancy. For example, gestational age sampling measures of centrality estimates for 8 of the 10 early sampled subset studies was in trimester 1, while two (Chen et al., 2021; Hjermitslev et al., 2020) studies were in early trimester 2 (Wright et al., 2023).
[c]The Robledo et al. (2015) study was the only study based on preconception PFNA exposure measures; thus, this was not included in the 10-study subset which targeted the most homogeneous study grouping all based on maternal sampling during early pregnancy.

Table 5-2. 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 derivation	Notes
Decreased postnatal (F1) survival, preweaning	Das et al. (2015), *medium*	Developmental (GD 1–17)	CD-1 mouse, sexes combined	Yes, PND 21	Statistically significant decreases in high dose group at each timepoint; Effects at high dose were greatest at weaning therefore this timepoint was selected.
	Wolf et al. (2010), *medium*	Developmental (GD 1–18)	129S1/SvlmJ wild type, sexes combined[24]	Yes, PND 21	Effects did not follow a monotonic dose-response gradient but were advanced for dose-response given the large reductions in survival and decreased survival in another mouse strain.
Reduced postnatal (F1)	Das et al. (2015), *medium*	Developmental (GD 1–17)	CD-1 mouse, sexes combined	Yes, PND 7 and 21	Consistent effects in two mouse strains with overlapping dose ranges but some sex dependence;

[24]Wolf et al. (2010) used both 129S1/SvlmJ wild type and PPARα null mice, table includes only wild type mice.

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derivation	Notes
body weight, preweaning	Wolf et al. (2010), *medium*	Developmental (GD 1–18)	129S1/SvlmJ wild type, males and females	Yes, PND 7 and, 21	dose-dependent effects in CD-1 mice and statistically significant decrements at the high dose in wild type mice; PND 7 selected as the timepoint with generally the largest deficits in both strains and wild type sexes; PND 21 selected to account for full preweaning period.
	Rogers et al. (2014) *medium*	Developmental (GD 1–20)	SD rat, males and females	No	Decrements observed that are consistent with effects in mice but in a single-dose design and with some evidence of maternal stress (decreased pregnancy weight gain).
Reduced postnatal (F1) body weight, post-weaning	Das et al. (2015), *high*	Developmental (GD 1–17)	CD-1 mouse, males and females	Yes, PND 24 (males, females), PND 287 (males), PND 42 (females)	Statistically significant decrements in males at up to PND 287 and in females at up to PND 42; PND 24 also selected as the first post-weaning timepoint with the largest reductions in both sexes.
Delayed developmental landmark (eye opening)	Das et al. (2015), *medium*	Developmental (GD 1–17)	CD-1 mouse, sexes combined	Yes, PND 15	Dose-dependent delays in achieving milestone in two mouse strains; Findings in CD-1 mice selected as delays were presented as number of days delayed relative to controls, which is the preferred output for this endpoint.
	Wolf et al. (2010), *medium*	Developmental (GD 1–18)	129S1/SvlmJ wild type, sexes combined	No	
Delayed developmental landmark (preputial separation)	Das et al. (2015), *medium*	Developmental (GD 1–17)	CD-1 mouse, males	Yes, PND 28	Dose-dependent delays in achieving milestone.
Delayed developmental landmark (vaginal opening)	Das et al. (2015), *medium*	Developmental (GD 1–17)	CD-1 mouse, females	Yes, PND 30	

Hepatic effects

The epidemiological evidence base for PFNA was found to provide *moderate* evidence of effects, specifically consistently increased serum ALT, AST, and GGT in adults (results in children were inconsistent). All nine *medium* confidence studies that evaluated ALT in adults found positive

associations between serum PFNA and ALT with statistical significance reported in six of the nine studies, making it the focus of dose-response modeling (see Section 3.2.3 and Table 3-14). Focusing on serum ALT was further supported as it is highly abundant in the liver and injury to the organ leads to increased serum ALT levels. It is present at relatively low levels in non-hepatic tissues and serum elevations related to non-hepatic injury are reported to be uncommon, although severe muscle injury may increase ALT levels in the blood (i.e., it is sensitive but not specific to liver injury) (Newsome et al., 2018; Thulin et al., 2014). While GGT and AST may also be increased in response to liver injury, these enzymes are abundant in many other tissues, making it possible for elevations to occur by non-hepatic pathways; therefore, GGT and AST were not advanced for dose-response analysis (Newsome et al., 2018; van Beek et al., 2013; Dufour et al., 2000).

Of the available studies reporting results for PFNA exposure and ALT outcomes in Table 3-14 (Kim et al., 2023; Liao et al., 2023; Cakmak et al., 2022; Liu et al., 2022; Attanasio, 2019; Jain and Ducatman, 2019d; Nian et al., 2019; Mora et al., 2018; Salihovic et al., 2018; Gleason et al., 2015; Lin et al., 2010), EPA focused on those studies which provided the greatest confidence that the quantitative results were unlikely to be due to confounding by other PFAS. As mentioned in Section 3.2.4, one study by Kim et al. (2023) conducted mixtures modeling to identify the independent effect of PFNA, and two other studies by Nian et al. (2019) and Cakmak et al. (2022) provided results for multiple PFAS where the effects of PFNA were among the strongest and therefore the least likely to be confounded by weaker effects of other PFAS. The studies selected for dose-response modeling were the studies by Kim et al. (2023) and Nian et al. (2019) because both studies had reported statistically significant dose-response analyses that would support deriving a POD for adverse liver effects based on ALT whereas the estimate reported in Cakmak et al. (2022) was imprecise (very wide confidence interval) (see Table 5-3).

The study by Kim et al. (2023) was determined to be the preferred choice for deriving a POD for adverse liver effects because this study was judged to have a "good" rating in the confounding domain during study review (see the heat map in Figure 3-40). Kim et al. (2023) used directed acyclic graphs (DAGs) to select potential confounders and all models included age, sex, education level, household income, smoking status, BMI, heavy drinking, regular exercise. The exposure distribution of PFNA was largely overlapping between Kim et al. (2023) and Nian et al. (2019), although Nian et al. (2019) has a wider distribution with a lower 25th-percentile. Details of the modeling for estimating the POD for PFNA are in Appendix D.1 for both Kim et al. (2023) and Nian et al. (2019). Selected POD results based on the preferred hybrid approach with two cutoff values are shown in Table 5-13 these two studies.

In support of the epidemiological evidence base, there was *robust* evidence in rodents of PFNA-induced adverse liver effects. The animal studies evaluating the effects of PFNA exposure on the liver (lower confidence studies were not considered) include six *high/medium* confidence, short-term studies in adult rats and mice (NTP, 2018; Das et al., 2017; Rosen et al., 2017; Hadrup et al., 2016; Wang et al., 2015a; Fang et al., 2012c), and three developmental toxicity studies that

reported liver weight endpoints in nonpregnant and pregnant mice, and offspring (Zhang et al., 2021; Das et al., 2015; Wolf et al., 2010). The *high* confidence 28-day gavage study in SD rats (NTP, 2018) was selected for subchronic candidate toxicity value derivation because it evaluated multiple hepatic endpoints that indicated dose-dependent and coherent evidence of PFNA-induced liver toxicity. Additionally, two *high/medium* confidence developmental toxicity studies in mice that reported liver enlargement (preference for relative liver weight) in nonpregnant and pregnant mice, as well as in offspring (Das et al., 2015; Wolf et al., 2010), and a *medium* confidence 14-day gavage study in male mice (Wang et al., 2015a) were selected for quantitative analysis in another murine species and lifestage. With respect to the offspring liver enlargements, relative liver weight increases at PND 1 and 24 in Das et al. (2015) and PND 21 in Wolf et al. (2010) were selected to model effects at birth (CD-1 mice only; not evaluated in 129S1/SvlmJ wild type mice) and at the end of the lactational term in both mouse strains. The other studies were not prioritized for modeling because there were more sensitive studies of the outcome available, or they examined only a single high dose or a shorter duration exposure. However, results of these studies were generally consistent with those selected for modeling. The consistent and dose-dependent increases in relative liver weights in adult rats and mice, as well as in rodent offspring, and the coherent hepatocyte lesions observed in adult rats in the 28-day study, were prioritized as candidates for dose-response modeling (see Section 3.2.4, "Animal Studies" and "Evidence Integration"). The clinical chemistry data reported in the 28-day study in rats and 14-day studies in mice provided generally corroborative evidence of liver injury but were ultimately not advanced for dose-response modeling due to somewhat inconsistent responses across species and sexes. The studies and endpoints selected for dose-response modeling are shown below in Tables 5-3 and 5-4.

Table 5-3. Endpoints considered for dose-response modeling and derivation of points of departure for liver effects in humans

Endpoint	Study reference and confidence	Population	POD derivation	Notes
Increased serum ALT	Kim et al. (2023), *medium*	Adults, male and female	Yes	Study examined a sub-population of the Korean National Environmental Health Survey (KoNEHS) and reported significant percentage changes in ln-ALT for log_2-unit increase in PFNA of 7.5% (95% CI: 2.3, 12.8) for men and 7.0% (95% CI: 2.2, 11.9) for women using multiple linear regression adjusted for age, sex, education, income, smoking, heavy drinking, exercise, and BMI. The regression coefficients β were calculated as 0.0723 (95% CI: 0.0227, 0.1204) ln-ALT(U/L) per log_2 (ng/mL) PFNA for

Endpoint	Study reference and confidence	Population	POD derivation	Notes
				men and 0.0677 (95% CI: 0.0218, 0.1124) ln-ALT(U/L) per $\log_2$ (ng/mL) PFNA for women.[25]
Increased serum ALT	Nian et al. (2019), *medium*	Adults, male and female	Yes	Study examined a large population of adults in Shenyang (one of the largest fluoropolymer manufacturing centers in China), part of the Isomers of C8 Health Project, and reported a significant percentage change in ln-ALT for ln-unit increase in PFNA of 6.2 (95% CI: 3.1, 9.4) using multiple linear regression adjusted for age, sex, career, income, education, alcohol consumption, smoking, giblet and seafood consumption, exercise and BMI. The regression coefficient β (for men and women combined) was calculated as 0.0602 (95% CI: 0.0305, 0.0898) ln-ALT(U/L) per ln-PFNA (ng/mL).
Increased serum ALT	Cakmak et al. (2022), *medium*	Adults and children, male and female	No	Study examined a large population of children and adults (ages 3–79 years) in Canada. And reported a nonsignificant percentage increase in log-ALT per geometric mean increase in log-PFNA of 3.6% (−1.7, 9.3) using generalized linear mixed models controlling for age, biological sex, ethnicity, BMI, smoking status (current/former/never), alcohol consumption, household education, and household income.

Table 5-4. 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, sex, and lifestage	POD derivation	Notes
Hepatocyte lesions	NTP (2018), *high* (hypertrophy, cytoplasmic alterations and vacuolization, necrosis)	Gavage, 28 d	SD rat, male and female, adult	Yes	Dose-dependent effects were generally consistent across sexes with some evidence of greater sensitivity in males, and coherent with increasing liver weight and hepatobiliary cholestasis markers.
Increased relative liver weight	NTP (2018), *high*	Gavage, 28 d	SD rat, male and female, adult	Yes	Dose-dependent effects were consistent across studies, species, sex, and lifestage

[25]Percentage increase = (eβ-1)*100) see Kim et al. (2023).

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, sex, and lifestage	POD derivation	Notes
	Fang et al. (2012c), *medium*	Gavage, 14 d	SD rat, male, adult	No	with generally greater effect magnitudes in mice (vs. rats), males, and nonpregnant mice (vs. pregnant). *High* confidence findings in adult male and female rats after 28-d exposures were selected over the 14-d and 7-d findings because they were the longest exposure. *High* and *medium* confidence findings in mouse adults and offspring were selected to consider effects in differing rodent models and lifestages. Effects in offspring at PND 1 (data available for CD-1 mice only) and around weaning were selected (PNDs 21 and 24) to compare results from similar timepoints, and to capture effects at birth.
	Hadrup et al. (2016), *medium*	Gavage, 14 d	Wistar-Hannover rat, male, adult	No	
	Wang et al. (2015a), *medium*	Gavage, 14 d	BALB/c mouse, male, adult	Yes	
	Rosen et al. (2017), *medium*	Gavage, 7 d	Wild type 29S1/SvlmJ mouse, male, adult	No	
	Das et al. (2017), *medium*	Gavage, 7 d	Wild type SV129 mouse, male, adult	No	
	Das et al. (2015), *high*	Gavage, GD 1–17	Crl:CD1 mouse, female, pregnant and nonpregnant	Yes (pregnant and nonpregnant)	
	Wolf et al. (2010), *high*	Gavage, GD 1–18	Wild type 29S1/SvlmJ mouse, female, pregnant and nonpregnant, adult	Yes (pregnant and nonpregnant)	
	Das et al. (2015), *medium*	Developmental GD 1–17	Crl:CD1 mouse, sexes combined, offspring	Yes, PND 1 and 24	
	Wolf et al. (2010), *medium*	Developmental GD 1–18	Wild type 29S1/SvlmJ mouse, sexes combined, offspring	Yes, PND 21	
	Zhang et al. (2021), *medium* (dams); *Low* (offspring)	Gavage, GD 1–18	ICR mouse, dam, and female offspring	Yes (dams); No (offspring)	

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, sex, and lifestage	POD derivation	Notes
Increased serum ALT/AST/ALP	NTP (2018), *high*	Gavage, 28 d	SD rat, male and female, adult	No	Effects in rats and mice were inconsistent. Effects in male and female rats were generally mild (<twofold increases) but pronounced in male mice at 5 mg/kg-d (7.4-fold and 3.1-fold increase in ALT and AST, respectively); however, the mouse data were not prioritized since other endpoints of longer exposure duration were available and more sensitive.
	Fang et al. (2012c), *medium*	Gavage, 14 d	SD rats, male, adult	No	
	Wang et al. (2015a), *medium*	Gavage, 14 d	BALB/c mice, male, adult	No	
Increased hepatobiliary markers (total bile acids, bilirubin)	NTP (2018), *high*	Gavage, 28 d	SD rat, male and female, adult	No	Dose-dependent effects were observed in both sexes but were not prioritized for modeling because the other liver endpoints evaluated are considered more direct measures.
Altered blood proteins (albumin, globulin, A/G ratio, total protein)	NTP (2018), *high*	Gavage, 28 d	SD rat, male and female, adult	No	Dose-dependent effects were inconsistent across sex for albumin and total protein although reduced globulin and elevated A/G ratio were aligned; however, these effects were not prioritized for modeling because the other liver endpoints evaluated are considered more direct measures.

Male reproductive effects

PFNA hazard conclusions for male reproductive effects are based primarily on *moderate* animal evidence of an effect. The epidemiological evidence base evaluating associations between PFNA exposures and male reproductive endpoints was inconsistent across studies and lacked precision for some observations. Thus, only *high/medium* confidence animal studies were considered candidates for dose-response modeling (lower confidence studies were not considered). Five *high/medium* confidence studies in rats and mice evaluated effects of PFNA exposure on male reproductive endpoints, including three in adult rats (NTP, 2018; Feng et al., 2010; Feng et al., 2009), one in juvenile rats (Hadrup et al., 2016), and one in gestationally exposed prepubertal mice (Das et al., 2015). The NTP 28-day bioassay in adult rats was found to be *high* confidence for most of the male reproductive endpoints evaluated and was prioritized for derivation of candidate

toxicity values (NTP (2018); see Table 5-5). There are some concerns in this study related to exposure insensitivity that resulted in a *low* confidence rating for the sperm measures (i.e., testicular sperm counts and motility) and the null findings for most sperm measures given that the 28-day exposure duration does not capture the entire period of spermatogenesis. There was also some residual uncertainty as to whether the sperm related effects observed were due to direct effects on sperm production/viability (suggested by some of the mechanistic evidence, e.g., Sertoli cell functioning, Leydig cell atrophy, sperm cell apoptosis) or secondary to overt effects on the organs (e.g., decreased epididymal weight). However, dose-dependent reductions in epididymal (cauda) sperm counts, while relatively modest (<40%), were observed at a dose similar (≥1.25 mg/kg-day) to other reproductive effects despite the potential insensitivity of the short-term exposure, making it suitable for quantitative analysis. The 28-day study also observed coherent testicular and epididymal histopathology and organ weight reductions that were advanced for modeling that corresponded to dose-dependent reductions in absolute epididymal (cauda and whole) and testicular weights in rats. Dose-dependent delays in preputial separation in gestationally exposed mice (also advanced as a developmental effect; see Section 3.2.2) and reductions in serum testosterone in the NTP 28-day study were also advanced for modeling, while other studies that evaluated serum and testicular testosterone in mice were not prioritized for quantitation as they were shorter exposures and/or low confidence findings.

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 lifestage	POD derivation	Notes
Decreased epididymal (cauda) sperm counts, absolute	NTP (2018), *low* due to concern for potential insensitivity that may bias results toward the null	Gavage, 28 d	SD rat, adult	Yes	Dose-dependent effects; absolute values preferred per recommendations of EPA male reproductive guidelines (U.S. EPA, 1996). Effects in the 90-d study corroborate effects in rats but were not prioritized for POD derivation because the study is *low* confidence due to inadequate reporting of methods and results presentation.
	Singh and Singh (2019b), *Low*	Gavage, 90 d	Parkes mouse, prepubertal to adult	No	

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and lifestage	POD derivation	Notes
Decreased testicular sperm counts, absolute	NTP (2018), *low* due to concern for potential insensitivity that may bias results toward the null	Gavage, 28 d	SD rat, adult	No	Effects were not dose-dependent.
	Feng et al. (2009), *low* due to concern for potential insensitivity that may bias results toward the null	Gavage, 14 d	SD rat, adult	No	Effects at higher doses than other male reproductive findings.
Reduced absolute epididymis (cauda and whole) and testis weights	NTP (2018), *high*	Gavage, 28 d	SD rat, adult (epididymis and testis)	Yes	Dose-dependent effects in epididymis (cauda and whole) and testis of rats that are coherent with other male reproductive effects.
	Singh and Singh (2019d), *medium*	Gavage, 14 d	Parkes mouse, prepubertal (testis)	No	Null findings in testis weights of mice across studies, but developmental and 14-d exposure studies provide corroborative findings of decreases of similar magnitude to rats.
	Singh and Singh (2019b), *medium*	Gavage, 90 d	Parkes mouse, prepubertal to adult (testis)	No	
	Singh and Singh (2019c), *low* confidence	Gavage, GD 12–21	Parkes mouse, PND 3	No	
Testis and epididymis lesions/ histopathology	NTP (2018), *high*	Gavage, 28 d	SD rat, adult	Yes	In adult rats (28-d), germ cell degeneration, spermatid retention in seminiferous tubules, Leydig cell atrophy in testes, and ductal germ cell exfoliation in epididymis. Consistent effects observed in other shorter duration (14-d) or *low* confidence studies, results from these studies were not selected.
	Feng et al. (2009), *low*	Gavage, 14 d	SD rat, adult	No	
	Feng et al. (2010), *low*	Gavage, 14 d	SD rat, adult	No	
	Singh and Singh (2019b), *low*	Gavage, 90 d	Parkes mouse, prepubertal to adult	No	
	Singh and Singh (2019d), *low*	Gavage, 14 d	Parkes mouse, prepubertal	No	

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and lifestage	POD derivation	Notes
Reduced serum and testicular testosterone	NTP (2018), *high* (serum)	Gavage, 28 d	SD rat, adult	Yes	Consistent and dose-dependent effects in adult rats (28-d) that are coherent with other reproductive effects. Generally consistent results in other studies but of shorter exposure duration (14-d) and/or *low* confidence so not selected.
	Singh and Singh (2019d), *medium* (serum and testes)	Gavage, 14 d	Parkes mouse, prepubertal	No	
	Singh and Singh (2019b), *low* (serum)	Gavage, 90 d	Parkes mouse, prepubertal to adult	No	
	Singh and Singh (2019c), *low* (testes)	Gavage, GD 12–21	Parkes mouse, PND 3	No	
Delayed reproductive development (preputial separation)	Das et al. (2015), *medium*	Developmental (GD 1–17)	CD-1 mouse, pubertal	Yes, PND 28	Dose-dependent delays in gestationally exposed mice (also selected as a developmental endpoint).

Immune effects

The overall hazard conclusion is that the ***evidence suggests*** but is not sufficient to infer that PFNA exposures may cause immune system effects in humans. This conclusion is based on the epidemiological evidence base that provides *slight* evidence of associations between PFNA exposures and immunosuppression related to reduced anti-tetanus and anti-diphtheria antibody concentrations in young children following vaccination. While there was considerable uncertainty with the epidemiological results due to a weaker pattern of results than other correlated PFAS, less consistency, a smaller magnitude of effect, and a greater potential for confounding with other co-occurring PFAS, the immunosuppression data were advanced for dose-response modeling given that this effect is observed with other PFAS (PFDA, PFOA, PFOS, PFHxS). The endpoints amenable to pursue BMD modeling were decreased serum antibody concentrations data from Grandjean et al. (2012) and Grandjean et al. (2017b). These published associations used continuous log-transformed antibody concentrations as well as continuous PFNA concentrations that had been log-transformed. Such analyses, while appropriate for hazard identification, are not statistically suitable for deriving BMDLs as PODs based on published results. If categorical results had been presented using the logged exposures, then a NOAEL or LOAEL approach for estimating a POD could have been undertaken, but those results were not reported. However, Budtz-Jørgensen and Grandjean (2018a) provided analyses using untransformed PFNA concentrations of the Grandjean et al. (2012) results that allowed for their use in deriving BMDLs as PODs in children (aged 5 and 7 years). The BMD modeling of the selected *medium* confidence epidemiology studies by Grandjean

et al. (2012) using untransformed PFNA concentrations by Budtz-Jørgensen and Grandjean (2018a) did not show statistically significant effects of PFNA on antibody concentrations in children aged 5 and 7 years in both the single-PFAS model and in the multi-PFAS model of PFNA controlling for PFOS and PFOA. Thus, BMDs and BMDLs for the effects of PFNA on childhood antibody concentrations to diphtheria and tetanus are provided in Appendix D for completeness and informational purposes but are not advanced as PODs for presentation alongside other PODs and are not considered for use in derivation of candidate toxicity values (see Table 5-6 and Appendix D.1). The animal toxicology evidence base was found to be *indeterminate* and generally limited to observational or *low* confidence studies that were not considered informative for modeling.

Table 5-6. Endpoints considered for dose-response modeling and derivation of points of departure for immune effects in humans

Endpoint	Study reference and confidence	POD derivation	Notes
Antibody concentrations for diphtheria and tetanus	Grandjean et al. (2012) and Budtz-Jørgensen and Grandjean (2018a); Grandjean et al. (2017b), *medium*	BMDs and BMDLs derived (see Appendix D.1), but these were not advanced as PODs	Effect of log-transformed PFNA on log-transformed diphtheria antibodies was consistently decreased across multiple time intervals in Grandjean et al. (2012) and was significant for PFNA and diphtheria measured at 5 yr. However, the untransformed results by Budtz-Jørgensen and Grandjean (2018a) indicated null effects of PFNA on antibody concentrations in children aged 5 and 7 yr. Thus, results were carried forward for BMD and BMDL calculations for possible future comparisons but are not advanced further.
Antibody concentrations for diphtheria and tetanus	Grandjean et al. (2017a), *medium*	No	While these results contribute to understanding the hazard for PFNA, 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.

Thyroid effects

The overall hazard conclusion is based primarily on the *high* confidence 28-day bioassay in adult rats showing large, dose-dependent reductions in serum free and total T4 in females and serum free T4 in males (see Table 5-7) (NTP, 2018). Although this study suggests that PFNA exposure may cause effects, it was not sufficient to infer a hazard (and the epidemiology evidence was found to be *slight* with largely inconsistent results across age groups.) However, given that other PFAS have been observed to affect the thyroid and concerns for potential downstream effects of thyroid hormone (TH) perturbations on fetal brain development and growth, and since a *high* confidence study was available, PODs were derived in females for comparison with other PFNA PODs and to inform UF selection. The free T4 reduction in males was not prioritized due to body weight loss at the higher end of the dose range that made interpretation difficult. Further, while

free T4 is the form that exerts cellular activity, the total T4 immunoassay results are considered to be more reliable than the free T4 results, as the latter used analog RIA methods that could have over-estimated the free T4 reductions (see Section 3.2.6, "Thyroid and adrenal hormones"). For this reason, total, but not free, T4 was prioritized for modeling.

Table 5-7. Endpoints considered for dose-response modeling and derivation of points of departure for thyroid effects in animals

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derivation	Notes
Decreased serum total T4	NTP (2018), *high*	Gavage, 28 d	SD rat, adult female	Yes	Dose-dependent and large decreases in free and total T4 in females and free T4 in males; effects in males were not prioritized due to weight loss at higher doses and free T4 was not prioritized in either sex due to uncertainty regarding the reliability of the free T4 measures.
Decreased serum free T4	NTP (2018), *high*	Gavage, 28 d	SD rat, adult female	No	
Decreased serum total T4	NTP (2018), *high*	Gavage, 28 d	SD rat, adult male	No	
Decreased serum free T4	NTP (2018), *high*	Gavage, 28 d	SD rat, adult male	No	

Estimation or Selection of Points of Departure (PODs) for Oral Reference Dose (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 guideline 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 guideline recommends alternative BMRs, specifically a BMR of 1 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). The BMRs selected for dose-response modeling of PFNA-induced health effects are listed in Table 5-8 along with the rationale for their selection. For effects on offspring body weights, a BMR of 5% relative deviation from the control mean is used for continuous data (and 5% extra risk for dichotomous data)(U.S. EPA, 2012a).

Table 5-8. Benchmark response levels selected for benchmark dose (BMD) modeling of PFNA health outcomes

Endpoint	BMR	Rationale
Developmental effects		
Decreased birth weights, humans	5% extra risk of exceeding adversity cutoff (hybrid approach)	A 5% extra risk is commonly used for dichotomous developmental endpoints as recommended by EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a). For birth weight, a public health definition of low birth weight exists, and the hybrid approach (see *EPA's Benchmark Dose Technical Guidance*; Section 1.3.3. Approaches to BMD Computation) was used to estimate the dose at which the extra risk of falling below that cutoff equaled 5%.
Decreased offspring body weight, mice	5% relative deviation	5% change was selected because the developmental effects were observed during a potentially sensitive lifestage. Additionally, a 5% change in markers of growth/development in gestational exposure studies has generally been 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, 2016c, 2012b, 2004, 2003).
Increased offspring mortality, mice	1% extra risk	Although 5% extra risk is generally supported for developmental and reproductive outcomes with dichotomous data (U.S. EPA, 2012a), a lower BMR of 1% ER was considered appropriate for modeling offspring mortality due to the severity of the effect.
Delays in attainment of developmental landmarks (eye opening, vaginal opening, preputial separation) and increased offspring relative liver weights, mice	5% relative deviation	A 5% change was selected because the developmental effects were observed during a potentially sensitive lifestage. Additionally, a 5% change in markers of growth/development in gestational exposure studies has generally been 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, 2021c, 2016c, 2012b, 2004, 2003).

Endpoint	BMR	Rationale
Hepatic effects		
Increased serum ALT, humans	10% extra risk exceeding adversity cutoff (hybrid approach)	Both extra risks of 5% and 10% were considered. A BMR of less than 10% can be supported for severe or debilitating health outcomes. Given the findings of associations between increased ALT and severe liver disease (Park et al., 2019), a BMR of 5% was considered. However, modest elevations in ALT are more likely associated with milder forms of liver injury, including steatosis and NAFLD. Due to uncertainties in measuring ALT, in selecting the most appropriate upper limit of normal (and the difficulty in interpreting specific elevations above the upper limit of normal as adverse), and in selecting the reference population, a BMR of 10% extra risk was selected as a "minimally adverse" effect and as a standard reporting level per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a).
Increased relative liver weight, rats (adults)	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 (U.S. EPA, 2010, 2009).
Increased hepatocyte lesions/histopathology, rats	10% extra risk	No information is available regarding the change in these responses that would be considered biologically significant. Given that the biological information is not sufficient to identify the BMR, a 10% extra risk for dichotomous data was selected for this endpoint, consistent with recommendations of EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a).
Male reproductive effects		
Increased testis and epididymis lesions/histopathology, rats	10% extra risk	No information is readily available that allows for determining a minimally biological significant response. Given that the biological information is not sufficient to identify the BMR, a 10% extra risk for dichotomous data was selected for this endpoint, consistent with recommendations of EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a).
Decreased absolute epididymal sperm count, rats	1 standard deviation	No information is available that allows for determining a minimally biological significant response. Given that the biological information is not sufficient to identify the BMR, a 1 SD for continuous data was selected for these endpoints, consistent with recommendations of EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a).
Decreased serum testosterone, rats		
Decreased absolute testicular weight, rats		
Decreased absolute epididymal weight, rats		
Thyroid effects		
Decreased serum total T4, adult female rats	1 standard deviation	Toxicological evidence to support identification of a minimally biologically significant response is lacking in adult animals. Additionally, the level of response in chemical-induced serum

Endpoint	BMR	Rationale
		T4 insufficiency associated with neurodevelopmental effects is unclear, with decreases of approximately 10–25% in serum T4 identified as potentially concerning in some human studies (e.g., (Korevaar et al., 2016; Haddow et al., 1999)) and rodent studies (e.g., (Gilbert et al., 2016; Gilbert, 2011)). Given that the biological information is not sufficient to identify the BMR and the decreases in serum T4 (up to 53%) in female rats exceed these values, a 1 SD for continuous data was selected for this endpoint, consistent with EPAs *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a).

Where modeling was feasible, the estimated BMDLs were used as PODs (see Tables 5-9 and 5-10). Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in Appendix D. Where dose-response modeling was not feasible, or adequate modeling results were not obtained, NOAEL or LOAEL values were identified on the basis of biological rationales when possible and used as the POD. For example, for 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 Tables 5-11 to 5-16.

Modeling results in humans (decreased birth weight)

The epidemiological studies selected for BMD modeling of mean reductions in birth weights are listed in Table 5-9. The internal doses reported in the human studies were converted to human equivalent doses (HEDs) using CL_H set to a value of 0.09 or 0.124 mL/kg-day, which are assumed to be appropriate mean values for all males and females of corresponding lifestages (see Table 5-9 and the associated discussion below on extrapolating between rodents and humans, and Section 3.1.8). The BMD modeling results of the human evidence are presented below and summarized in further detail in Appendix D.1.

Three *high* confidence studies reported decreased birth weight in infants whose mothers were exposed to PFNA with exposure sampling in early pregnancy were selected as described above (Wikström et al., 2020; Sagiv et al., 2018; Manzano-Salgado et al., 2017a). BMD modeling was performed for these studies (see Appendix D.1.3). Table D-9 in Appendix D.1.3 presents the BMDs and BMDLs for the individual studies considered for POD derivation using two methods. The results under the column of "Exact Percentage" in Table D-9 were derived by using 8.27% as the probability of an adverse response at zero dose and a BMR of 5% extra risk. The 8.27% value was calculated from the CDC Wonder site (https://wonder.cdc.gov/natality.html) and represents the percentage of live births in 2018 that fall below the standard public health definition from WHO of

low birth weight (i.e., <2,500 g) (WHO, 2004). BMDLs among these studies were 0.56 to 1.25 ng/mL using this method. The probability of an adverse response at zero dose (8.27%) in the above method was calculated without accounting for the existence of background PFNA exposure in the U.S. population. EPA performed sensitivity analyses accounting for the existence of background PFNA exposure (0.4 ng/mL from America's Children and the Environment (ACE) Biomonitoring on Perfluorochemicals) in the U.S. population using an alternative assumption on the control group response distribution for the cutoff of low birth weight on BMDLs (see Appendix D.1.3). This alternative approach, presented under the column of "Alternative Tail Probability" in Table D-9, was very similar to the main results, presented under the column of "Exact Percentage." The range of BMDLs among the three selected individual studies was 0.95 to 1.67 ng/mL using this alternative approach.

In addition to these five studies, epidemiologic data were also available on another 22 studies examining mean birth weight deficits in relation to PFNA. Meta-analyses were conducted on all 27 studies and different strata based on study confidence and sample timing (see Appendix D.1.4). BMDs and BMDLs were calculated using the same approach as above for the summary estimates from meta-analyses. The BMDLs for the 11 earlier sampled study subsets (1.87 ng/mL), the 10 earlier sampled study subsets (1.81 ng/mL), and the *high* and *medium* confidence study subsets (1.70 ng/mL) were very comparable to that from the overall full set of studies (1.68 ng/mL) (see Table D-11).

Table 5-9. BMDs and BMDLs for effect of PFNA on decreased birth weight, by using percentage (8.27%) of live births falling below the public health definition of low birth weight, or alternative tail probability

Study and Confidence	Exposure median (IQR) (ng/mL)	Reported β (95% CI)	Re-expressed β (95% CI) (g/ng/mL)	Exact percentage[a] $P(0) = 8.27\%$)		Alternative tail probability[b]	
				BMD (ng/mL)	BMDL (ng/mL)	BMD (ng/mL)	BMDL (ng/mL)
Sagiv et al. (2018), *high*	0.7 (0.5–1.0)	−28.2 (−52.0, −4.4) g/IQR (ng/mL)	−56.4 (−104.0, −8.8)	2.04	1.19	2.47	1.45
Valvi et al. (2017)[c], *high*	0.59 (0.46–0.79)	−42.0 (−108.0, 25.0) g/log$_2$(ng/mL)	−101.0 (−259.8, 60.1)	1.31	0.56	1.43	0.62
Manzano-Salgado et al. (2017a), *high*	0.66 (0.36)	−10.3 (−38.1, 17.6) g/log$_2$(ng/mL)	−24.9 (−92.5, 42.7)	4.11	1.25	5.46	1.67
Starling et al. (2017)[c], *high*	0.4 (0.3–0.6)	−57.6 (−104.1, −11.2) g/ln(ng/mL)	−140.2 (−253.3, −27.3)	1.06	0.63	1.07	0.64

Study and Confidence	Exposure median (IQR) (ng/mL)	Reported β (95% CI)	Re-expressed β (95% CI) (g/ng/mL)	Exact percentage[a] $P(0) = 8.27\%$)		Alternative tail probability[b]	
				BMD (ng/mL)	BMDL (ng/mL)	BMD (ng/mL)	BMDL (ng/mL)
Wikström et al. (2020)	0.53 (0.39–0.73)	−46.0 (-89.0, −4.0) g/ln(ng/mL)	-84.9 (−164.3, −7.4)	1.49	0.84	1.68	0.95
Meta-analysis; all 27 birth weight studies	–	−32.9 (−47.0, −18.7) g/ln(ng/mL)	−48.9 (−69.9, −27.8)	2.29	1.68	2.83	2.08
Meta-analysis; 22 *medium* and *high* confidence birth weight studies	–	−32.9 (−48.0, −17.8) g/ln(ng/mL)	−47.3 (−69.1, −25.6)	2.35	1.70	3.93	2.11
Meta-analysis; 11 early pregnancy birth weight studies (all *medium* or *high* confidence)	–	−22.0 (−40.1, −4.0) g/ln(ng/mL)	−33.5 (−61.0, −6.1)	3.16	1.87	4.09	2.42
Meta-analysis; 10 early pregnancy birth weight studies (all *medium* or *high* confidence)	–	−22.8 (−41.0, −4.6) g/ln(ng/mL)	−35.2 (−63.3, −7.1)	3.03	1.81	3.90	2.33

– = no data available.

[a]The exact percentage (8.27%) of live births in the U.S. in 2018 that fell below the cutoff of 2,500 g as the tail probability to represent the probability of extreme ("adverse") response at zero dose ($P(0)$).

[b]The alternative tail probability method of live births falling below the public health definition of low birth weight.

[c]Included for comparison purposes. These results are discussed more fully in Appendix D.1.3.

For decreased birth weight associated with PFNA exposure, the POD selected from the available epidemiologic literature is 1.81 ng/mL based on the 10-study meta-analysis of *high* and *medium* confidence studies with maternal sampling collected predominately during early pregnancy that helped mitigate concerns for potential bias due to pregnancy-related hemodynamic effects (see Appendix C.1.4). Another "early pregnancy" study by Robledo et al. (2015) was excluded from this subset as it examined a different critical exposure period, namely measurement of maternal serum PFNA preconception (up to 12 months prior to pregnancy). Thus, EPA considered the 10 studies of early maternal sampling during pregnancy to represent the most appropriate and homogeneous (study design-wise) study grouping.

EPA evaluated using a POD based on the meta-analysis compared with use of a single study, weighing the benefit provided by evidence from the combined studies against the additional

uncertainty and bias introduced by the re-expression necessary to calculate the meta-analysis POD but not the individual study POD. Calculation of the meta-analysis POD required the application of re-expression in two stages. First, the effect estimates from all 10 studies needed to be on the same scale. Since eight effect estimates were based on log-transformed exposure, the two effect estimates that were on the natural scale needed to be re-expressed from the natural scale to the log scale. The meta-analysis resulted in a summary effect estimate of the effects of exposure on the log scale. In order to conduct the BMD modeling, a second stage of re-expression of the summary effect estimate from the log scale to the natural scale of exposure was performed. A recent study by Linakis et al. (2024) examined the uncertainty introduced by the re-expression method and found a systemic bias in the direction of a larger effect estimate that is related to the magnitude of the variance of the exposure (i.e., an overestimation of the true effect estimate when converting from the log scale to the natural scale). Converting from the natural to the log scale is expected to have a similar systemic bias but in the direction of a smaller effect estimate. Specifically, EPA estimated that the average systemic bias from re-expression of log scale to the natural scale based on an exposure distribution similar to that used in the POD derivation (sigma = 0.52) would be approximately 30% on the summary effect estimates (see Figure 2 and S1, Panel B in Linakis et al. (2024)). While the magnitude of this bias on the BMD and BMDL cannot be estimated with confidence, it is expected to be approximately 30% if the bias on the effect estimates from re-expression is 30%. In this instance, bias away from the null in the effect estimate would result in a lower BMD and BMDL than would be estimated without this bias from re-expression.

The resulting PODs for the meta-analysis and individual studies are relatively close together giving confidence that either choice is suitable to inform the RfD for this endpoint (see Table 5-9). PODs from the meta-analyses of all studies combined (1.68–1.87 ng/mL), including the 10-study early pregnancy subset (1.81 ng/mL) were consistent in relative magnitude to PODs from the individual studies with early sampling subsets (0.56 to 1.25 ng/mL). Of the individual studies for which PODs were derived, there was less uncertainty in two studies with the earliest collected sampling (Wikström et al., 2020; Sagiv et al., 2018); the PODs for Sagiv et al. (2018) and Wikström et al. (2020) were 1.19 and 0.84 ng/mL, respectively. Despite modestly lower BMDLs in the individual studies, these results were not selected for the POD since less uncertainty is anticipated from the meta-analysis result of 10 studies using maternal biomarkers collected early in pregnancy across a set of studies able to capture greater data diversity (e.g., increased heterogeneity in populations and PFNA exposure distributions as compared with individual studies). The limitation of the POD from meta-analysis, compared to the single study PODs, is bias introduced from the re-expression. This bias is expected to result in a lower POD than the value that would be calculated without bias. Therefore, it is not justified to select a still lower value for the POD from single studies in order to avoid the limitation of bias from re-expression. EPA determined that the large amount of additional data supporting the meta-analysis of 10 early sampling time studies outweighed the bias

introduced by the re-expression method, supporting its selection for the POD (see meta-analyses results and methodology in Section C.1.5 and Wright et al. (2023)).

For details on the meta-analysis methods for evaluating decreased birth weight, including study inclusion criteria, data scaling, and statistical and sensitivity analyses, see Appendix C.1. For details on the BMD analysis using the meta-analysis and individual study results, see Appendix D.1.

Modeling results in humans (increased serum ALT)

The epidemiological studies selected for BMD modeling of increased serum ALT are listed in Table 5-3. The internal doses reported in the human studies were converted to HEDs using CL_H set to a value of 0.090 mL/kg-day, using the estimated clearance for men and women above age 40. The summarized BMD modeling results of the human evidence are presented in Table 5-10 and are provided in further detail in Appendix D.1.

Two *medium* confidence studies, Nian et al. (2019) and Kim et al. (2023), were selected for the POD derivation. EPA derived multiple estimates of the POD from these two studies, for men and women, applying different benchmark responses (BMRs) and different approaches to define adverse changes. EPA used three approaches from the EPA Benchmark Technical Guidance (U.S. EPA, 2012a) to estimate PODs: 1) the hybrid approach which uses either a biologically based cutoff in the distribution of ALT concentrations to define a level above which ALT may be interpreted as abnormal, or a percentile-based approach to define such a level, and a BMR of 10% (or 5%) extra risk beyond that cutoff to estimate a magnitude of exceedance above this cutoff that is (minimally) adverse (see Tables D-14 and D-15 in Appendix D.1.5); 2) the standard deviation approach which defines the BMR as a change in the mean of one standard deviation (SD) (or ½ SD) (see Table D-16 in Appendix D.1.5); and 3) the NOAEL/LOAEL approach.

Tables D-14, D-15, and D-16 in Appendix D.1.5 show the BMDs and the BMDLs for men and women based on Kim et al. (2023) using all three approaches and two options of defining the hybrid cutoffs from Valenti (2021). The range of BMDL values is 1.3 ng/mL to 16.1 ng/mL where the lower limit is based on a BMR of 5% and the upper limit is based on the BMR of 1 SD, the latter of which was equivalent to an extra risk of 38% using the hybrid approach. Even with a wide range of different methods to derive a BMDL, there is only a one order of magnitude difference across BMD methodologies within one sex and one study. Table 5-10 shows the BMD and the BMDL from Appendix Table D-14 for men and women based on Kim et al. (2023).

Table 5-10. BMDs and BMDLs for effect of PFNA on increased serum ALT in humans using the hybrid approach and a BMR of 10% extra risk of for liver effects defined as increased risk of liver disease.

Study reference and confidence	Gender	Exposure median (IQR) (ng/mL)	Slope of dose-response β (95% CI)[a]	BMD (ng/mL)	BMDL (ng/mL)
Kim et al. (2023), medium	Men	2.24 (1.65, 3.23)	0.0723 (95% CI: 0.0227, 0.1204) ln-ALT (U/L) per log_2 (ng/mL) PFNA	3.45	2.20
	Women	1.79 (1.21, 2.66)	0.0677 (95% CI: 0.0218, 0.1124) ln-ALT (U/L) per log_2 (ng/mL) PFNA	2.99	2.02
Nian et al. (2019), *Medium*	Men	2.19 (1.33, 3.24)	0.0602 (95% CI: 0.0305, 0.0898) ln-ALT (U/L) per ln (ng/mL) PFNA	9.20	4.81
	Women	1.31 (0.68, 2.23)	0.0602 (95% CI: 0.0305, 0.0898) ln-ALT (U/L) per ln (ng/mL) PFNA	7.09	4.00

[a] See Appendix D.1 for details on how the published dose-response in Kim et al. (2023) was transformed from units of percentage change in ln-ALT per doubling in log_2 PFNA as shown in Table 3-14.

Modeling results in animals

The experimental animal endpoints selected for BMD modeling are listed in Tables 5-2, 5-4, 5-5, and 5-7. Tables 5-12, 5-14, 5-15, and 5-16 present each endpoint's POD derived from either BMD modeling or NOAEL/LOAEL characterization, their corresponding HED or POD_{HED}) along with the data-derived extrapolation factor (DDEF) used to calculate the HED. The pharmacokinetic (PK) modeling approach used to calculate the DDEFs, described in detail in the following section, assigns constant DDEFs among dose groups for all endpoints, therefore it was appropriate to convert BMD-derived PODs to HEDs after modeling results based on the animal doses (U.S. EPA, 2012a) The BMD modeling results of the animal evidence are presented in Appendix D.2.

Approach for pharmacokinetic modeling and extrapolating PFNA between rats, mice and humans

EPA's preferred approach for animal-human extrapolation and interpretation of human dosimetric data is by physiologically based pharmacokinetic (PBPK) modeling, provided that a sufficiently reliable model is available. In the absence of such a model, the second-tier approach is to apply chemical-specific PK data that establish the relationship between exposure and internal dose, although not formally integrated into a model. For oral exposures interspecies scaling can also be accomplished by the default method of $BW^{0.75}$ scaling if neither a PBPK model nor the data to inform extrapolation are available.

As explained more fully in Section 3.1.6, the published PBPK models for PFNA were not considered suitable for application due to uncertainty in the underlying assumptions and model structure and concerns with estimation of parameters for humans. Because clearance of PFNA in humans is significantly underpredicted by allometric scaling ($BW^{3/4}$) that option was also not considered adequate. Three other options evaluated for animal-human extrapolation were use of the custom PK model described in Appendix E.4.1, interpolation of blood concentrations measured in toxicity studies, and use of a DDEF. (Derivation of the DDEF is described in Appendix E.5 with resulting DDEFs for various animal-human extrapolations provided in Table E-8.) The custom PK model was found to predict available PK data for male and female mice and male rats better than an assumption of steady state, which is implicit in use of a DDEF. However, the best option for estimating internal doses of rats in the NTP bioassay was determined to be direct analysis of measured end-of-study PFNA concentrations in that study, with the interpolation approach informed by the PK model results. Hence animal-to-human extrapolation of internal dose PODs to HEDs will be conducted using a hybrid PK approach for all endpoints, where:

1) internal doses (average serum concentrations) for endpoints obtained in male and female mice are estimated using the PK model,

2) internal doses in rats are interpolated from the measured end-of-study concentrations as described in Appendix E.4.1, and

3) HEDs are then calculated from the corresponding internal dose PODs using lifestage appropriate values of human CL.

For the purpose of comparison, the HED calculated via DDEF is provided for of the endocrine endpoint in Table 5-16 below. However, the largest impact is on HED calculation for developmental endpoints observed in mice (values by interpolation in Table 5-12), where the PK model predictions of internal dose are significantly lower than the steady-state values and demonstrably closer to measured plasma concentrations (see Appendix E.4.1, Figure E-8).

Whether or not the PK model is used to estimate internal doses for animal toxicity studies, the human version of the PK model was considered too uncertain for direct application. However, results with the model indicate that humans are likely to be within a factor of 1.2 of their steady-state concentration for most of their lifetimes given chronic exposure and while some exceedance of this range is predicted in children, they are still expected to be within a factor of 1.5 (see Section 3.1.6, "Classical pharmacokinetic modeling in humans" and Appendix E.4.2). When an internal dose POD (POD_{int}), specifically a serum concentration, is obtained from human epidemiological studies for birth weight effects correlated with maternal serum levels measured during or at the end of pregnancy, the HED_{DDEF} was calculated as:

$$HED_{CL} = POD_{int} \times CL_{H,} \quad (5\text{-}1)$$

using the estimate for human clearance in women of reproductive age, CL_H = 0.124 mL/kg-day.

When the POD_{int} is a serum concentration obtained from human epidemiological studies with observations outside of the perinatal period (e.g., in 7-year-old children), the HED was similarly calculated using the CL_H for males and females <12.4 (or >40) years of age, CL_H = 0.090 mL/kg-day. Likewise, CL_H = 0.090 mL/kg-day is used for reproductive effects in adult males and hepatic effects in men and women, since hepatic effects can occur in older women for whom the lower level of CL_H applies. Hepatic effects are also considered relevant to women below age 40 but a lower clearance is needed to obtain an HED that is protective for all women.

For gestational and lactational exposure, EPA evaluated exposure to fetuses and young offspring (mouse pups up to 7 days of age and human infants) based on predicted or measured serum levels in the mouse dam or human mother. The human maternal clearance for women of reproductive age was used for the corresponding endpoints since the majority of the perinatal data in women indicate that their blood concentrations will be at or below their pre-pregnancy concentrations, which are determined by that clearance level. This approach assumes that if human maternal serum levels remain at or below the corresponding average serum concentrations in the mouse dam (calculated from the start of gestation through the time of endpoint observation up to PND 7), then the exposure to the human child will likewise be below those in the mouse pups where the endpoint was observed.

For endpoints observed in older mouse pups (≥ PND 15), the average serum concentration predicted using the PK model in the mouse pup from the start of gestation to the day of observation was used as POD_{int}, assuming the concentration in the mouse fetus is equal to the mouse dam. (Details of the PK simulations for mice are as described in Appendix E.4.1.) In this case the serum concentration in the offspring is assumed to determine the potential health effect and clearance in children was assumed to equal that estimated for human males and nonreproductive age females. Since PK model simulations predict that a breastfed human child may experience serum concentrations greater than steady state, but for a limited period of time (given the same dose as the mother, Appendix E.4.2), the implicit assumption of steady state in the child is judged by EPA to be a reasonable method of estimating the average internal dose among children.

Analysis of uncertainty in the pharmacokinetic extrapolation for PFNA

Uncertainty in the estimated clearance in experimental animals and humans is discussed in detail in Sections 3.1.7 and 3.1.8, respectively. For experimental animals, the uncertainty in the species- and sex-specific mean values is judged to be less than a factor of 2 while for humans the uncertainty in the sex- and lifestage-specific values is judged to be less than a factor of 3. For both rats and humans, the clearance values estimated in Section 3.1.4 and resulting extrapolation approaches described above are intended to predict species- or population-average differences in PFNA dosimetry for relevant lifestages. There is also interindividual variability within each group. For example, differences in BW growth over time are expected to have a direct impact on the blood concentration of PFNA. Such variability is often grouped together with "uncertainty," such as when

applying the "uncertainty factor" for intra-human variability (UF_H), which is done in conjunction with dosimetric extrapolation because it is known that humans vary in their pharmacokinetic parameters and pharmacodynamic sensitivity. But for the purpose of estimating population-average dosimetry, such variability is not a source of uncertainty. For example, uncertainty in the population-average CL for women was evaluated in Section 3.1.8, but the difference between the average CL in women and the CL for any individual is not considered a source of uncertainty with regard to the average CL estimate. Instead, interindividual variability is a matter of uncertainty in the application of the estimated HEDs when predicting risk for the most sensitive individuals. The later uncertainty is addressed by application of the UF_H.

Analysis of general uncertainty in the use of compartmental pharmakinetic (PK) modeling or measured concentrations in rats versus DDEFs

While the probability may be low that both (1) the true clearance in any species and sex for rats or mice is twofold higher than the estimated mean values and (2) the corresponding human population mean clearance is threefold lower than the values selected for HED calculation, if both of those were the case then the currently estimated HEDs could be overpredict by a factor of 6. As shown in Appendix E.4.1, Figure E-7 and Table E-8, use of the male rat CL and assumption of steady-state concentration, which is implicit in use of the DDEF, overpredicted the observed concentrations observed at the end of the NTP bioassay in males by as much as 2.5-fold. Hence, the direction and extent of uncertainty for application of the DDEF to endpoints from male rats could be high. Given that the two-compartment (2-C) PK model more closely matches the male rat data, strongly indicating that the serum concentrations in male rats accumulates throughout the exposure (see Appendix E.4.1, Figure E-7), use of the 2-C PK model for male rats is considered to have lower uncertainty than the DDEF. The error in the 2-C PK model versus the NTP data in Figure E-7 indicates that the PK model would err in the direction of health protection by estimating a lower POD_{int}, while the DDEF would not.

As discussed above and in Section 3.1.6, "Classical pharmacokinetic modeling in experimental animals," linear interpolation among the measured concentrations at the end of the NTP study was used for corresponding POD_{int} values in rats. Since the relationship between the end-of study serum concentrations and exposure level in male rats had modest nonlinearity up to a dose of 2.5 mg/kg-day while that in female rats was quite linear up to 6.25 mg/kg-day (see Appendix E, Figure E-7), the coefficient of variation for the measured concentration at a dose of 2.5 mg/kg-day in male rats, 13%, and 6.25 mg/kg-day, 28%, is considered a reasonable estimate of the uncertainty in the interpolated end-of-study average values. It is difficult to then estimate the additional uncertainty in assuming that C_{avg} is equal to these end-of-study values for female rats, or one half the end-of-study values for male rats. But given the *robust* evidence that the half-life of PFNA in female rats is between 2 and 3 days, while that for male rats is between 23 and 68 days (see Table 3-2), it seems unlikely that C_{avg} is more than 50% above or below the end-of-study concentration. in female rats or departs by more than that factor from linear accumulation with

time in male rats over the 28 days of exposure. Together these uncertainties suggest that the approach for interpolating the measured concentrations to obtain POD_{int} has an uncertainty of less than 1.5-fold and can be considered more accurate than use of the 2-C PK model.

For mice evaluated in a developmental bioassay the 2-C PK model overpredicted the observed concentrations to a large extent (see Appendix E.4.1, Figure E-8) but not nearly as much as the estimated steady-state concentrations. The 2-C PK model also allows one to evaluate internal dose for toxicity endpoints observed at different gestational and postnatal times in a way that would be complicated using only the empirical serum concentration data. Decisions would be required on how to interpolate the PK measurements for each time point (GD 19, PND 7, PND 15) if one wished to use that approach. For mouse developmental studies use of the 2-C PK model has lower uncertainty than use of the DDEF approach. While predicted concentrations in PND 21 (study day 42) pups exceeded measured levels by as much as an order of magnitude, the model allows calculation of C_{avg} in a rational manner that is consistent across dose levels and endpoint observation days when attempting to identify the sensitivity of various endpoints on the basis of internal dose.

For nonpregnant female mice, while simulations of 3 mg/kg-day repeated dosing overpredicted the observed serum concentration significantly, predictions for 1 and 5 mg/kg-day predicted the observed data quite well (see Appendix E.4.1, Figure E-8). Hence, the 2-C PK model was also judged by EPA to have lower uncertainty than the DDEF for interpreting endpoints in adult mice outside of pregnancy and to be preferable over interpolation for comparisons across endpoints.

In summary, uncertainty in use of the interpolated NTP plasma concentration data for rats should be less than a factor of 1.5 and this approach should give a fairly unbiased estimate of internal dose, rather than using the steady-state values calculated from the estimated CL values. While there is more uncertainty in use of the mouse PK model to estimate internal doses for the mouse developmental studies, the model predictions are clearly better than using the steady-state concentration estimated from CL in mice. The dosing regimen and limited PK data for mouse developmental studies make the option of interpolating the data complex and also uncertain. Hence, use of the PK model to estimate internal doses is the best option for the mouse developmental studies, and endpoints in nonpregnant adult mice, despite an uncertainty of perhaps an order of magnitude.

Analysis of uncertainty from pharmacokinetic (PK) extrapolation for chronic and developmental exposures

The results described in Appendix E.4.1 indicate that use of PK parameters estimated from single-dose PK studies using classical PK analyses, albeit with adjustment for observed changes in body weight over the course of those experiments, did not predict dosimetry during the NTP rat bioassay with good accuracy (see Figure E-7). Further, there were large discrepancies between predictions and many measured serum levels for pregnant mice and their pups, as well as poor

prediction of one of three dose levels for nonpregnant female mice (see Appendix E.4.1, Figure E-8). On the other hand, the PK model predictions capture time dependence that otherwise would be difficult to estimate and, except for female rats, better predicted the observed serum concentrations than assuming steady state. These prediction uncertainties cannot be disentangled from the uncertainty in the individual PK parameters and model structure for rats and mice. Given that the PK model mostly underpredicted the observed data, which were fairly linear with dose, it seems unlikely that these discrepancies are due to the PK model not including saturable renal clearance, which is a recognized mechanism for PFAS in general. But other mechanisms related to internal dose, exposure duration, and lifestage cannot be ruled out. For example, liver concentration data from Das et al. (2015) (see Appendix E.4.1, Figure E-9) indicate some saturation of PFNA binding in the liver—which could produce nonlinearity in the relationship between dose and blood serum concentrations—but show a much more linear relationship with dose than the serum concentration data. Likewise, it is unclear why simulations for nonpregnant female mice evaluated by Das et al. (2015) significantly overpredicted the measured serum concentrations after the 3 mg/kg-day dose while predicting well the concentration after 5 mg/kg-day and only slightly overpredicting the concentration after 1 mg/kg-day (see Appendix E.4.1, Figure E-8). It is possible that saturable resorption in the mouse kidney results in less accumulation in the serum at 3 mg/kg-day than at 1 mg/kg-day, but then a second mechanism must become active at 5 mg/kg-day, which creates a nonlinearity in the other direction.

Controlled PK studies to determine the separate impacts of dosing duration and lifestage are needed to address questions raised by the existing data and failure of the PK model. Other research is needed to better understand tissue distribution to enable success of full PBPK models, which are the best option for incorporation of multiple PK mechanisms. In the absence of a complete understanding of the various PK data, the ability to accurately predict internal doses from chronic exposures is compromised. But despite the shortcomings of the current state, PK modeling can still inform the relationship between short-term bioassays and chronic exposure in humans in important ways.

All endpoints extrapolated from animal studies were observed in animals exposed to PFNA for 28 days or less, including the NTP bioassay (NTP, 2018) and responses in nonpregnant females exposed as part of developmental studies (e.g., (Das et al., 2015)). While the PK modeling may not have accurately predicted the measured serum concentrations, given that the half-life in male rats and male and female mice is 23–136 days, it is very unlikely that the concentrations in mice and male rats reached steady state by the time of observation. For male rats, the body burden predicted after 28 days is only 36% of steady state and continued exposure could lead to PFNA concentrations almost 3 times higher than observed. This potential for further accumulation represents a significant uncertainty for extrapolation to chronic exposure. However, use of the PK interpolation for these endpoints in rats accounts for the limited accumulation by providing an internal dose equivalent to what is likely given an exposure duration of 3 months at approximately

one-third of the daily dose. The PK analysis used effectively adjusts for the difference in PK accumulation with duration as well as current understanding allows. Estimation of HEDs assuming steady-state levels in humans then accounts for the PK of chronic human exposure. Since the human clearance value used to calculate HEDs was estimated from human bioassay data where the subjects were chronically exposed (Chiu et al., 2022), the HED calculation is not based on an extrapolation from acute PK data. Finally, use of the subchronic-to-chronic UF, $UF_S = 10$, is expected to address the potential that continued exposure of a tissue to the same average concentration could induce greater toxicity due to pharmacodynamic mechanisms. Therefore, uncertainties in the PK analysis are offset by the fact that it adjusts for accumulation potential in the animal bioassays for the extrapolation to chronic exposures.

Besides effects observed in adult animals (including dams in developmental studies, for which effects are presumed relevant to any adult) there are the developmental effects observed in mice (Das et al., 2015; Wolf et al., 2010). Since these effects result from exposure to the fetus or offspring during gestation and early development, they generally do not require adjustment for extrapolation from subchronic-to-chronic exposure. But given the estimated half-life of 47 days in female mice, it is likely that had dosing of the dams been started weeks or months before mating, the body burden would have been higher at the time of mating and throughout gestation than in the studies as conducted. Both of the studies ended dosing on gestation day 18, and while lactational transfer to the pups would have occurred from the maternal body burden accumulated during dosing, the concentration in maternal serum and hence milk would almost certainly have been higher throughout the nursing period had dosing continued. In short, the same levels of response would likely have been observed given chronic dosing at much lower doses. However, as with the male rat dose-response, the PK model adjusts for the duration of dosing, returning predictions equivalent to what would occur given long-term dosing at lower dose levels, thereby offsetting the potential uncertainty.

For human dosimetry, Goeden et al. (2019) used a one-compartment PK model (one compartment for the mother and fetus, two compartments for the mother and child) like that described in Appendix E.4.2 to evaluate the developmental dosimetry of PFOA. Assuming ongoing exposure to the mother (assumed to be at steady state at conception), Goeden et al. (2019) predicted a very large bolus in the exclusively breastfed child, reaching a peak 6 times higher than the steady-state level in adults at 1 year of age when weaning was assumed to occur. EPA evaluated the Goeden et al. (2019) model and concluded that there were mass balance errors leading to some overprediction of dosimetry in the child. But when these were corrected and the model was parameterized for PFNA in humans with a child ingesting breastmilk at an average rate, rather than an upper percentile, a bolus in the child was still predicted, although the peak was only about 30% higher than the steady state predicted for men and nonreproductive age women (see Appendix E.4.2, Figure E-11). When lactational transfer was predicted, with the upper percentile milk ingestion rate for 12 months, the peak was still within a factor of 2 of the steady state (results not

shown) and breastfeeding for shorter durations result in proportionately lower peak levels. Thus, it seems likely that breastfed infants of women who are chronically exposed to PFNA experience higher than steady-state concentrations, although more likely within a factor of 2 of that level. So, while the PK model parameterized for humans was deemed too uncertain for estimation of HEDs based on the exact time-course predicted in a human child, qualitatively the PK model predictions are interpreted as showing that serum concentrations in most children will remain within 30% of steady state. Even allowing for uncertainty in the parameter choices and PK model results, serum concentrations in the child are judged unlikely to exceed the estimated steady state by more than threefold, given chronic exposure. In combination with the applied UF_H 10 the resulting RfDs should be sufficiently protective, given chronic exposure to the mother and child.

Finally, it is noted that uncertainty in animal-to-human extrapolation of less than a factor of 10 is expected to have negligible impact on the RfD value ultimately selected given that the POD_{HED} values estimated from developmental mouse studies were 4–5 orders of magnitude higher than those estimated from epidemiological studies (human birth weight vs. maternal serum concentration). Use of the human PK model to estimate dosimetry in the human infant vs. mouse pup, compared with the approach used here, would not change the outcome enough to change the ranking of HEDs.

Application of human equivalent doses (HEDs) for animal-human extrapolation and dosimetric interpretation of epidemiological endpoints

Tables 5-11 and 5-13 display the estimated internal PODs and POD_{HEDs} for developmental effects and hepatic effects from human studies, and Tables 5-12, 5-14, and 5-15 summarize POD and POD_{HEDs} for developmental, liver, and male reproductive effects, respectively, from animal studies selected for the derivation of candidate values. As described above, these POD_{HED} values apply a combined PK analysis of internal doses in animal studies (using the PK model or observed concentrations) and the human clearance, as described above with further details below. For several PODs extrapolated from animal studies, HEDs calculated by the DDEF approach are shown for comparison purposes in the table footnotes. PODs for thyroid effects are also presented for comparison against the PODs for other effects (e.g., to inform uncertainty, Table 5-16). Given that the available *high* and *medium* confidence studies tested the free acid form of PFNA, normalization from a salt to the free acid using a molecular weight conversion was unnecessary. Formulas for conversions from the acid to the salts of PFNA with available data are provided, as follows (note that the same formula can be used for other salts of PFNA):

$$PFNA\ (ammonium) = \left(\frac{MW\ ammonium\ salt}{MW\ free\ acid}\right) = \left(\frac{481}{464}\right) = 1.037 \quad (5\text{-}2)$$

$$PFNA\ (\text{sodium salt}) = \left(\frac{MW\ sodium\ salt}{MW\ free\ acid}\right) = \left(\frac{486}{464}\right) = 1.047 \quad (5\text{-}3)$$

Table 5-11. PODs from epidemiological evidence of fetal growth restriction considered for the derivation of PFNA candidate toxicity values

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD internal dose (mg/L)	POD_{HED} (mg/kg-d)[a]
Decreased birth weight	Meta-analysis; 10 early pregnancy birth weight studies	Human, male and female	$BMDL_{ER5}$, Hybrid	1.81×10^{-3}	2.2×10^{-7}
	Meta-analysis; 11 early pregnancy birth weight studies	Human, male and female	$BMDL_{ER5}$, Hybrid	1.87×10^{-3}	2.3×10^{-7}
	Sagiv et al. (2018), *High* confidence	Human, male and female	$BMDL_{ER5}$, Hybrid	1.19×10^{-3}	1.5×10^{-7}
	Manzano-Salgado et al. (2017a), *High confidence*	Human, male and female	$BMDL_{ER5}$, Hybrid	1.25×10^{-3}	1.6×10^{-7}
	Wikström et al. (2020), *High confidence*	Human, male and female	$BMDL_{ER5}$, Hybrid	0.84×10^{-3}	1.0×10^{-7}

[a]POD_{HED} = POD internal dose (mg/L) × 0.124 mL/kg-day × 10^{-3} L/mL, i.e., using the clearance estimated for women of reproductive age since the POD internal dose was determined from maternal serum levels.

Table 5-12. PODs from the animal evidence considered for the derivation of PFNA candidate toxicity values for developmental effects

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD timepoint	POD (mg/kg-d)	POD_{int}[a] (mg/L)	POD_{HED}[b] (mg/kg-d)
Decreased postnatal (F1) survival, preweaning	Das et al. (2015), *Medium*	CD-1 mouse, sexes combined	$BMDL_{1RD}$, Hill NCV	PND 21	2.688	51.03	4.6×10^{-3}
	Wolf et al. (2010), *Medium*	129S1/SvlmJ wild type mouse, sexes combined	$BMDL_{1RD}$, NOAEL[c]	PND 21	0.830	17.3	1.6×10^{-3}
Reduced postnatal (F1) body weight, preweaning	Das et al. (2015), *High*	CD-1 mouse, sexes combined	$BMDL_{5RD}$, Poly3 NCV	PND 7	0.562	11.9	1.5×10^{-3}
			$BMDL_{5RD}$, Linear CV	PND 21	0.448	8.12	7.3×10^{-4}
	Wolf et al. (2010), *Medium*	129S1/SvlmJ wild type	$BMDL_{5RD}$, Poly4 CV (m), EXP3 CV (f)	PND 7	0.654 (m); 0.246 (f)	15.7 (m); 5.91 (f)	1.9×10^{-3} (m); 7.3×10^{-4} (f)

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD timepoint	POD (mg/kg-d)	POD_{int}[a] (mg/L)	POD_{HED}[b] (mg/kg-d)
		mice, males and females	$BMDL_{5RD}$, NA[d] (m); $BMDL_{5RD}$, POLY4 CV (f)	PND 21	NA (m)[d]; 0.977 (f)	20.3 (f)	NA (m)[d];1.8×10^{-3} (f)
Reduced (F1) body weight, post-weaning	Das et al. (2015), *High*	CD-1 mouse, males and females	$BMDL_{5RD}$, Linear CV (m), EXP4 CV (f)	PND 24	0.442 (m); 0.456 (f)	7.73 (m); 7.98 (f)	7.0×10^{-4} (m); 7.2×10^{-4} (f)
		CD-1 mouse, females	$BMDL_{5RD}$, Hill CV	PND 42	0.194	2.66	2.4×10^{-4}
		CD-1 mouse, males and females	$BMDL_{5RD}$, EXP3 CV (m,f)	PND 287	0.944 (m); 1.027 (f)	3.12 (m); 3.40 (f)	2.8×10^{-4} (m); 3.1×10^{-4} (f)
Delayed eye opening	Das et al. (2015), *Medium*	CD-1 mouse, sexes combined	$BMDL_{5RD}$, POLY2 NCV	PND 15	1.095	21.1	1.9×10^{-3}
Delayed preputial separation[e]		CD-1 mouse, males	$BMDL_{5RD}$, POLY2 CV	PND 28	1.758	30.0	2.7×10^{-3}
Delayed vaginal opening[e]		CD-1 mouse, females	$BMDL_{5RD}$, EXP2 CV	PND 30	1.080	17.7	1.6×10^{-3}

[a]For each POD the PK analysis was used to estimate a corresponding POD_{int} (mg/L), i.e., the estimated average serum concentration obtained using the PK model for mice to predict the average serum concentration in the mouse fetus/pup calculated from conception to the time of observation.

[b]$POD_{int} \times CL_H \times 10^{-3}$ L/mL for each endpoint; for endpoints observed on or before PND 7 the value of CL_H for women of reproductive age was used since this is presumed to determine the serum levels in the mother, fetus and neonate; for endpoints observed on or after PND 15 the value of CL_H for males and nonreproductive age women was used since the serum concentration in the young child is presumed to be a factor and that depends on CL in the child.

[c]NOAEL/LOAEL values chosen as POD when BMD modeling fails.

[d]NA= not applicable. The data for this endpoint does not exhibit a dose-response relationship and thus was not considered appropriate for POD derivation (see Table 3-10 and Appendix D.2).

[e]PFNA effects on delayed preputial separation and vaginal opening are considered indicators of effects on developmental landmarks and reproductive system development.

Table 5-13. PODs from the epidemiological evidence considered for the derivation of PFNA candidate toxicity values for hepatic effects

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD_{int} (mg/L)[a]	POD_{HED} (mg/kg-d)[b]
Increased ALT representing increased risk of liver effects	Kim et al. (2023), *Medium*	Human, female	$BMDL_{ER5}$, Hybrid with cutoff of 30	1.34×10^{-3}	1.2×10^{-7}
	Kim et al. (2023), *Medium*	Human, female	$BMDL_{ER10}$, Hybrid with cutoff of 30	2.02×10^{-3}	1.8×10^{-7}

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD_{int} (mg/L)[a]	POD_{HED} (mg/kg-d)[b]
	Kim et al. (2023), *Medium*	Human, male	$BMDL_{ER5}$, Hybrid with cutoff of 42	1.48×10^{-3}	1.3×10^{-7}
	Kim et al. (2023), *Medium*	Human, male	$BMDL_{ER10}$, Hybrid with cutoff of 42	2.20×10^{-3}	2.0×10^{-7}
	Nian et al. (2019), *Medium*	Human, female	$BMDL_{ER5}$, Hybrid with cutoff of 30	1.91×10^{-3}	1.7×10^{-7}
	Nian et al. (2019), *Medium*	Human, female	$BMDL_{ER10}$, Hybrid with cutoff of 30	4.00×10^{-3}	3.6×10^{-7}
	Nian et al. (2019), *Medium*	Human, male	$BMDL_{ER5}$, Hybrid with cutoff of 42	2.23×10^{-3}	2.0×10^{-7}
	Nian et al. (2019), *Medium*	Human, male	$BMDL_{ER10}$, Hybrid with cutoff of 42	4.81×10^{-3}	4.3×10^{-7}

[a]Units for the POD_{int} were changed from Tables D-14, D-15, and D-16 where they are ng/mL (to match the concentrations reported in the studies) to mg/L because EPA uses units of mg/kg-d for POD(HED)s. The conversion factor is (ng/mL)*(mg/10^6 ng)*(1,000 mL/L).

[b]POD_{HED} = POD internal dose (mg/L) × 0.090 mL/kg-day × 10^{-3} L/mL, using the estimated clearance for men and nonreproductive age women.

Table 5-14. PODs from the animal evidence considered for the derivation of PFNA candidate toxicity values for hepatic effects

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD timepoint	POD (mg/kg-d)	POD_{int}[a] (mg/L)	POD_{HED}[b] (mg/kg-d)
Increased liver weight (relative)	28-d study, NTP (2018), *High*	SD rat, male and female	$BMDL_{10RD}$, EXP2 CV (m), EXP4 CV (f)	Adult	0.237 (m); 0.470 (f)	10.8 (m); 8.02 (f)	9.8×10^{-4} (m); 7.2×10^{-4} (f)[c]
Hepatic hypertrophy/ histopathology		SD rat, male and female	$BMDL_{10ER}$, LOAEL (m), Logistic (f)	Adult	0.625 (m); 2.065 (f)	28.4 (m); 35.5 (f)	2.6×10^{-3} (m); 3.2×10^{-3} (f)
Increased liver weight (relative)	14-d study, Wang et al. (2015a), *Medium*	BALB/c mouse, male	$BMDL_{10RD}$, Hill CV	Adult	0.105	1.20	1.1×10^{-4}
Increased liver weight (relative)	Das et al. (2015), *High*	CD-1 mouse, females, nonpregnant	$BMDL_{10RD}$, LOAEL	Adult	1	18.0	1.6×10^{-3}
Increased liver weight (relative)	Das et al. (2015), *High*	CD-1 mouse, females, pregnant	$BMDL_{10RD}$, EXP3 CV	Adult	0.43	8.08	7.3×10^{-3}

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD timepoint	POD (mg/kg-d)	POD_{int}[a] (mg/L)	POD_{HED}[b] (mg/kg-d)
Increased liver weight (relative)	Wolf et al. (2010), *High*	129S1/SvlmJ wild type, females, nonpregnant	$BMDL_{10RD}$, LOAEL	Adult	1	33.3	3.0×10^{-3}
Increased liver weight (relative)	Wolf et al. (2010), *High*	129S1/SvlmJ wild type, females, nonpregnant	$BMDL_{10RD}$, Power CV	Adult	0.372	12.4	1.1×10^{-3}
Increased liver weight (relative)	Das et al. (2015), *High*	CD-1 mouse, F1, sexes combined	$BMDL_{5RD}$, LOAEL	PND 1	1	19.8	2.5×10^{-3}
Increased liver weight (relative)	Das et al. (2015), *High*	CD-1 mouse, F1, sexes combined	$BMDL_{5RD}$, LOAEL	PND 24	1	17.7	1.6×10^{-3}
Increased liver weight (relative)	Wolf et al. (2010), *High*	129S1/SvlmJ wild type, F1, sexes combined	$BMDL_{5RD}$, LOAEL	PND 21	0.83	17.3	1.6×10^{-3}

[a]For each POD a PK analysis was used to estimate a corresponding POD_{int} (mg/L). For adult mice, the average serum concentration over the duration of each study, from the start of dosing to the time of observation, was obtained using the PK model. For rats, the POD_{int} was estimated by interpolation of measured end-of-study PFNA serum concentrations from the NTP bioassay as described in "Approach for pharmacokinetic modeling and extrapolating PFNA between rats, mice and humans." For the mouse pups, POD_{int} was the average serum concentration from conception time of observation, calculated using the PK model. See Appendix E.4.1 for further details.

[b]POD_{HED} = POD_{int} (mg/L) × 0.090 mL/kg-day × 10^{-3} L/mL, using the human clearance for men and nonreproductive age women, i.e., assuming hepatic endpoints are relevant to those lifestages.

[c]For comparison, POD_{HED} calculated by the DDEF approach is POD × DDEF. For extrapolation from male rats, DDEF = 2.10×10^{-2} and for extrapolation from female rats, DDEF = 1.19×10^{-3} (f) (see Appendix E, Table E-8). The resulting POD_{HED} values are 5.0×10^{-3} mg/kg-d and 5.6×10^{-4} mg/kg-d, respectively.

Table 5-15. PODs from the animal evidence considered for the derivation of PFNA candidate toxicity values for male reproductive effects

Endpoint	Study/ confidence	Strain/ species	POD type/model	POD timepoint	POD (mg/kg-d)	POD_{int}[a] (mg/L)	POD_{HED}[b] (mg/kg-d)
Testis and epididymis lesions/ histopathology[c]	28-d study, NTP (2018), *High*	SD rat	$BMDL_{10ER}$, NOAEL	Adult	1.25	80.5	7.2×10^{-3}
Testis, Leydig cell atrophy/ histopathology			$BMDL_{10ER}$, Logistic		0.961	56.4	5.1×10^{-3}
Decreased serum testosterone			$BMDL_{1SD}$, Poly2 NCV		1.855	133	12×10^{-3}
Decreased epididymis weight, cauda and whole (absolute)			$BMDL_{1SD}$, Linear NCV (cauda), Linear CV (whole)		0.687 (cauda) 0.493 (whole)	33.5 (cauda) 22.4 (whole)	3.0×10^{-3} (cauda) 2.0×10^{-3} (whole)
Decreased testis weight (absolute)			$BMDL_{1SD}$, Poly2 CV (rt), Linear CV (lft)		0.901 (rt) 0.631 (lft)	51.4 (rt); 28.9 (lft)	4.6×10^{-3} (rt) 2.6×10^{-3} (lft)
Decreased cauda epididymal sperm counts (absolute)[d]			$BMDL_{1SD}$, Linear CV		0.945	55.1	5.0×10^{-3}
Delayed preputial separation[e]	Das et al. (2015), *Medium*	CD-1 mouse, males	$BMDL_{5RD}$, POLY2 CV	PND 28	1.758	30.0	2.7×10^{-3}

[a]For rats the POD_{int} (mg/L) was the average serum concentration over the duration of the NTP study, estimated by interpolation of measured end-of-study PFNA serum concentrations from the NTP bioassay as described in "Approach for pharmacokinetic modeling and extrapolating PFNA between rats, mice and humans." For the mouse pups, POD_{int} was the average serum concentration from conception to PND 28 (time of observation), calculated using the PK model. See Appendix E.4.1 for further details.

[b]$POD_{HED} = POD_{int}$ (mg/L) × 0.090 mL/kg-day × 10^{-3} L/mL, using the human clearance for men. Using the DDEF approach (not shown) POD_{HED} = POD × DDEF values were three to 4 times higher than those obtained using the PK approach. For example, for testis and epididymis lesions/ histopathology the DDEF approach yielded $POD_{HED} = 2.6 \times 10^{-2}$ mg/kg-d vs. 7.2×10^{-3} from the PK approach.

[c]Testis lesions: Germinal epithelium degeneration, spermatid retention (seminiferous tubules); Epididymis lesions: Ductal germ cell exfoliation. Note that modeling of other epididymal histopathology (i.e., hypospermia, epithelial apoptosis) observed at higher dose levels (≥5 mg/kg-d) are included in Appendix E.2.7).

[d]PFNA effects on relative sperm counts were null. Absolute values are preferred in this instance given that sperm contribute to epididymal weight, which was reduced in this study, and so expressing the data as a ratio could mask declines in sperm number (although whether effects are due to impacts on sperm production/viability or secondary to overt toxicity is an uncertainty) (U.S. EPA, 1996).

[e]PFNA effects on delayed preputial separation are considered indicators of effects on developmental landmarks and reproductive system development.

Table 5-16. PODs from the animal evidence for thyroid effects shown for comparative purposes

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD timepoint	POD (mg/kg-d)	POD_{int} or DDEF[a]	POD_{HED} (mg/kg-d)
Thyroid effects							
Serum total thyroxine (T4)	28-d study, NTP (2018), *High*	SD rat, female	$BMDL_{1SD}$, EXP4 NCV	Adult	0.837	14.2 mg/L (POD_{int})	1.3×10^{-3} [(b)]
						1.19×10^{-3} (DDEF)	1.0×10^{-3} [(c)]

[a]The POD_{int} value was estimated by interpolation of measured end-of-study PFDA serum concentrations from the NTP bioassay as described in "Approach for pharmacokinetic modeling and extrapolating PFNA between rats, mice and humans." DDEF = F_{abs}(female rat) × CL_H(men & older women)/CL_A(female rat), values in Table 3-3, shown for comparison.
[b]$POD_{HED} = POD_{int} \times CL_H$, where $CL_H = 9 \times 10^{-5}$ L/kg-d (0.09 mL/kg-day, value for men and older women, Table 3-3).
[c]POD_{HED} = POD × DDEF, shown for comparison.

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

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b) 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 PFNA. The identified potential areas of susceptibility to PFNA exposure-induced health effects, including in infants and 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 Tables 5-17.

For male reproductive effects, quantitative information is limited to studies in which animals were exposed for ≤28 days. For each of these identified hazards, there is little information available to assess the extent to which the specific changes caused by PFNA exposure for 28 days might be expected to worsen with longer subchronic or chronic exposures. Separately, human equivalent PODs for these endpoints were much less sensitive (several orders of magnitude) than the PODs for developmental or hepatic effects from the epidemiological studies (see Tables 5-11, 5-13, and 5-15). EPA methodology and guidelines establish that the minimum database requirement for the derivation of a lifetime reference value from animal toxicity data is a subchronic bioassay (U.S. EPA, 1994). Therefore, derivation of candidate lifetime toxicity values for male reproductive effects was not performed given the high degree of uncertainty when using PODs from a 28-day 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).

While developmental effects in mice from the Das et al. (2015) and Wolf et al. (2010) studies were observed during a sensitive lifestage, these effects were also not advanced further for derivation of candidate values due to the preference for human evidence and the greater sensitivity

reported in the *high* confidence epidemiological studies. Similarly, the short-term testing in adult rats and mice indicating hepatic toxicity were also not advanced further for derivation of candidate values given the greater sensitivity reported in the *medium* confidence human studies. Specifically, HEDs for fetal birth weight deficits and increased serum ALT in adults were several orders of magnitude more sensitive than HED values from the developmental toxicity studies in mice and the short-term testing in rats and mice (see Tables 5-11 to 5-14).

Overall, the selected epidemiological studies of PFNA associations with reduced fetal birth weight and increased serum ALT in adults were advanced. For the fetal birth weight endpoint, given the available POD_{HEDs} were of similar magnitude (ranging from 1.0×10^{-7} to 2.3×10^{-7}), the $BMDL_{5ER\ (HED)}$ of 2.2×10^{-7} mg/kg-day from EPA's meta-analysis of 10 early sampling studies was chosen on the basis of reduced birth weight in males and females as the studies used were representative of the entire human population (both sexes combined), and evaluated PFNA serum concentrations primarily in early pregnancy (thus reducing uncertainty relating to pregnancy hemodynamics). Additionally, these 10 early sampling studies are all *high* or *medium* confidence. An uncertainty of the meta-analysis related to the re-expression of some effect estimates that was necessary to perform the calculations, but EPA determined that the large amount of additional data supporting the meta-analysis of 10 early sampling time studies outweighed the bias introduced by the re-expression method. Therefore, the POD_{HED} from the 10-study meta-analyses was ultimately advanced for reference value derivation (see Section 5.2.1, "Modeling results in humans (decreased birth weight)," Section C.1.5, and Wright et al. (2023) for meta-analyses results and methodology).

For increased serum ALT, women were found to have lower PODs and so the final PODs for HEDs were computed just for women as the more sensitive sex. EPA selected the PODs based on the *medium* confidence (Kim et al., 2023) study over those derived from the *medium* confidence (Nian et al., 2019) study because the dose-response function from Kim et al. (2023) was based on mixture modeling using multiple methods showing that PFNA is the strongest driver of the positive association with ALT. Therefore, it is unlikely that this association is confounded by other PFAS. It is noted that PODs from Nian et al. (2019) were comparable and only modestly higher than the PODs derived based on the selected study, (Kim et al., 2023) (see Appendix D.1.5). Further, even with a wide range of different methods to derive a POD_{HEDs}, there is only a one order of magnitude difference (ranging from 1.2×10^{-7} to 1.5×10^{-6}) from the {Kim, 2023, 10754695@@author-year} study. EPA considered the BMR of 10% extra risk to be the most appropriate as ALT concentrations above the 95th percentile would predominantly be considered to be (minimally) adverse. The POD_{HED} of 1.8×10^{-7} mg/kg-day was chosen on the basis of increased serum ALT using the hybrid approach with a BMR of 10% extra risk for ALT concentrations greater than the 95th percentile in the healthy subset in Valenti (2021) among women based on the dose-response function from {Kim, 2023, 10754695@@author-year}.

Table 5-17. Uncertainty factors for the development of the candidate lifetime RfD values for PFNA

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied as the developmental and liver effects are reported in epidemiological studies.
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 of PFNA exposures in humans. In particular, UF_H is viewed as constituted from two sub-factors of approximately three each, $UF_{H,PK}$ and $UF_{H,PD}$, representing the PK and PD uncertainty and variability.
UF_S	1	A UF_S of 1 is applied to the developmental and liver effects observed in the selected epidemiological studies (i.e., decreased birth weight from a meta-analysis and increased ALT representing increased risk of liver injury from Kim et al. (2023)). For the developmental effects, the developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). For the liver effects, implicit in the PK modeling is the assumption of a long-term chronic exposure that reaches steady state, supporting the application of a UF_S of 1. Additionally, the cross-sectional epidemiology study by Kim et al. (2023) is interpreted to reflect a long-term PFNA exposure duration, noting that the median age of participants in this study ranged from approximately 51 to 54. Declining trends of PFNA over time in the U.S. population (see Table 1-3; NHANES biomonitoring of PFNA from 2011 to 2018) and in the Korean cohort evaluated by Kim et al. (2023) also indirectly support application of a UF_S of 1.
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 the developmental and liver effects from epidemiological studies advanced for candidate lifetime value derivation.
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the evidence base. Although limited, the evidence base in experimental animals includes *high/medium* confidence short-term exposure studies in rodents, two overall *high* confidence developmental studies in mice, and mechanistic evidence to inform the liver effects. The evidence base for PFNA also includes several *high/medium* confidence epidemiological studies most informative for developmental and liver effects (as well as immune effects that were not advanced for RfD derivations[b]). However, uncertainties remain for the developmental and liver effects. The evidence base for development effects lacked studies examining effects in fetuses, infants, and children (e.g., thyroid toxicity, neurotoxicity, mammary gland development, and multigenerational effects). For the liver effects, there remain uncertainties in the data underlying the use of cutoff values for increased ALT and variability in upper limit of normal (ULN) values for ALT depending on laboratory practices, population demographics, and the health status of reference populations. In all, the data are too limited to conclude with certainty that the quantified developmental and liver effects are likely to be the most sensitive; thus, a UF_D of 1 was not selected. A UF_D of 10 was also not selected given the availability of data from well-conducted studies across a range of health outcomes in multiple species, including sensitive evaluations of developmental, liver, and immune endpoints in humans. See discussion below for additional details.
UF_C	See Table 5-18	Composite Uncertainty Factor = $UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

[a]PK = pharmacokinetic; PD = pharmacodynamic; UF_A = interspecies uncertainty factor, UF_H = interhuman uncertainty factor, UF_s = extrapolating from subchronic-to-chronic uncertainty factor, UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor, UF_D = database uncertainty factor.

[b]The overall evidence was interpreted to suggest but not infer that PFNA exposure might cause these effects; thus, these data were not considered for use in RfD derivation. Additionally, although these studies were prioritized for development of PODs for comparative purposes (e.g., to inform uncertainty), BMD modeling was null for immune-related antibody reductions in epidemiology studies with PFNA and not advanced further (see Appendix D.1).

As described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b) the database uncertainty factor (UF_D) is applied to account for the potential of deriving an underprotective reference value as a result of incomplete characterization of a chemical's toxicity (U.S. EPA, 2002b). For PFNA, both a $UF_D = 10$ and a $UF_D = 3$ were considered due to the limited evidence bases. The developmental effects evidence base lacked studies for several important outcomes in fetuses, infants and children (e.g., thyroid toxicity, neurotoxicity, mammary gland development, and multigenerational effects). For the liver effects, there were evidence base uncertainties related to the use of cutoff values for increased ALT and variability in upper limit of normal (ULN) values for ALT depending on laboratory practices, population demographics, and the health status of reference populations. Ultimately, a $UF_D = 3$ was applied to both evidence bases as a UF_D of 10 was not considered to be supported given that there are high-quality studies across multiple outcomes, endpoints, and species, including *high/medium* confidence epidemiological studies examining developmental and liver effects.

The lack of evaluation of thyroid effects in early lifestages is an area of uncertainty in the PFNA evidence base. THs are essential mediators of many physiological processes and are especially important for brain development, before and after birth (see reviews by (O'Shaughnessy and Gilbert, 2019; Bernal, 2015; Gilbert et al., 2012; Morreale de Escobar et al., 2004; Zoeller and Rovet, 2004)). While the epidemiological evidence of PFNA effects on THs was mostly inconsistent across age groups and pregnant women, large reductions in serum T4 were observed in adult female and male rats exposed to PFNA for 28 days (NTP, 2018) (see Section 3.2.7, "Thyroid and adrenal hormones"). It is plausible that serum T4 reductions may also have been observed in the developmental toxicity studies had they been examined. This data gap is notable given observations in both *high/medium* confidence studies in mice (Das et al., 2015; Wolf et al., 2010) of delayed eye opening that is a well-known outcome of TH insufficiency in rodents (see Section 3.2.2, "Postnatal developmental landmarks" and Section 3.2.7, "Evidence Integration"). Reductions in serum T4 are also health outcomes of concern for other PFAS (e.g., PFBS, PFBA, PFHxA). Thus, the lack of data evaluating the potential effects of PFNA on THs in developing animals is a source of uncertainty. However, because the POD_{HED} values of $1.0–1.3 \times 10^{-3}$ mg/kg-day (see Table 5-16) based on reduced serum T4 in adult female rats are approximately four orders of magnitude less sensitive than the developmental POD_{HED} (1.5×10^{-7} mg/kg-day for reduced fetal birth weight), this helps to mitigate some uncertainty that the reduced serum T4 in animals and potential thyroid-mediated effects on development may be more sensitive. However, this quantitative difference does not fully address the evidence base uncertainty in early lifestages that may be at heightened sensitivity to thyroid effects because thyroid hormone reserves are lower than in adults and feedback systems are absent or incompletely formed (Morreale de Escobar et al., 2004; Zoeller and Rovet, 2004). It is also the case that while there is *slight* epidemiological evidence of developmental neurotoxicity for PFNA, specifically for ADHD and related behaviors, this evidence base is highly uncertain and no developmental neurotoxicity studies or mechanistic evidence are available in animals (see

Section 3.2.8). Another source of uncertainty in the PFNA evidence base is the *slight* evidence of immune effects based primarily on reductions in antibody responses in children following vaccinations. While these findings are observed in several well-conducted epidemiological studies, there are nevertheless uncertainties in the findings, particularly given the weaker pattern of results compared with other correlated PFAS with less consistency and smaller magnitude of effects that increases the potential for confounding across PFAS sharing this effect (PFDA, PFOA, PFOS, PFHxS) (see Section 3.2.6). Further, the single-PFAS and multi-PFAS BMD modeling of the immune-related reductions in antibody responses showed poor model fit for PFNA and model instability resulting in several BMD estimates being statistically 'undefined' (see Appendix D.1). Thus, the weaker pattern of effects and BMD modeling does not suggest the potential for greater sensitivity than the available candidate toxicity values and did not warrant increasing the UF_D.

Finally, database uncertainties exist as to whether PFNA may affect mammary gland morphology and development as has been observed in multigenerational testing in mouse dams and offspring exposed to PFOA (White et al., 2007), (White et al., 2009), (White et al., 2011a), (White et al., 2011b). While there are *high/medium* confidence developmental toxicity studies in mice and rats by Das et al. (2015), Wolf et al. (2010), and Rogers et al. (2014) that corroborated some findings of the *high/medium* confidence epidemiological studies, none of these studies evaluate potential transgenerational impacts of longer-term exposures, and there is no two-generation study that would typically evaluate these endpoints. The 1994 reference concentration guideline (U.S. EPA, 1994) and 2002 reference dose report (U.S. EPA, 2002b) support applying a UF_D in situations when such a study is missing. The EPA Reference Dose Report 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 PFNA, PFOA, and PFOS evidence bases together, however, diminishes the concern that the availability of a multigenerational reproductive testing would result in reference values lower than those currently derived for PFNA. Although limited in their ability to assess reproductive health or function, measures of male reproductive toxicity, including the reproductive development endpoints (i.e., delayed preputial separation); (Das et al., 2015), occurred at doses equal to or higher than those that resulted in effects in other organ systems (e.g., liver and thyroid) when measured after exposure to PFNA for 28 days (NTP, 2018). Similar results were observed for the animal evidence base for PFOA and PFOS indicating reproductive effects were not more sensitive markers of toxicity (ATSDR, 2021). Therefore, when considering the limited chemical-specific information alongside information from structurally related compounds, the lack of a multigenerational reproductive study is not considered a major concern relative to UF_D selection. However, the lack of testing on whether PFNA may affect mammary gland development is a data gap and source of database uncertainty.

For the liver effects, EPA applied the recently updated International Federation of Clinical Chemistry ULN values from Valenti (2021) to define a biologically based cutoff in the ALT

distribution for purposes of modeling (i.e., upper 95th percentile of ALT in a population of apparently healthy blood donors). These ULNs were determined by EPA to be a reasonable boundary for detecting liver injury for purposes of the quantitative analysis herein. However, there remain uncertainties in the evidence base related to the precision of the ULN cutoff values for ALT as a definitive cutoff for detecting liver injury based on variability in interlaboratory methodologies, as well as differing demographic and anthropometric characteristics of studied populations (Pacifico et al., 2013).

Taken together, given the residual concerns for potentially more sensitive effects outlined above, a UF_D is considered necessary. A value of 3 was selected for the UF_D to account for the uncertainty surrounding the lack of an evaluation of the potential for: data gaps in the developmental evidence base (i.e., thyroid and neurodevelopmental effects in susceptible early lifestages, mammary gland effects during pregnancy/lactation and development, and multigenerational effects in animals), and uncertainty in the data underlying use of ALT cutoff values with variability in ULN values depending on laboratory practices, population demographics, and the health status of reference populations.

The UFs described in Table 5-17 and the text above were applied and the resulting candidate values are shown in Table 5-18. The candidate values are derived by dividing the POD_{HED} by the composite uncertainty factor (UF_C) as shown below in Equation 5-4.

$$\text{Candidate values for PFNA} = POD_{HED} \div UF_C \quad (5\text{-}4)$$

Table 5-18. Selected and candidate RfD values for PFNA from *high*/*medium* confidence epidemiological studies evaluating developmental effects on decreased birth weight and hepatic effects on increased serum ALT in adults[a]

Study/ confidence	Species/sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)
Selected study									
Meta-analysis; 10 studies[b]	Human, male and female	2.2×10^{-7}	1	10	1	1	3	30	7×10^{-9}
Kim et al. (2023)[c]	Human, female	1.8×10^{-7}	1	10	1	1	3	30	6×10^{-9}

[a]UF_A = interspecies uncertainty factor, UF_H = interhuman uncertainty factor, UF_S = extrapolating from subchronic-to-chronic uncertainty factor, UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor, UF_D = database uncertainty factor.

[b]The meta-analysis of 10 early sampling timepoint studies was selected as all are *high* and *medium* confidence and assessed maternal PFNA serum concentrations primarily during early pregnancy, minimizing concerns surrounding potential bias due to pregnancy-related hemodynamic effects.

[c]Kim et al. (2023) selected as it was a *medium* confidence study that used DAGs to select potential confounders with all models including age, sex, education level, household income, smoking status, BMI, heavy drinking, and exercise.

Selection of Lifetime Toxicity Value(s)

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

Table 5-18 summarizes the selected osRfD for developmental effects identified in Section 3.2.2 and for hepatic effects identified in Section 3.2.3. The confidence decisions about the study, evidence base, quantification of the POD, and overall RfD for these organ/system-specific values are described in Table 5-19, along with the rationales for selecting those confidence levels. In deciding an overall confidence designation, confidence in the evidence base is prioritized over the other confidence decisions. No lifetime osRfDs are derived for male reproductive effects because the only animal data that would be suitable for deriving those values comes from developmental studies and the "developmental" osRfD is intended to be used for all effects of developmental exposures. The overall confidence in the osRfDs for developmental effects is *medium-high* and for liver effects is *medium*. Selection of the overall RfD is described in the following section.

Table 5-19. Confidence in the lifetime organ/system-specific osRfDs for PFNA

Confidence categories	Designation	Discussion
Developmental osRfD = 7×10^{-9} mg/kg-d		
Confidence in studies[a] used to derive osRfD	*High*	Confidence in the meta-analysis was rated as *high*. The subset of 10 studies in the meta-analysis was selected for dose-response analysis due to low overall risk of bias and reliable early pregnancy exposure measurements.
Confidence in evidence base supporting this hazard	*Medium-high*	Confidence in the evidence base for developmental effects is *medium-high*. There was *robust* evidence of reduced birth weight among multiple human studies, including *high* and *medium* confidence studies. The human evidence base also showed some coherence across other fetal and postnatal endpoints (e.g., postnatal weight and height), although some of the fetal growth endpoints were less consistent. The evaluations (detailed in Appendix C.1) do not show a consistent direction or impact of confounding from PFAS co-exposure across the available studies. While there was some attenuation in meta-analytical results in early sampled studies, the evidence was *robust* across all sampling periods and study confidence levels, suggesting there are demonstrated birth weight deficits as PFNA exposure levels increase. However, some residual uncertainty remains regarding potential bias in epidemiological studies due to biomarker sample timing and/or some general potential confounding by exposure to other co-occurring PFAS (e.g., as noted in Appendix C.1, PFDA had the strongest and most consistent correlations across all studies with PFNA) in some epidemiological studies but this did not substantially reduce confidence in the evidence base. The PFNA evidence base for fetal growth restriction and developmental epidemiological effects in general was considerably stronger than that seen for PFDA (which had a *slight* judgment for developmental epidemiological evidence). Additional cross-stream coherence was observed in three *high/medium* developmental toxicity studies in gestationally exposed mice and rats that observed consistent and dose-dependent effects on postnatal growth endpoints (see Table 5-12 for POD_{HED} values).

Confidence categories	Designation	Discussion
Confidence in quantification of the POD_{HED}	*Medium-high*	Confidence in the quantification of the POD and osRfD is *medium-high* given the POD was based on a meta-analysis of 10 early pregnancy studies modeled using a BMD hybrid approach within the range of the observed data and dosimetric adjustment using PFNA-specific pharmacokinetic information, both of which introduce some uncertainty (see above discussions on the analysis of uncertainty regarding the data re-expression necessary for the meta-analysis and the pharmacokinetic modeling of PFNA). Confidence is increased given the consistency of the selected POD with PODs identified from individual human studies (i.e., (Wikström et al., 2020; Sagiv et al., 2018; Manzano-Salgado et al., 2017a)) and meta-analyses of various sample timing and study confidence stratifications.
Overall confidence in osRfD	*Medium-high*	The overall confidence in the osRfD is *medium-high* and is primarily driven by *medium-high* confidence in the evidence base supporting developmental effects (birth weight deficits), as well as the *high* confidence in the study selected to derive the osRfD and the *medium-high* confidence in the quantification of the POD_{HED}.
Hepatic osRfD = 6×10^{-9} mg/kg-d		
Confidence in study[a] used to derive osRfD	*Medium*	Confidence in the Kim et al. (2023) study is rated as *medium*. The study was selected for deriving a POD for adverse liver effects because it was judged to have a "good" rating in the confounding domain during study review (see the heat map in Figure 3-40). Kim et al. (2023) used directed acyclic graphs (DAGs) to select potential confounders, and all models included age, sex, education level, household income, smoking status, BMI, heavy drinking, and exercise. Mixture modeling using multiple methods showed that PFNA from among all the examined PFAS was the strongest driver of the positive association with ALT and GGT, the latter of which provides additional support for a hepatic origin of increased serum enzymes than ALT alone (Newsome et al., 2018; van Beek et al., 2013; Dufour et al., 2000). Additionally, the exposure distribution of PFNA was largely overlapping between Kim et al. (2023) and a second study advanced for modeling by Nian et al. (2019). However, some residual uncertainty remains due to the cross-sectional design of the study and other minor limitations that are not expected to have resulted in selection bias.

Confidence categories	Designation	Discussion
Confidence in evidence base supporting this hazard	*Medium*	Confidence in the evidence base is *medium*. There was *moderate* evidence of consistent positive associations between increased serum enzymes (ALT, GGT, AST) and PFNA exposures in multiple medium confidence human studies. The available evidence further suggests that the associations are unlikely due to confounding by other PFAS based on mixture modeling in a subset of studies. However, some residual uncertainty remains regarding potential bias in epidemiological studies due to some general potential confounding by exposure to other co-occurring PFAS that cannot be entirely ruled out. It is unlikely that PFAS co-exposures would explain the observed associations given that PFNA was a top contributor across several PFAS based on multipollutant modeling in three of five studies. In further evidence of liver effects, there was additional cross-stream coherence from animal and mechanistic studies, including *robust* evidence of liver effects based on consistent and coherent treatment-related increases in liver weight, histopathology, hepatobiliary cholestasis, and some clinical chemistry markers (e.g., increased ALT that was modest in rats but pronounced in mice) across multiple studies, species, rodent strains, sexes, and lifestages. Although uncertainties remain (e.g., lack of longer duration exposures), the animal and mechanistic findings were found in this assessment to meet the criteria set forth by Hall et al. (2012) for adversity (see Section 3.2.4. Hepatic Effects, Consideration for potential adaptive versus adverse responses). Overall, however, uncertainties in the available evidence base, particularly the studies on serum enzymes ultimately used to derive the selected quantitative estimate, best support a confidence level of *medium*.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium*. The POD was based on a BMD hybrid approach within the range of the observed data. Uncertainty remains regarding the use and selection of the cutoff applied in the hybrid approach as well as the lack of a clear basis for BMR selection. However, this concern is reduced because three different methods, each examining multiple BMRs, all yielded PODs within a narrow range, and the PODs from the critical study (Kim et al., 2023) were similar to those identified from another human study by Nian et al. (2019). Dosimetric calculation of the HED using the PFNA-specific clearance also introduces some uncertainty, however the clearance used is expected to provide appropriate coverage for the majority of adults when used in combination with $UF_A = 3$ (see discussion of "Analysis of uncertainty in the pharmacokinetic modeling of PFNA," above).
Overall confidence in osRfD	*Medium*	The overall confidence in the osRfD is *medium* driven by *medium* confidence in the study, evidence base, and quantification of the POD_{HED}.

[a]Study evaluation details can be found in HAWC.

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

The osRfD value for PFNA selected in the previous section is summarized in Table 5-20. From the identified human health effects of PFNA and the derived osRfD for developmental effects, an overall ***RfD of 7 × 10^{-9} mg/kg-day based on decreased birth weights in humans*** was selected. As described in Table 5-19, confidence in the RfD is *medium-high*, based on *medium-high* confidence in the developmental osRfD. The developmental osRfD is based on a meta-analysis of 10 studies.

The developmental osRfD is expected to be protective across all lifestages and is based on effects observed in males and females indicating that the overall RfD would be protective for both sexes. Additional support for the developmental osRfD comes from the nearly identical *medium* confidence hepatic osRfD of 6×10^{-9} mg/kg-day based on increased ALT in adult females from a *medium* confidence epidemiological study. The negligibly higher developmental osRfD was selected over the hepatic osRfD due to greater overall confidence in the value, including higher confidence in the precision of the POD (see Table 5-19).

Table 5-20. Selected RfD for PFNA

System	Basis	POD_{HED} (mg/kg-d)	UF_C	osRfD (mg/kg-d)	Confidence
Developmental	Decreased birth weight	2.2×10^{-7} based on $BMDL_{ER5}$ Hybrid from meta-analysis	30	7×10^{-9}	*Medium-high*

5.2.2. Subchronic Oral Reference Dose (RfD) Derivation

In addition to providing an RfD for lifetime exposure (see Table 5-20), this document also provides an RfD for less-than-lifetime ("subchronic") exposure. Candidate subchronic toxicity values are based on endpoints advanced for consideration in deriving the RfD in Tables 5-11 to 5-14. Data sets found to be sufficient for derivation of candidate subchronic RfDs were available for the following hazards: developmental, hepatic, and male reproductive effects. As described in the previous section, PODs were derived for immune and thyroid effects for comparative purposes and to inform uncertainty (see UF_D discussion above) but were judged to be insufficient to infer a hazard and not advanced for subchronic toxicity value derivation.

Given that developmental effects (fetal growth restriction) were observed in humans exposed to PFNA during susceptible lifestages, this endpoint was also considered for the derivation of the candidate subchronic osRfD, applying identical UFs to those used for the lifetime osRfD (see Table 5-20). As described above, the $BMDL_{ER5(HED)}$ of 2.2×10^{-7} mg/kg-day based on decreased birth weight from a meta-analysis of 10 studies was selected as the POD for the derivation of the developmental subchronic osRfD. Similar to the derivation of the lifetime osRfD, developmental effects observed in mice from Das et al. (2015) and Wolf et al. (2010) were not advanced for the derivation of candidate subchronic values because the developmental POD_{HEDs} from human studies are substantially more sensitive than the POD_{HEDs} from the mouse studies and were therefore prioritized (see Tables 5-11 to 5-14).

Endpoints for hepatic and male reproductive toxicity observed in short-term exposure studies in rodents were also considered for derivation of subchronic osRfDs. As compared with the large uncertainty in extrapolating short-term exposures of 28 days or less to lifetime PFNA exposures in the context of the RfD, it was considered reasonable to try to extrapolate these data sets for the purposes of deriving subchronic candidate values. While supported for deriving the

lifetime hepatic osRfD, the epidemiological evidence of increased serum ALT was not advanced for subchronic derivations as the pharmacodynamics leading to liver effects in humans at shorter exposure durations are unknown and could not be reliably inferred from the human evidence base of chronic exposures and potential effects. The PODs calculated in Tables 5-15 and 5-16 were selected for each health effect for the derivation of the candidate subchronic osRfDs on the basis of 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 the liver effects, the $BMDL_{10RD\ (HED)}$ of 7.2×10^{-4} mg/kg-day based on the rat PK approach and human clearance (5.6×10^{-4} mg/kg-day based on the DDEF approach) for increased relative liver weight in adult female rats (NTP, 2018) was prioritized since it is among the more sensitive POD_{HED} values of relatively longer exposure. Further support comes from a constellation of coherent effects, including histopathological evidence of hepatocellular hypertrophy and necrotic changes, hepatic steatosis, and hepatobiliary cholestasis, which together with results from other short-term rodent studies, met the criteria set forth by Hall et al. (2012) for adversity, and thus serves as the hepatic subchronic osRfD (see Table 5-14).

For male reproductive effects, endpoints with a *high* confidence rating (i.e., testis and epididymis lesions, decreased serum testosterone, decreased testis weight, and decreased epididymis weight [whole and cauda]) were prioritized over endpoints that had potential sensitivity issues due to short-term study exposure (i.e., decreased epididymal sperm counts). The PODs of the prioritized endpoints were similar to one another (HEDs ranging from 2.0 to 12×10^{-3} mg/kg-day) using the rat PK approach (POD_{int}, see Table 5-15) and were consistent with mechanistic evidence that PFNA may target Sertoli cell and Leydig cell functioning leading to decreased spermatogenesis and testosterone (see Section 3.2.4). Therefore, the most sensitive POD based on a $BMDL_{1SD(HED)}$ 2.0×10^{-3} mg/kg-day for decreased whole epididymis weight was chosen for the derivation of the subchronic male reproductive osRfD.

The use of animal data for hepatic and male reproductive endpoints required the application of different default uncertainty factor values than those used for developmental effects in humans and can be found in Table 5-21.

Table 5-21. Uncertainty factors for the development of candidate subchronic RfD values for PFNA

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied as the developmental effects are reported in epidemiological studies.
	3	For the hepatic and male reproductive effects, a UF_A of 3 is applied to the experimental animal evidence to account for uncertainty in characterizing the pharmacokinetic and pharmacodynamic differences between mice or rats and humans following oral PFNA exposures. Some aspects of the cross-species extrapolation of pharmacokinetic and pharmacodynamic processes have been accounted for by using pharmacokinetic data, modeling and DDEF approaches to extrapolate internal doses in rodents to serum levels in humans. However, residual uncertainty related to potential pharmacokinetic and pharmacodynamic differences remains. 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 PFNA exposure. This applies to all animal and human PODs.
UF_S	1	A UF_S of 1 is applied to the developmental effects observed in the selected epidemiological studies (i.e., decreased birth weight from a meta-analysis). The developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). OR
	10	A UF_S of 10 is applied to adult liver and male reproductive effects (increased liver weight and decreased epididymis (whole) weight, respectively) for POD derivation due to the short exposure duration of the study (28 d). 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.
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the evidence base. Although limited, the evidence base in experimental animals includes *high/medium* confidence short-term exposure studies in rodents and two overall *high* confidence developmental studies in mice. The evidence base for PFNA also includes several *high/medium* confidence epidemiological studies most informative for immune,[b] liver,[c] and developmental effects. However, for the developmental effects, uncertainties remain regarding the lack of studies examining effects in fetuses, infants, and children (e.g., thyroid toxicity, neurotoxicity, mammary gland development, and multigenerational effects). In all, the data are too limited 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. A UF_D of 10 was also not selected given the availability of data from well-conducted studies across a range of health outcomes in multiple species, including sensitive evaluations of developmental and immune endpoints in humans.
UF_C	See Table 5-22	Composite Uncertainty Factor = $UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

[a]UF_A = interspecies uncertainty factor, UF_H = interhuman uncertainty factor, UF_S = extrapolating from subchronic-to-chronic uncertainty factor, UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor, UF_D = database uncertainty factor.

[b]The overall evidence was interpreted to suggest but not infer that PFNA exposure might cause these effects; thus, these data were not considered for use in RfD derivation. Additionally, although these studies were prioritized for development of PODs for comparative purposes (e.g., to inform uncertainty), BMD modeling was null for immune-related antibody reductions in epidemiology studies with PFNA and not advanced further (see Appendix D.1).

[c]The epidemiological evidence of increased serum ALT was not advanced for subchronic derivations as the pharmacodynamics leading to liver effects in humans at shorter exposure durations are unknown and could not be reliably inferred from the available evidence.

As described above under *Derivation of Candidate Lifetime Toxicity Values for the RfD*, and in U.S. EPA (2002b), five possible areas of uncertainty and variability were considered in deriving the candidate subchronic values for PFNA. 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 subchronic POD_{HED} values are listed in Table 5-21, including the UF_D, which is 3 due to data gaps and database uncertainties discussed previously in the derivation of the lifetime RfD.

A $UF_A = 3$ is applied to account for uncertainties surrounding pharmacokinetic and pharmacodynamic differences between rodents and humans following oral PFNA exposure for the derivation of subchronic values for liver and male reproductive effects in animal studies. As is usual in the application of this UF, the pharmacokinetic uncertainty is mostly addressed through application of dosimetric approaches for estimating HEDs (see "Approach for pharmacokinetic modeling and extrapolating PFNA between rats, mice, and humans," above). This leaves residual uncertainty regarding the pharmacokinetics and uncertainty surrounding the pharmacodynamics. Typically, a threefold UF is applied for this uncertainty and in the absence of chemical-specific information. Therefore, this UF_A is applied to hepatic and male reproductive endpoints, with additional consideration of the available mechanistic data for liver effects.

As described in Section 3.2.3, the mechanistic evidence base for PFNA-induced liver effects (hepatomegaly, hepatic hypertrophy, hepatobiliary cholestasis, liver lipid accumulation) includes multiple in vivo and in vitro bioassays in rodents, with some comparative in vitro testing in human cell lines, that support a MOA that involves activation of PPARα pathways. A limited number of in vitro studies provide supportive evidence of less sensitivity of PFNA toward human PPARα than mouse PPARα (Wolf et al., 2012; Wolf et al., 2008a) and other mammalian species (Baikal seals (Nagasaka et al., 2006)). If PPARα were the only biologically plausible MOA for noncancer liver effects, it could be argued that a value of one for the remaining portion of the UF_A may be supported given the evidence of reduced PPARα sensitivity in humans. However, as described in Section 3.2.3, in vivo studies in PPARα null mice and in vitro human and rodent models support that PFNA effects on the liver occur at least partially independent of PPARα, with multiple lines of evidence coalescing around activation of CAR/PXR pathways. While it is possible that study methodologies and model selection played a role in the observed differences, there is also the possibility of differential sensitivity in human liver cells to non-PPARα pathways that is unclear for this chemical. An MOA that plausibly involves both PPARα-dependent and -independent pathways for liver effects is also supported by some of the transcriptional studies herein that included other structurally related PFAS (C7, C8, C10), and in studies for these PFAS in null and humanized animal models (Das et al., 2017; Rosen et al., 2017; Rosen et al., 2013; Nakagawa et al., 2012). Thus, while there is support that PPARα is important to the mechanism of PFNA-induced liver effects, evidence exists to support other pathways, such as CAR/PXR and potentially other PPAR isoforms, as contributing to the noncancer liver MOA. In consideration of the uncertainty surrounding the potential involvement of multiple pathways beyond PPARα in the liver MOA for PFNA, and the currently

unknown potential for PFNA activation of PPARα to influence lipid metabolism pathways (a liver effects endpoint for PFNA), the UF_A has been set to three for purposes of deriving toxicity values for liver effects. This is consistent with the general practice that the toxicodynamic portion of the UF_A is typically assigned a value of 3 if there is no evidence sufficient to reasonably rule out that responses in humans could be more sensitive than those in rodents.

With regard to the UF_S, EPA guideline states that for "short-term and longer-term reference values, the application of a UF_S 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, 2002b). This is the case for liver and male reproductive endpoints derived from the NTP 28-day study. Although there is no chemical-specific information to evaluate the potential for increased sensitivity with exposures longer than 28-days (e.g., a standard 90-day subchronic rodent study), the following considerations are outlined to inform the application of the UF_S for duration extrapolation. For a subset of liver effects, the increase in relative liver weight demonstrated time dependency across the short-term exposures. Specifically, relative liver weights in adult male SD rats exposed for 14 days increased from 31% at 1 mg/kg-day (Fang et al., 2012c) to 60% at 1.25 mg/kg-day following a 28-day exposure (NTP, 2018). However, it was not possible to compare short-term results in adult male rats at the lower or higher ends of the dose ranges tested due to non-overlapping dose levels and overt toxicity, respectively. The low doses in the 14-day studies (Hadrup et al., 2016; Fang et al., 2012c) were ~threefold lower than the low dose in the NTP 28-day study. At the higher end of the dose range of 2.5 and 5 mg/kg-day, there was overt toxicity reported in male rats in all three studies that prevented reliable comparisons (i.e., high mortality in NTP (2018) at 5 and 10 mg/kg-day and high body weight loss in Hadrup et al. (2016) and Fang et al. (2012c) at 5 mg/kg-day, and NTP (2018) at 2.5 mg/kg-day). These data uncertainties at the low and high end of the dose range made it difficult to decipher liver effects with exposure duration, and there are no longer-term exposure data in male rats >28 days. However, the roughly doubling of liver enlargement in male rats from 14 to 28 days of exposure at the middle dose of approximately 1 mg/kg-day where there was no overt toxicity suggests the potential for increasing sensitivity with increasing exposure duration. For male reproductive effects, the 28-day study duration was insufficient to cover the entire period of spermatogenesis in rats (~8 weeks), raising concerns about reduced sensitivity and the possibility of more severe effects with longer exposures, and uncertainties concerning PFNA disposition (pharmacokinetic) at longer exposures. Thus, considering the potential for these health outcomes to worsen with increasing exposure durations, and the large uncertainty associated with the lack of a chemical-specific subchronic study, application of a UF_S of 10 is supported for deriving subchronic RfDs from the NTP 28-day toxicity study. The candidate subchronic osRfD values are shown in Table 5-22.

Table 5-22. Candidate values for the subchronic osRfD for PFNA[a]

Endpoint	Study/ confidence	Species, lifestage, Sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)
Developmental effects										
Decreased birth weight	Meta-analysis; 10 studies[b], High	Human, male and female	2.2×10^{-7}	1	10	1	1	3	30	7×10^{-9}
Hepatic effects										
Increased relative liver weight	NTP (2018), High	SD rat, adult female	7.2×10^{-4} [PK approach]	3	10	10	1	3	1,000	7×10^{-7}
			5.6×10^{-4} [DDEF approach]	3	10	10	1	3	1,000	6×10^{-7}
Male reproductive effects										
Decreased absolute whole epididymis weight	NTP (2018), High	SD rat, adult male	2.0×10^{-3} [PK approach]	3	10	10	1	3	1,000	2×10^{-6}
			1.0×10^{-2} [DDEF approach]	3	10	10	1	3	1,000	1×10^{-5}

[a]UF_A = interspecies uncertainty factor, UF_H = interhumans uncertainty factor, UF_S = extrapolating from subchronic-to-chronic uncertainty factor, UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor, UF_D = database uncertainty factor.

[b]The meta-analysis of 10 early sampling timepoint studies was selected as all are *high* and *medium* confidence and assessed maternal PFNA serum concentrations primarily during early pregnancy, minimizing concerns surrounding potential bias due to pregnancy-related hemodynamic effects.

Selection of Subchronic Toxicity Value(s)

As described above, subchronic osRfDs associated with each health effect are presented as they may be useful for certain decision purposes (i.e., site-specific risk assessments with less-than-lifetime exposures). Like with the lifetime osRfD, the developmental subchronic osRfD is based on the meta-analysis of 10 studies showing decreased birth weights in males and females combined. The other subchronic osRfD values selected were based on the NTP (2018) study indicating increased relative liver weight in adult female rats for hepatic effects and decreased absolute epididymis (whole) weight for male reproductive effects. For male rats, the PK model was considered the most accurate approach for estimating the average serum concentration over the 28-day NTP study, while for female rats EPA's analysis indicated that the measured end-of-study concentration was a better predictor of the average serum concentration than the PK model (details in Appendix E.4.1). Hence the PK model (male rats) or a linear interpolation of the measured concentrations from the NTP study (female rats) was used to estimate the internal doses at the corresponding PODs. Then the HEDs were calculated using the human clearance for men and women outside of reproductive age, which should assure that all members of the population remain below the internal dose PODs given uncertainties in the human PK due to limited data. Confidence

in the studies used to derive the quantitative estimate for each osRfD are described in Table 5-23, considering confidence in the study, the specific evidence base for that health effect, and the quantitative POD_{HED} estimate for each subchronic osRfD.

Table 5-23. Confidence in the subchronic organ/system-specific RfDs for PFNA

Confidence categories	Designation[a]	Discussion
Developmental subchronic osRfD = 7×10^{-9} mg/kg-d		
Confidence in studies[a] used to derive osRfD	*High*	Confidence in the meta-analysis was rated as *high*. The subset of 10 studies in the meta-analysis was selected for dose-response analysis due to low overall risk of bias and reliable early pregnancy exposure measurements.
Confidence in evidence base supporting this hazard	*Medium-high*	Confidence in the evidence base for developmental effects is *medium-high*. There was *robust* evidence of reduced birth weight among multiple human studies, including *high* and *medium* confidence studies. The human evidence base also showed some coherence across other fetal and postnatal endpoints (e.g., postnatal weight and height), although some of the fetal growth endpoints were less consistent. The evaluations (detailed in Appendix C.1) do not show a consistent direction or impact of confounding from PFAS co-exposure across the available studies. While there was some attenuation in meta-analytical results in early sampled studies, the evidence was *robust* across all sampling periods and study confidence levels, suggesting there are demonstrated birth weight deficits as PFNA exposure levels increase. However, some residual uncertainty remains regarding potential bias in epidemiological studies due to biomarker sample timing and/or some general potential confounding by exposure to other co-occurring PFAS (e.g., as noted in Appendix C.1, PFDA had the strongest and most consistent correlations across all studies with PFNA) in some epidemiological studies but this did not substantially reduce confidence in the evidence base. The PFNA evidence base for fetal growth restriction and developmental epidemiological effects in general was considerably stronger than that seen for PFDA (which had a *slight* judgment for developmental epidemiological evidence). Additional cross-stream coherence was observed in three *high/medium* developmental toxicity studies in two strains of gestationally exposed mice that observed consistent and dose-dependent effects on postnatal growth endpoints (see Table 5-12 for POD_{HED} values).
Confidence in quantification of the POD_{HED}	*Medium-high*	Confidence in the quantification of the POD and osRfD is *medium-high* given the POD was based on a meta-analysis of 10 early pregnancy studies modeled using a BMD hybrid approach within the range of the observed data and dosimetric adjustment using PFNA-specific pharmacokinetic information, both of which introduce some uncertainty (see above discussions on the analysis of uncertainty regarding the data re-expression necessary for the meta-analysis and the pharmacokinetic modeling of PFNA). Confidence is increased given that the selected POD was consistent with the PODs that were identified from individual human studies (i.e., (Wikström et al., 2020; Sagiv et al., 2018; Manzano-Salgado et al., 2017a))and meta-analyses of various sample timing and study confidence stratifications.

Confidence categories	Designation[a]	Discussion
Overall confidence in osRfD	*Medium-high*	The overall confidence in the osRfD is *medium-high* and is primarily driven by *high* confidence in the evidence base supporting developmental effects (birth weight deficits), as well as the *high* confidence in the study selected to derive the osRfD and the *medium-high* confidence in the quantification of the POD_{HED}.
Hepatic subchronic osRfD = 7×10^{-7} mg/kg-d (PK approach)		
Confidence in study[a] used to derive osRfD	*High*	Confidence in the NTP (2018) study is rated *high* based on good or adequate ratings for most study quality domains (https://hawc.epa.gov/rob/study/100517686/) and characteristics that make it suitable for deriving toxicity values, including the appropriateness 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, clinical chemistry markers, and organ weights were observed across short-term rodent studies (primarily one *high* confidence 28-d study) that are supported by mechanistic studies of biological plausibility and possible human relevance. There was also some human evidence of serum increased ALT in adults and generally consistent increases in serum AST and bilirubin that offered some coherence to the animal and mechanistic evidence base. Uncertainties remain due to the absence of longer-term animal 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 PFNA 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 the range of the observed data and dosimetric adjustment was based on PFNA-specific pharmacokinetic information, the latter of which introduces some uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFNA 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 = 2×10^{-6} mg/kg-d (PK approach)		
Confidence in study[a] used to derive osRfD	*Medium-high*	Confidence in the NTP (2018) study is rated *high* (https://hawc.epa.gov/rob/study/100517686/) for most male reproductive measures, 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 for most study quality domains) and characteristics that make it suitable for deriving toxicity values (appropriateness of the exposure paradigm, use of a relevant species, and the study size and design).

Confidence categories	Designation[a]	Discussion
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 were observed in a *high* confidence 28-d rat study. The findings are supported by coherent evidence from a limited number of mechanistic studies. Despite the available evidence, outstanding uncertainties in the evidence base remain, including the absence of adequate epidemiology studies and longer-term animal exposure studies (>28 d) that capture the entire period of spermatogenesis (~8 wk), and multigenerational studies that evaluate effects in both adults and developing humans and animals. Given these evidence base uncertainties, it is not possible to fully evaluate that the osRfD is 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 PFNA-specific pharmacokinetic information, the latter of which introduces some uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFNA 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 uncertainties associated with the *medium-low* evidence base descriptor.

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

Selection of subchronic RfD and confidence statement

Organ/system-specific subchronic RfD values for PFNA selected in the previous section are summarized in Table 5-24.

Table 5-24. Subchronic osRfD values and the selected subchronic RfD for PFNA

Selected subchronic RfD					
Basis		**RfD (mg/kg-d)**		**Confidence**	
Developmental effects		7×10^{-9}		*Medium-high*	
Subchronic organ/system-specific RfDs					
System	**Basis**	**POD_{HED} (mg/kg-d)**	**UF_C**	**osRfD (mg/kg-d)**	**Confidence**
Developmental	Decreased birth weight, human, males and females	2.2×10^{-7} based on $BMDL_{ER5}$ from meta-analysis of 10 studies	30	7×10^{-9}	*Medium-high*
Hepatic	Increased relative liver weight, adult female rat	7.2×10^{-4} based on $BMDL_{10RD}$, from NTP (2018)	1,000	7×10^{-7}	*Medium*
Male reproductive	Decreased epididymal weight (whole), adult rat	2.0×10^{-3} based on $BMDL_{1SD}$ from NTP (2018)	1,000	2×10^{-6}	*Medium-low*

RfD = oral reference dose; osRfD = organ/system-specific reference dose; POD = point of departure; UF_c = composite uncertainty factor.

From the identified targets of PFNA toxicity and derived subchronic osRfDs (see Table 5-22), a ***subchronic RfD of 7 × 10⁻⁹ based on developmental effects (decreased birth weight)*** is selected for less-than-lifetime exposures. Confidence in the subchronic RfD is *medium-high*, based on *medium-high* confidence in the subchronic developmental osRfD, as described in Table 5-23. The decision to select the subchronic developmental osRfD as the subchronic RfD was based on all the available osRfDs in addition to overall confidence and composite uncertainty for those subchronic osRfDs.

5.2.3. Inhalation Reference Concentration (RfC)

One acute, single-dose inhalation exposure study was identified (Kinney et al., 1989), but it was considered *low* confidence and provided **inadequate evidence** to draw conclusions regarding any potential health effects. Thus, the evidence was insufficient for reference value derivation, so an RfC was not calculated.

5.3. CANCER TOXICITY VALUES

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

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