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IRIS Toxicological Review of Perfluorodecanoic Acid (PFDA) and Related Salts

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Supplemental Information

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CONTENTS

TABLES

FIGURES

ABBREVIATIONS AND ACRONYMS

AC50	activity concentration at 50%
ADME	absorption, distribution, metabolism, and excretion
AIC	Akaike's information criterion
ALT	alanine aminotransferase
AOP	adverse outcome pathway
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
$BW^{3/4}$	body weight scaling to the 3/4 power
CA	chromosomal aberration
CAA	Clean Air Act
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service registry number
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CHO	Chinese hamster ovary (cell line cells)
CI	confidence interval
CL	confidence limit
CNS	central nervous system
COI	conflict of interest
CPAD	Chemical and Pollutant Assessment Division
CPHEA	Center for Public Health and Environmental Assessment
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	extra risk
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GLP	Good Laboratory Practice
GSH	glutathione
GST	glutathione-*S*-transferase
HAP	hazardous air pollutant
HAWC	Health Assessment Workspace Collaborative
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HBCD	hexabromocyclododecane
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
i.p.	intraperitoneal
i.v.	intravenous
IAP	IRIS Assessment Plan
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
MeSH	Medical Subject Headings
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MPS	mononuclear phagocyte system
MTD	maximum tolerated dose
NCI	National Cancer Institute
NMD	normalized mean difference
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OAR	Office of Air and Radiation
OECD	Organization for Economic Co-operation and Development
OLEM	Office of Land and Emergency Management
ORD	Office of Research and Development
OSF	oral slope factor
PBPK	physiologically based pharmacokinetic
PECO	populations, exposures, comparators, and outcomes
PK	pharmacokinetic
PND	postnatal day
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RD	relative deviation

RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
ROBINS I	Risk of Bias in Nonrandomized Studies of Interventions
SAR	structure-activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase, also known as AST
SGPT	serum glutamic pyruvic transaminase, also known as ALT
SRBC	sheep red blood cell
TDAR	T-Dependent Antibody Response
TK	toxicokinetics
TSCATS	Toxic Substances Control Act Test Submissions
TWA	time-weighted average
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOS	Web of Science

APPENDIX A. SYSTEMATIC REVIEW PROTOCOL FOR THE PFAS IRIS ASSESSMENTS

A single systematic review protocol was used to guide the development of five separate IRIS per- and polyfluoroalkyl substance (PFAS) assessments (i.e., perfluorobutanoic acid [PFBA], perfluorohexanoic acid [PFHxA], perfluorohexane sulfonate [PFHxS], perfluorononanoic acid [PFNA], and perfluorodecanoic acid [PFDA]). This *Systematic Review Protocol for the PFAS IRIS Assessments* was released for public comment and subsequently updated. The updated protocol and prior revisions can be found at the following location: https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=345065.

APPENDIX B. ADDITIONAL DETAILS OF SYSTEMATIC REVIEW METHODS AND RESULTS

Table B-1. Summary of detailed search strategies for perfluorodecanoic acid and related salts (PubMed, Web of Science, Toxline, TSCATS, Toxcenter)

Search	Search strategy	Dates of search
PubMed		
Search terms	335-76-2[rn] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluorodecanoic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-Decanoic acid"[tw] OR "Decanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-"[tw] OR "Decanoic acid, nonadecafluoro-"[tw] OR "Perfluorodecanoate"[tw] OR "PFDeA"[tw] OR "PFDcA"[tw] OR ("PFDA"[tw] AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR B-1orB-1luoro*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw] OR PFOS[tw] OR PFOA[tw]))	No date limit–7/26/2017
Literature update search terms	((335-76-2[rn] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluorodecanoic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-Decanoic acid"[tw] OR "Decanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-"[tw] OR "Decanoic acid, nonadecafluoro-"[tw] OR "Perfluorodecanoate"[tw] OR "PFDeA"[tw] OR "PFDcA"[tw] OR ("PFDA"[tw] AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR B-1orB-1luoro*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw] OR PFOS[tw] OR PFOA[tw])) AND ("2017/08/01"[Date – Publication] : "2018/03/01"[Date – Publication])	Updates performed: February 2018, May 2019, May 2020, April 2021, April 2022, and April 2023 (this last update reflected in Appendix I)
Web of Science		
Search terms	TS="PFDeA" OR TS="PFDcA" OR TS="Ndfda" OR TS="Nonadecafluoro-n-decanoic acid" OR TS="Nonadecafluorodecanoic acid" OR TS="Perfluoro-n-decanoic acid" OR TS="Perfluorodecanoic acid" OR TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-Decanoic acid" OR TS="Decanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-" OR TS="Decanoic	No date limit–7/26/2017

Search	Search strategy	Dates of search
	acid, nonadecafluoro-" OR TS="Perfluorodecanoate" OR (TS=PFDA AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR B-2orB-2luoro* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated)) OR (TS=PFDA AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR B-2orB-2luoro* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA	
Literature update search terms	TS="PFDeA" OR TS="PFDcA" OR TS="Ndfda" OR TS="Nonadecafluoro-n-decanoic acid" OR TS="Nonadecafluorodecanoic acid" OR TS="Perfluoro-n-decanoic acid" OR TS="Perfluorodecanoic acid" OR TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-Decanoic acid" OR TS="Decanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-" OR TS="Decanoic acid, nonadecafluoro-" OR TS="Perfluorodecanoate" OR (TS=PFDA AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR B-2orB-2luoro* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated)) OR (TS=PFDA AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR B-2orB-2luoro* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA)) AND PY=2017-2018	Updates performed: February 2018, May 2019, May 2020, April 2021, April 2022, and April 2023 (this last update reflected in Appendix I)
Toxline		
Search terms	(335-76-2 [rn] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluorodecanoic acid" OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-decanoic acid" OR "decanoic acid 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-" OR "decanoic acid nonadecafluoro-" OR "nonadecafluoro-n-decanoic acid" OR "nonadecafluorodecanoic acid" OR "perfluoro-1-nonanecarboxylic acid" OR "perfluoro-n-decanoic acid" OR "perfluorocapric acid" OR "perfluorodecanoate" OR "perfluorodecanoic acid" OR "ndfda" OR "PFDeA" OR "PFDcA" OR (pfda AND (fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro* OR perfluorinated OR fluorinated OR pfas OR pfos OR pfoa))) AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]	No date limit–7/21/2017
Literature update search terms		Updates performed: February 2018 and May 2019
TSCATS		

Search	Search strategy	Dates of search
Search terms	335-76-2[rn] AND TSCATS [org]	No date limit–7/21/2017

APPENDIX C. BENCHMARK DOSE MODELING RESULTS

C.1. BENCHMARK DOSE MODELING RESULTS FROM HUMAN STUDIES

The endpoints selected for benchmark dose (BMD) modeling include decreased serum antibody concentrations (Budtz-Jørgensen and Grandjean, 2018a; Grandjean et al., 2012) and decreased birth weight (Luo et al., 2021; Yao et al., 2021; Wikström et al., 2020; Valvi et al., 2017; Lenters et al., 2016). The internal doses reported in the human studies were used in the BMD modeling and then converted to human equivalent doses (HEDs) using the estimated human clearance as described in Section 3.1.7 of the main document, the modeling results are presented in this appendix.

C.1.1. Benchmark Dose Modeling Approaches or Immune Effects

Modeling Results for Decreased Tetanus Antibody Concentrations at 7 Years of Age and PFDA Measured at 5 Years of Age

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of perfluorodecanoic acid (PFDA) measured at age 5 years, against $\log_2$-transformed antitetanus antibody concentrations measured at the 7-year-old examination controlling for sex, exact age at the 7-year-old examination, and booster type at age 5 years. Models were evaluated with additional control for perfluorooctane sulfonic acid (PFOS) (as $\log_2$[PFOS]) and perfluorinated alkylated substances (PFOA) (as $\log_2$[PFOA]), and without PFOS and PFOA. Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model, a piecewise-linear model with a knot at the median PFDA concentration, and a logarithmic function. The logarithmic functions fit no better than the piecewise-linear functions (Budtz-Jørgensen and Grandjean, 2018a). The piecewise-linear model did not fit better than the linear model for the PFDA exposure without adjustment for PFOS and PFOA using a likelihood ratio test (p = 0.51; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOS and PFOA ($\log_2$[PFOS] and $\log_2$[PFOA]) (p = 0.40). The U.S. Environmental Protection Agency (EPA) used the linear model results that were the best fitting of the three models.

Table C-1 summarizes the results from Budtz-Jørgensen and Grandjean (2018b) for PFDA at age 5 years and tetanus antibodies at age 7 years. These regression coefficients (β), their standard errors (SE), p-values, and the 90% lower confidence bounds were provided by Budtz-Jørgensen and Grandjean (2018b).

Table C-1. Results specific to the slope from the linear analyses of PFDA measured in serum at age 5 years and $\log_2$(tetanus antibody concentrations) measured at age 7 years in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b)

Exposure	Model shape	PFOS and PFOA adjusted	Slope (β) per ng/mL in serum	SE(β) ng/mL in serum	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
PFDA at age 5 yr	Linear	No	−1.55	0.602	p = 0.01	−2.55
PFDA at age 5 yr	Linear	Yes	−0.98	0.681	p = 0.15	−2.10

Interpretation of results in Table C-1:

- PFDA is a significant predictor in the single-PFAS model (β = −1.55; p = 0.01).
- Effects of PFDA in the single-PFAS model are attenuated when log2[PFOS] and log2[PFOA] are included in the model (β = −0.98; p = 0.15).
- The point estimate results for PFDA (β) in the single-PFAS model are potentially confounded by PFOS and/or PFOA since there was a 37% reduction in the effect size for PFDA from −1.55 to −0.98 when controlling for PFOS and PFOA.
    - One explanation is that PFOS and/or PFOA were confounders of the PFDA effect and controlling for those coexposures removed confounding.
    - Another possibility is that controlling for coexposures like PFOS and PFOA actually induced confounding (Weisskopf et al., 2018; Weisskopf and Webster, 2017).
    - The reasons for the change in main effect size for PFDA are unknown. For this reason, there is uncertainty in knowing which point estimate is the best representation of any effect of PFDA.
- However, the lower bound on the point estimate (β_{LB}) for the single-PFAS is 18% lower than the multi-PFAS model estimate for PFDA.
    - The definition of the RfD, which is based on the β_{LB}, includes allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate, and the uncertainty for potential confounding in the BMD from including or excluding PFOS and PFOA here is about 37%, while the uncertainty for potential confounding in the BMDL is about 18%.

Additional details about other potential confounders follows:

- PFNA is not a significant predictor in the single-PFAS model (β = −0.227; p = 0.16) and the association is just 15% of the strength of the PFDA association from (Budtz-Jørgensen and Grandjean, 2018b), thus PFNA could not have been a meaningful confounder even though PFNA was highly correlated with PFDA (r = 0.78) (Grandjean et al., 2012)).
- PCBs had a weak correlation with PFDA (r = 0.14; Grandjean et al. (2012)) meaning that PCBs could not have been a meaningful confounder of the PFDA effect estimate.

Selection of the Benchmark Response

The BMD approach involves dose-response modeling to obtain BMDs, i.e., dose levels corresponding to specific response levels near the low end of the observable range of the data and the lower limit of the BMD (BMDLs) to serve as potential PODs for deriving quantitative estimates below the range of observation (U.S. EPA, 2012). Selecting a BMR to estimate the BMDs and BMDLs involves making judgments about the statistical and biological characteristics of the dataset and about the applications for which the resulting BMDs and BMDLs will be used. An extra risk of 10% is recommended as a standard reporting level for quantal data for toxicological data. Biological considerations may warrant the use of a BMR of 5% or lower for some types of effects as the basis of the POD for a reference value. However, a BMR of 1% has typically been used for quantal human data from epidemiological studies (U.S. EPA, 2012), although this is more typically used for epidemiological studies of cancer mortality within large cohorts of workers that can support the statistical estimation of small BMRs.

A blood concentration for tetanus antibodies of 0.1 IU/mL is sometimes cited in the tetanus literature as a "protective level." (Grandjean et al., 2017) noted that the Danish vaccine producer, Statens Serum Institut, recommended the 0.1 IU/mL "cutoff" level "to determine whether antibody concentrations could be considered protective"; and Galazka and Kardymowicz (1989) mentions the same concentration but Galazka et al. (1993) argues:

> *"The amount of circulating antitoxin needed to ensure complete immunity against tetanus is not known for certain. Establishment of a fixed level of tetanus antitoxin does not take into consideration variable conditions of production and adsorption of tetanus toxin in the anaerobic area of a wound or a necrotic umbilical stump. A given serum level could be overwhelmed by a sufficiently large dose of toxin. Therefore, there is no absolute protective level of antitoxin and protection results when there is sufficient toxin-neutralizing antibody in relation to the toxin load (*Passen and Andersen, 1986*)."*

Without a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, a default BMR of 1-SD change from the control mean may be selected, as suggested in EPA's draft Benchmark Dose Technical Guidance Document (U.S. EPA, 2012). As noted above, a lower BMR can also be used if it can be justified on a biological and/or statistical basis. Figure C-1 replicates a figure in the technical guidance (page 23; (U.S. EPA, 2012) to show that in a control population in which 1.4% are considered to be at risk of having an adverse effect, a downward shift in the control mean of 1-SD results in an approximately 10% extra risk of being at risk of having an adverse effect.

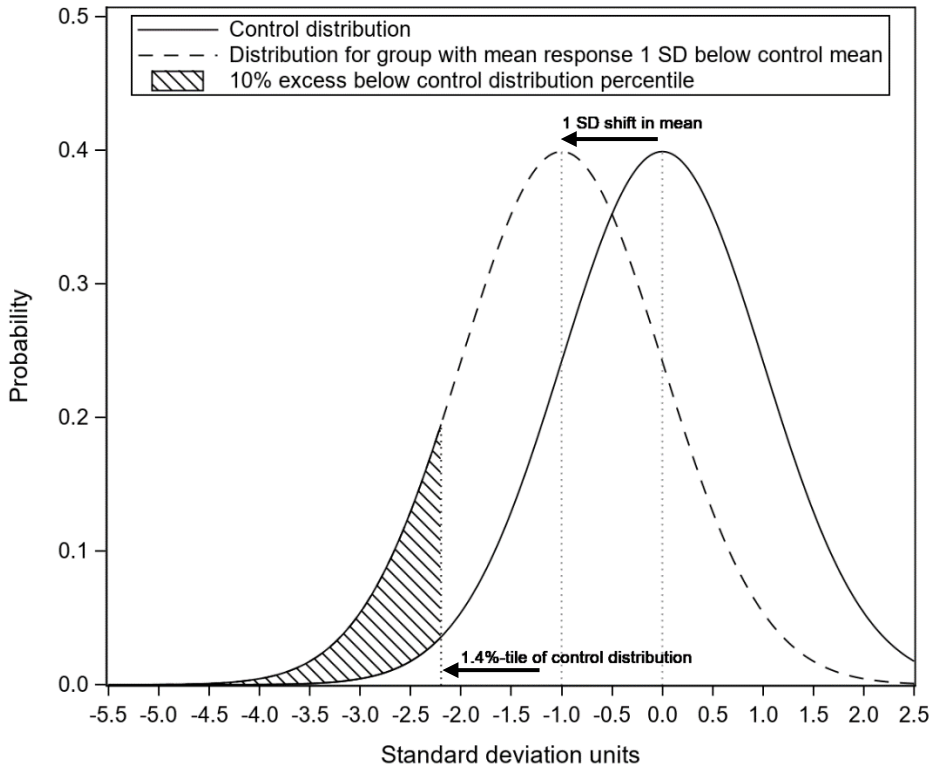


Figure C-1. Difference in population tail probabilities resulting from a one-standard deviation shift in the mean from a standard normal distribution, illustrating the theoretical basis for a baseline BMR of 1 SD.

Statistically, the technical guidance additionally suggests that studies of developmental effects can support lower BMRs. Biologically, a BMR of ½ SD is a reasonable choice as antitetanus antibody concentrations prevent against tetanus, which is a rare, but severe and sometimes fatal infection, with a case-fatality rate in the United States of 13% during 2001–2008 (Liang et al., 2018). The case-fatality rate can be more than 80% for early lifestage cases (Patel and Mehta, 1999). Selgrade (2007) suggests that specific immunotoxic effects observed in children may be broadly indicative of developmental immunosuppression impacting their ability to protect against a range of immune hazards—which could be a more adverse effect than just a single immunotoxic effect. Thus, decrements in the ability to maintain effective levels of tetanus antitoxins following immunization may be indicative of wider immunosuppression in these children exposed to PFDA. By contrast, a BMR of 1-SD may be more appropriate for an effect that would be considered "minimally adverse." A BMR smaller than ½ SD is generally selected for severe effects (e.g., 1% extra risk of cancer mortality); decreased antibody concentrations offer diminished protection from severe effects but are not themselves severe effects.

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a one-SD change in the distribution of $\log_2$(tetanus antibody concentrations), and ½-SD change in the distribution of $\log_2$(tetanus antibody concentrations). The SD of the

$\log_2$(tetanus antibody concentrations) at age 7 years was estimated from the distributional data presented in Grandjean et al. (2012), as follows: The interquartile range (IQR) of the tetanus antibody concentrations at age 7 years in IU/mL was (0.65, 4.6). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (−0.62, 2.20). Assuming that these $\log_2$-transformed values are reasonably represented by a normal distribution, the width of the IQR is approximately 1.35 SDs. Thus, SD = IQR/1.35, and the SD of tetanus antibodies in $\log_2$(IU/mL) is (2.20 – (−0.62))/1.35 = 2.09 $\log_2$(IU/mL). To show the impact of the BMR on these results, Table C-2 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

While there was no clear definition of the size of an adverse effect for a continuous endpoint like antibody concentrations, the value of 0.1 IU/mL is sometimes cited. As a check, EPA evaluated how much extra risk would have been associated with a BMR set at a cutoff value of 0.1 IU/mL. Using the observed distribution of tetanus antibodies at age 7 years in $\log_2$(IU/mL), EPA calculated that 2.8% of those values would be below the cutoff value of 0.1 IU/mL which is −3.32 $\log_2$(IU/mL). A BMR of ½ SD resulted in 7.9% of the values being below that cutoff, which is 5.1% extra risk and demonstrates the generic guidance that a BMR of ½ SD can provide a reasonably good estimate of 5% extra risk. Figure C-2 shows an example of this.

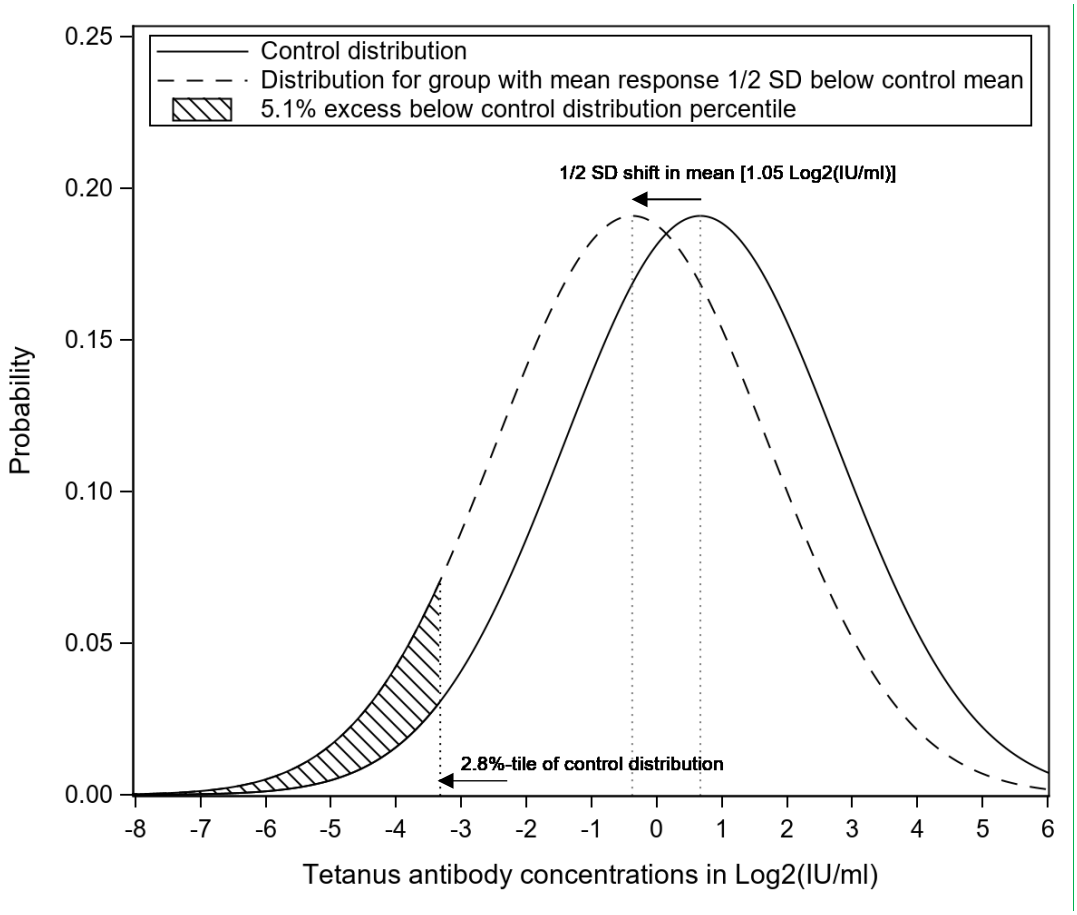


Figure C-2. Difference in population tail probabilities resulting from a ½-standard deviation shift in the mean from an estimation of the distribution of $\log_2$(tetanus antibody concentrations at age 7 years).

To provide context for the size of the two BMRs evaluated, the BMR (½ SD) is used in the derivation of the BMDL. The SD of the $\log_2$(tetanus antibody concentration in IU/mL) is 2.09 $\log_2$(IU/mL), and thus ½ SD is 1.05 $\log_2$(IU/mL). Exponentiating this to the natural scale, a ½-SD change in $\log_2$(IU/mL) is equivalent to a 2.07-IU/mL change (i.e., $2^{1.05}$). The interquartile range of PFDA in the serum of 5-year-olds was (0.65 IU/mL, 4.60 IU/mL), so a ½-SD change of 2.07 IU/mL is approximately equal to half the interquartile rage or about a 25% change in the distribution.

Table C-2. BMDs and BMDLs for effect of PFDA at age 5 years on antitetanus antibody concentrations at age 7 years using a BMR of ½-SD change in $\log_2$(tetanus antibodies concentration) and a BMR of 1-SD change in $\log_2$(tetanus antibodies concentration)

	Estimated without control of PFOS and PFOA		Estimated with control of PFOS and PFOA	
BMR	**BMD (ng/mL in serum)** β = −1.55 per ng/mL	**BMDL (ng/mL in serum)** β_{LB} = −2.55 per ng/mL	**BMD (ng/mL in serum)** β = −0.98 per ng/mL	**BMDL (ng/mL in serum)** β_{LB} = −2.10 per ng/mL
½ SD	0.673	**0.411**[a]	1.067	0.497
1 SD	1.346	0.821	2.135	0.994

[a]Denotes the selected POD.

The lowest serum PFDA concentration measured at age 5 years was 0.05 ng/mL, the 5th percentile was 0.1 ng/mL, and the 10th percentile was 0.2 ng/mL (Grandjean and Bateson, 2021), so the estimated BMDL for a BMR of ½ SD ($BMDL_{½\ SD}$) in the single-PFAS model is above the 10th percentile of the observed distribution. No information was available to judge the fit of the model in the range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFDA well.

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 59% higher than the $BMD_{½\ SD}$ estimate from the models with just PFDA, and the $BMDL_{½\ SD}$ estimate is 21% higher. The change in BMD estimates may or may not reflect control for any potential confounding of the regression effect estimates. While which PFAS model provided a "better" estimate of the point estimate of the effect of PFDA is not clear, the two $BMDL_{½\ SD}$ estimates are similar (0.411 ng/mL vs. 0.497 ng/mL) and EPA advanced the derivation on the basis of results that did not control for PFOS and PFOA because this model appeared to fit PFDA better (p = 0.01 vs. 0.15) and there was low uncertainty due to potential confounding in the BMDL. However, confidence was somewhat diminished by the potential confounding in the main effect—even though there was low confounding of the BMDL. Overall confidence in the BMDLs for tetanus was judged *medium*.

For immunotoxicity related to tetanus associated with PFDA exposure measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 0.411 ng/mL in serum.

Modeling Results for Decreased Diphtheria Antibody Concentrations at 7 Years of Age and PFDA Measured at 5 Years of Age

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFDA measured at age 5 years, against $\log_2$-transformed antidiphtheria antibody concentrations measured at the 7-year-old examination controlling for sex, exact age at the 7-year-old examination, and booster type at age 5 years. Models were evaluated with additional control for PFOS (as $\log_2$[PFOS]) and PFOA (as $\log_2$[PFOA]), and without PFOS and PFOA. Three model shapes were evaluated by Budtz-Jørgensen

and Grandjean (2018a) using likelihood ratio tests: a linear model of PFDA, a piecewise-linear model with a knot at the median, and a logarithmic function. The logarithmic functions fit no better than the piecewise-linear functions (Budtz-Jørgensen and Grandjean, 2018a). The piecewise-linear model fit no better than the linear model for the PFDA exposure without adjustment for PFOS and PFOA using a likelihood ratio test (p = 0.55; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOS and PFOA ($\log_2$[PFOS] and $\log_2$[PFOA]) (p = 0.73). Table C-3 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for diphtheria in this exposure window. These regression coefficients (β), their standard errors (SE), p-values, and the 90% lower confidence bounds were provided by (Budtz-Jørgensen and Grandjean, 2018b).

Table C-3. Results specific to the slope from the linear analyses of PFDA in serum measured at age 5 years and $\log_2$(diphtheria antibodies) measured at age 7 years from Table 1 in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b)

Exposure	Model shape	PFOS and PFOA adjusted	Slope (β) per ng/mL in serum	SE(β) ng/mL in serum	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
PFDA at age 5 yr	Linear	No	−0.894	0.561	p = 0.11	−1.82
PFDA at age 5 yr	Linear	Yes	−0.297	0.635	p = 0.64	−1.35

Interpretation of results in Table C-3:

- PFDA is a nonsignificant predictor in the single-PFAS model (β = −0.894; p = 0.11).
- Effects are attenuated when $\log_2$[PFOS] and $\log_2$[PFOA] are included in the model (β = −0.297; p = 0.64).
- The point estimate results for PFDA are *potentially* confounded by PFOS and/or PFOA since there was a 67% reduction in the effect size for PFDA from −0.894 to −0.297 when controlling for PFOS and PFOA.
    - One explanation is that PFOS and/or PFOA were confounders of the PFDA effect and controlling for those coexposures removed confounding.
    - Another possibility is that controlling for coexposures like PFOS and PFOA induced confounding (Weisskopf et al., 2018; Weisskopf and Webster, 2017).
    - The reasons for the change in main effect size for PFDA are unknown. For this reason, there is uncertainty in knowing which point estimate is the best representation of any effect of PFDA.
- However, the lower bound on the point estimate (β_{LB}) for the single-PFAS model is 26% lower than the multi-PFAS model estimate for PFDA.
    - The definition of the RfD, which is based on the β_{LB}, includes allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate and the uncertainty for

potential confounding in the BMD from including or excluding PFOS and PFOA here is about 67%, while the uncertainty for potential confounding in the BMDL is about 35%.

Selection of the Benchmark Response

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1-SD change in the distribution of $\log_2$(diphtheria antibody concentrations), and ½-SD change in the distribution of $\log_2$(diphtheria antibody concentrations). A blood concentration for diphtheria antibodies of 0.1 IU/mL is sometimes cited in the diphtheria literature as a "protective level." Grandjean et al. (2017) noted that the Danish vaccine producer, Statens Serum Institut, recommended the 0.1 IU/mL "cutoff" level; and Galazka et al. (1993) mentions the same concentration), but Galazka et al. (1993) argues:

> *"However, it has also been shown that there is no sharply defined level of antitoxin that gives complete protection from diphtheria (Ipsen, 1946). A certain range of variation must be accepted; the same degree of antitoxin may give an unequal degree of protection in different persons. Other factors may influence the vulnerability to diphtheria including the dose and virulence of the diphtheria bacilli and the general immune status of the person infected (*Christenson and Böttiger, 1986*). Thus, an antibody concentration between 0.01 and 0.09 IU/ml may be regarded as giving basic immunity, whereas a higher titer may be needed for full protection. In some studies that used in vitro techniques, a level of 0.1 IU/ml was considered protective (*Cellesi et al., 1989*; *Galazka and Kardymowicz, 1989*)."*

Statistically, the technical guidance suggests that studies of developmental effects can support lower BMRs. Biologically, a BMR of ½ SD is a reasonable choice as antidiphtheria antibody concentrations prevent against diphtheria, which is very rare in the United States but can cause life-threatening airway obstruction or cardiac failure (Collier, 1975). Among 13 cases reported in the United States during 1996–2016, no deaths were mentioned (Liang et al., 2018). However, diphtheria remains a potentially fatal disease in other parts of the world. (Galazka et al., 1993) mentions a case-fatality rate of 5%–10%) and PFDA-related changes in antidiphtheria antibody concentrations cannot be considered "minimally adverse," given the historic lethality of diphtheria in the absence of vaccination. Selgrade (2007) suggests that specific immunotoxic effects observed in children may be broadly indicative of developmental immunosuppression impacting their ability to protect against a range of immune hazards—which could be a more adverse effect that just a single immunotoxic effect.

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1-SD change in the distribution of $\log_2$(diphtheria antibody concentrations) as a standard reporting level, and ½-SD change in the distribution of $\log_2$(diphtheria antibody concentrations). The SD of the $\log_2$(diphtheria antibody concentrations) at age 7 years was estimated from the distributional data presented in Grandjean et al. (2012) as follows: the interquartile range (IQR) of the diphtheria antibody concentrations at age 7 years in IU/mL was (0.4, 1.6). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (−1.32, 0.68). Assuming

that these log_2-transformed values are similar to the normal distribution, the width of the IQR is approximately 1.35 SDs, thus SD = IQR/1.35, and the SD of tetanus antibodies in log_2(IU/mL) is (0.68 – (−1.32))/1.35 = 1.48 log_2(IU/mL). To show the impact of the BMR on these results, Table C-4 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table C-4. BMDs and BMDLs for effect of PFDA at age 5 years on antidiphtheria antibody concentrations at age 7 years using a BMR of ½-SD change in log_2(diphtheria antibodies concentration) and a BMR of 1-SD log_2(diphtheria antibodies concentration)

	Estimated without control of PFOS and PFOA		Estimated with control of PFOS and PFOA	
BMR	**BMD (ng/mL in serum) β = −0.894 per ng/mL**	**BMDL (ng/mL in serum) β_{LB} = −1.82 per ng/mL**	**BMD (ng/mL in serum) β = −0.297 per ng/mL**	**BMDL (ng/mL in serum) β_{LB} = −1.35 per ng/mL**
½ SD	0.827	**0.407**[a]	2.488	0.550
1 SD	1.655	0.813	4.976	1.100

[a]Denotes the selected POD.

The lowest serum PFDA concentration measured at age 5 years was 0.05 ng/mL, the 5th percentile was 0.1 ng/mL, and the 10th percentile was 0.2 ng/mL (Grandjean and Bateson, 2021), so the estimated BMDL for a BMR of ½ SD ($BMDL_{½\ SD}$) in the single-PFAS model is at the 10th percentile of the observed distribution. No information was available to judge the fit of the model in the range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFDA well.

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is threefold higher than the $BMD_{½\ SD}$ estimate from the model with just PFDA, and the $BMDL_{½\ SD}$ is 35% higher. This may or may not reflect control for any potential confounding of the regression effect estimates. While which PFAS model provided the "better" estimate of the point estimate of the effect of PFDA is not clear, the two $BMDL_{½\ SD}$ estimates that serve as the PODs are comparable (0.407 ng/mL vs. 0.550 ng/mL), and EPA advanced the POD on the basis of results that did not control for PFOS and PFOA because this model appeared to fit PFDA better (p = 0.11 vs. 0.64) and there was low uncertainty due to potential confounding in the BMDL. However, confidence was diminished by the nonsignificant fit for PFDA (p = 0.11) and stronger potential confounding in the main effect—even though there was low confounding of the BMDL—and overall confidence in the BMDLs for diphtheria was judged *low*.

For immunotoxicity related to diphtheria, associated with PFDA measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 0.407 ng/mL in serum.

Modeling Results for Decreased Tetanus Antibody Concentrations at 5 Years of Age and Perinatal PFDA

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFDA measured perinatally in maternal serum, against log_2-transformed antitetanus antibody concentrations

measured at the 5-year-old examination controlling for sex, and exact age at the 5-year-old examination, cohort, and interaction terms between cohort and sex and between cohort and age. Models were evaluated with additional control for PFOS (as $\log_2$[PFOS]) and PFOA (as $\log_2$[PFOA]), and without PFOS and PFOA. Three model shapes of PFDA were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model, a piecewise-linear model with a knot at the median, and a logarithmic function. The logarithmic functions fit no better than the piecewise-linear functions Budtz-Jørgensen and Grandjean (2018a). Compared to the linear model, the piecewise-linear model fit no better than the linear model for either the PFDA exposure without adjustment for PFOS and PFOA using a likelihood ratio test (p = 0.81; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOS and PFOA ($\log_2$[PFOS] and $\log_2$[PFOA]) (p = 0.84).

Table C-5 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for tetanus in this exposure window. These regression coefficients (β), their standard errors (SE), p-values, and the 90% lower confidence bounds were provided by Budtz-Jørgensen and Grandjean (2018b).

Table C-5. Results of the linear analyses of PFDA measured perinatally in maternal serum and tetanus antibodies measured at age 5 years in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b)

Exposure	Model shape	PFOS and PFOA adjusted	Slope (β) per ng/mL in serum	SE(β) ng/mL in serum	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
Perinatal PFDA	Linear	No	−0.343	0.462	p = 0.46	−1.10
Perinatal PFDA	Linear	Yes	0.038	0.554	p = 0.95	−0.874

Interpretation of results in Table C-5:

- PFDA is a nonsignificant predictor in the single-PFAS model (β = −0.34; p = 0.46).
- Effects are completely attenuated when $\log_2$[PFOS] and $\log_2$[PFOA] are included in the model (β = 0.038; p = 0.55).
- Nevertheless, these data can be used to estimate a BMDL for completeness and to allow comparisons across PFAS.

Selection of the Benchmark Response

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1-SD change in the distribution of $\log_2$(tetanus antibody concentrations), and ½-SD change in the distribution of $\log_2$(tetanus antibody concentrations). The SD of the $\log_2$(tetanus antibody concentrations) at age 5 years was estimated from two sets of distributional data presented from two different cohorts of 5-year-olds that were pooled in Budtz-Jørgensen and

Grandjean (2018a). Grandjean et al. (2012) reported on 587 5-year-olds from the cohort of children born during 1997–2000 and in Grandjean et al. (2017) reported on 349 5-year-olds from the cohort of children born during 2007–2009. The means and SDs were computed separately and then pooled to describe the common SD. The IQR of the tetanus antibody concentrations in the earlier birth cohort at age 5 years in IU/mL was (0.1, 0.51). Log_2-tranforming these values provides the IQR in log_2(IU/mL) as (−3.32, −0.97). Assuming that these log_2-transformed values are similar to the normal distribution, the width of the IQR is approximately 1.35 SDs, thus SD = IQR/1.35, and the SD of tetanus antibodies in log_2(IU/mL) is (−0.97 – (−3.32))/1.35 = 1.74 log_2(IU/mL). The IQR of the tetanus antibody concentrations in the later birth cohort at age 5 years in IU/mL was (0.1, 0.3). Log_2-tranforming these values provides the IQR in log_2(IU/mL) as (−3.32, −1.74), and the SD of tetanus antibodies in log_2(IU/mL) is (−1.74 – (−3.32))/1.35 = 1.17 log_2(IU/mL). The pooled variance is a weighted sum of the independent SDs, and the pooled SD was estimated as 1.55 log_2(IU/mL).[1] To show the impact of the BMR on these results, Table C-6 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table C-6. BMDs and BMDLs for effect of PFDA measured perinatally and antitetanus antibody concentrations at age 5 years

	Estimated without control of PFOS and PFOA		Estimated with control of PFOS and PFOA	
BMR	**BMD (ng/mL in serum) β = −0.343 per ng/mL**	**BMDL (ng/mL in serum) β_{LB} = −1.103 per ng/mL**	**BMD (ng/mL in serum) β = 0.038 per ng/mL**	**BMDL (ng/mL in serum) β_{LB} = −0.874 per ng/mL**
½ SD	2.260	**0.702**[a]	–	0.886
1 SD	4.520	1.405	–	1.773

[a]Denotes the POD that corresponds to the analyses of PFDA concentrations perinatally and tetanus antibodies at age 5 years; – = values cannot be determined.

The lowest perinatal maternal serum PFDA concentration measured was 0.03 ng/mL, the 5th percentile was 0.1 ng/mL, and the 10th percentile was 0.2 ng/mL (Grandjean, 2021), so the estimated BMDLs for a BMR of ½ SD ($BMDL_{½\ SD}$ = 0.702 ng/mL) in the single-PFAS model are well above the 10th percentile of the observed distribution. No information was available to judge the fit of the model in the range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFDA well. The $BMDL_{½\ SD}$ estimate from the single-PFAS models was 0.702 ng/mL in serum. The BMDL estimates from the multi-PFAS models were about 26% higher than for the single-PFAS model.

Confidence in the BMDLs from the PFDA-only model (0.702 ng/mL in serum) and in the multi-PFAS model (0.886 ng/mL in serum) was judged *low*. Confidence is diminished by the low quality of the model fit for PFDA in either model compared with the PFDA results from tetanus in

[1]Pooled variance for tetanus in 5-year-olds = $[(502 - 1)(1.74)^2 + (298 - 1)(1.17)^2]/[502 + 298 - 2] = 2.41$. The pooled SD is the square root of 2.41, which is 1.55 log_2(IU/mL).

the 5-year to 7-year exposure-outcome window of time, and there is some uncertainty regarding potential confounding.

For immunotoxicity related to tetanus associated with PFDA measured perinatally, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 0.702 ng/mL in serum. Note this result is based on a poorly fit PFDA regression parameter (β) estimated as −0.343 per ng/mL in serum (90% CI: −1.103, 0.417; $p = 0.46$) Budtz-Jørgensen and Grandjean (2018b), and thus this POD is identified with *low* confidence.

For immunotoxicity related to tetanus associated with PFDA exposure measured at age 5 years, the POD estimated for comparison purposes is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 0.702 ng/mL in serum.

Modeling Results for Decreased Diphtheria Antibody Concentrations at 5 Years of Age and Perinatal PFDA

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFDA measured perinatally against log_2-transformed antidiphtheria antibody concentrations measured at the 5-year-old examination controlling for sex and age. Models were evaluated with additional control for PFOS (as log_2[PFOS]) and PFOA (as log_2[PFOA]), and without PFOS and PFOA. Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model of PFDA, a piecewise-linear model with a knot at the median, and a logarithmic function. The logarithmic functions fit no better than the piecewise-linear functions Budtz-Jørgensen and Grandjean (2018a). There was evidence that the piecewise-linear model fit better than the linear model for the PFDA exposure without adjustment for PFOS and PFOA ($p = 0.05$; see in Budtz-Jørgensen and Grandjean (2018a), Table 3), but not for the model that adjusted for PFOS and PFOA (log_2[PFOS] and log_2[PFOA]) ($p = 0.12$). Table C-7 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for diphtheria in this exposure window. These regression coefficients (β) and their standard errors (SE) were computed by EPA from the published BMDs and BMDL on the basis of a BMR of 5% change in diphtheria antibody concentrations in Table 2 of Budtz-Jørgensen and Grandjean (2018a).[2]

[2](Budtz-Jørgensen and Grandjean, 2018a) computed BMDs and BMDLs using a BMR of 5% decrease in the antibody concentrations. Their formula, BMD = log_2(1-BMR)/β, can simply be reversed to solve for β = log_2(1−BMR)/BMD. For negative dose response when more exposure results in lower antibody concentration, the BMDL is based on the lower bound of β, (β_{LB}). Thus, the $\beta_{LB} = log_2(1-BMR)/BMDL$. The $SE(\beta) = (\beta - \beta_{LB})/1.645$. The *p*-value is the two-sided probability that $Z \leq SE(\beta)/\beta$.

Table C-7. Results of the analyses of PFDA measured perinatally in maternal serum and diphtheria antibodies measured at age 5 years in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b)

Exposure	Model shape	PFOS and PFOA adjusted	Slope (β) per ng/mL in serum	SE(β)	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
Perinatal PFDA	Piecewise	No	−3.70	2.25	p = 0.100	−7.40
Perinatal PFDA	Piecewise	Yes	−2.47	0.750	p = 0.001	−3.700

Interpretation of results in Table C-7:

- PFDA is a nonsignificant predictor in the single-PFAS model (β = −3.700; p = 0.10).
- Effects of PFDA are attenuated when PFOS and PFOA are in the model (β = −2.467; p = 0.001).
- The point estimate results for PFDA are *potentially* confounded by PFOS and/or PFOA since there was a 33% change in the effect size for PFDA from −3.700 to −2.467 when controlling for PFOS and PFOA.
    - One explanation is that PFOS and/or PFOA were confounders of the PFDA effect and controlling for those coexposures removed confounding.
    - Another possibility is that controlling for coexposures like PFOS and PFOA actually induced confounding (Weisskopf et al., 2018; Weisskopf and Webster, 2017).
    - The reasons for the change in main effect size for PFDA are unknown. For this reason, there is uncertainty in knowing which point estimate is the best representation of any effect of PFDA.
- However, the lower bound on the point estimates (β_{LB}) for the single-PFAS model for PFDA is 100% lower than the multi-PFAS model effect estimate for PFDA.
    - The definition of the RfD, which is based on the β_{LB}, includes allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate and the uncertainty for potential confounding in the BMD from including or excluding PFOS and PFOA here is about 33%, while the uncertainty for potential confounding in the BMDL is about 100%.

Selection of the Benchmark Response

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1-SD change in the distribution of $\log_2$(tetanus antibody concentrations) as a standard reporting level, and ½-SD change in the distribution of $\log_2$(tetanus antibody concentrations). The SD of the $\log_2$(diphtheria antibody concentrations) at age 5 years was estimated from two sets of distributional data presented from two different birth cohorts of 5-year-olds that were pooled in Budtz-Jørgensen and Grandjean (2018a). Grandjean et al. (2012)

reported on 587 5-year-olds from the cohort of children born during 1997–2000 and Grandjean et al. (2017) reported on 349 5-year-olds from the cohort of children born during 2007–2009. The means and SDs were computed separately and then pooled to describe the common SD. The IQR of the diphtheria antibody concentrations in the earlier birth cohort at age 5 years in IU/mL was (0.05, 0.4). Log_2-tranforming these values provides the IQR in log_2(IU/mL) as (−4.32, −1.32). Assuming that these log_2-transformed values are similar to the normal distribution, the width of the IQR is approximately 1.35 SDs, thus SD = IQR/1.35, and the SD of diphtheria antibodies in log_2(IU/mL) is (−1.32 – (−4.32))/1.35 = 2.22 log_2(IU/mL). The IQR of the diphtheria antibody concentrations in the later birth cohort at age 5 years in IU/mL was (0.1, 0.3). Log_2-tranforming these values provides the IQR in log_2(IU/mL) as (−3.32, −1.74), and the SD of diphtheria antibodies in log_2(IU/mL) is (−1.74 – (−3.32))/1.35 = 1.17 log_2(IU/mL). The pooled variance is a weighted sum of the independent SDs, and the pooled SD was estimated as 1.90 log_2(IU/mL).[3] To show the impact of the BMR on these results, Table C-8 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table C-8. BMDs and BMDLs for effect of PFDA measured perinatally and antidiphtheria antibody concentrations at age 5 years

	Estimated without control of PFOS and PFOA		Estimated with control of PFOS and PFOA	
BMR	**BMD (ng/mL in serum) β = −3.700 per ng/mL**	**BMDL (ng/mL in serum) β_{LB} = −7.400 per ng/mL**	**BMD (ng/mL in serum) β = −2.467 per ng/mL**	**BMDL (ng/mL in serum) β_{LB} = −3.700 per ng/mL**
½ SD	0.257	0.128	0.385	**0.257[a]**
1 SD	0.514	0.257	0.770	0.514

[a]Denotes the POD that corresponds to the analyses of PFDA concentrations perinatally and diphtheria antibodies at age 5 years.

The lowest serum PFDA concentration measured perinatally was 0.03 ng/mL, the 5th percentile was 0.1 ng/mL, and the 10th percentile was 0.2 ng/mL (Grandjean and Bateson, 2021), so the estimated BMD for a BMR of ½ SD ($BMDL_{½\ SD}$) in the single-PFAS model is well within the observed range. No information was available to judge the fit of the model in the range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFDA well.

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 50% higher than the $BMD_{½\ SD}$ estimated from the model with just PFDA, and the $BMDL_{½\ SD}$ is 100% higher. This may or may not reflect control for any potential confounding of the regression effect estimates. The BMDLs that serve as the PODs are twofold different (0.128 ng/mL vs. 0.257 ng/mL) and EPA advanced the derivation on the basis of results that did control for PFOS and PFOA because this model appeared to fit PFDA well (p = 0.001 vs. 0.10) and there was low uncertainty due to potential confounding in the BMD and moderate uncertainty in the BMDL. Confidence in the BMDLs from PFDA linear model

[3]Pooled variance for diphtheria in 5-year-olds = $[(502 - 1)(2.22)^2 + (298 - 1)(1.17)^2]/[502 + 298 - 2] = 3.60$. The pooled SD is the square root of 2.41, which is 1.90 log_2(IU/mL).

(0.257 ng/mL in serum) with control of PFOS and PFOA is judged *medium* since the model fit reasonably well and these BMDLs show moderate uncertainty about confounding.

For immunotoxicity related to diphtheria, associated with PFDA measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 0.257 ng/mL in serum.

Summary of Modeling Results for Decreased Antibody Responses in Children

Table C-9 presents the BMDs and BMDLs from Budtz-Jørgensen and Grandjean (2018a) considered for POD derivation for reduced antibody responses across different combinations of exposure timing and outcome measurement, as detailed above. The BMDLs across the studies and methods ranged from 0.257 to 0.702 ng/mL.

Table C-9. Selected BMDs and BMDLs and associated uncertainty for effect of PFDA on decreased antibody responses in children from Budtz-Jørgensen and Grandjean (2018a)

Endpoint	$BMD_{1/2SD}$ (ng/mL)	$BMDL_{1/2SD}$ (ng/mL)	Confidence
Decreased serum tetanus antibody concentrations at 7 yr of age and PFDA measured at 5 yr of age[a]	0.673	0.411	Medium
Decreased serum diphtheria antibody concentrations at 7 yr of age and PFDA concentrations at 5 yr of age[a]	0.827	0.407	Low
Decreased serum tetanus antibody concentrations at 5 yr of age and perinatal PFDA (pregnancy wk 32–2 wk postpartum)[a]	2.260	0.702	Low
Decreased serum diphtheria antibody concentrations at 5 yr of age and perinatal PFDA (pregnancy wk 32–2 wk postpartum)[b]	0.385	0.257	Medium

[a]Estimated without control for PFOS and PFOA.
[b]Estimated with control for PFOS and PFOA.

C.1.2. Benchmark Dose Modeling Approaches for Developmental Effects

Modeling Results for Decreased Birth Weight

Five *high* confidence studies (Luo et al., 2021; Yao et al., 2021; Wikström et al., 2020; Valvi et al., 2017; Division of Environmental Epidemiology et al., 2016) reported decreased birth weight in infants whose mothers were exposed to PFDA. All studies reported exposure metrics in units of ng/mL and reported the β coefficients per ln(ng/mL) or per $\log_2$(ng/mL), along with 95% confidence intervals, estimated from linear regression models. The logarithmic transformation of exposure yields a negative value for low numbers, which can result in implausible results from

dose-response modeling (i.e., estimated risks are negative and responses at zero exposure cannot be determined). EPA first re-expressed the reported β coefficients in terms of per ng/mL according to Dzierlenga et al. (2020). Then EPA used the re-expressed β and the lower limit on the confidence interval to estimate BMD and BMDL values using the general equation $y = mx + b$, where y is birth weight and x is exposure, substituting the re-expressed β values from these studies for m. The intercept b represents the baseline value of birth weight in an unexposed population, and it can be estimated through $\overline{y} = m\overline{x} + b$ using an average birth weight from an external population as $\overline{y}$, an average exposure as $\overline{x}$, and a re-expressed β from the studies as m.

The CDC Wonder site (https://wonder.cdc.gov/natality.html) provides vital statistics for babies born in the United States. There were 3,791,712 live births in the United States in 2018 according to final natality data. The mean and standard deviation for birth weight were 3,261.6 ± 590.7 g (7.19 ± 1.30 lb), with 8.27% of live births falling below the public health definition of low birth weight (i.e., <2,500 g, or 5.5 lb). The full natality data for the U.S. data on birth weight was used as it is more relevant for deriving toxicity values for the U.S. public than the study-specific birthweight data. Also, the CDC Wonder database may be queried to find the exact percentage of the population falling below the cutoff value for clinical adversity. The CDC National Health and Nutrition Examination Survey (NHANES) Biomonitoring Data for Environmental Chemicals (https://www.cdc.gov/exposurereport/data_tables.html) provides the median serum PFDA concentrations (0.19 ng/mL) among NHANES females in 2011–2012. These values are subsequently used in the estimation of BMD and BMDL values from the available five epidemiological studies.

Valvi et al. (2017) reported a β coefficient of −41 g per $\log_2$(ng/mL) (95% CI: −102, 18) for the association between birth weight and maternal PFDA serum concentrations in a Denmark cohort. The reported β coefficient can be re-expressed in terms of per ng/mL according to (Dzierlenga et al., 2020). Given the reported study-specific median (0.28 ng/mL) and interquartile range (IQR) (0.22–0.38 ng/mL) of the exposure from (Valvi et al., 2017), EPA estimated the distribution of exposure by assuming the exposure follows a log-normal distribution with mean and standard deviation as:

$$\mu = ln(q_{50}) = ln(0.28) = -1.27 \quad \text{(C-1)}$$

$$\sigma = ln(q_{75}/q_{25})/1.349 = ln(0.38/0.22)/1.349 = 0.41 \quad \text{(C-2)}$$

Then, EPA estimated the 25th–75th percentiles at 10 percentile intervals of the exposure distribution and corresponding responses of reported β coefficient. The re-expressed β coefficient is determined by minimizing the sum of squared differences between the curves generated by the re-expressed β and the reported β. This resulted in a re-expressed β coefficient of −207.7 g per ng/mL (95% CI: −516.8, 91.2 g per ng/mL).

Typically, for continuous data, the preferred definition of the benchmark response (BMR) is to have a basis for what constitutes a minimal level of change in the endpoint that is biologically

significant. For birth weight, there is no accepted percentage change that is considered adverse. However, there is a clinical measure for what constitutes an adverse response: babies born weighing less than 2,500 g are considered to have low birth weight, and further, low birth weight is associated with a wide range of health conditions throughout life (Tian et al., 2019; Reyes and Mañalich, 2005; Hack et al., 1995). Given this clinical cutoff for adversity and that 8.27% of live births in the United States in 2018 fell below this cutoff, the hybrid approach can be used to define the BMR. The hybrid approach is advantageous in that it harmonizes the definition of the BMR for continuous data with that for dichotomous data.[4] Essentially, the hybrid approach involves the estimation of the dose that increases the percentile of responses falling below (or above) some cutoff for adversity in the tail of the response distribution. Application of the hybrid approach requires the selection of an extra risk value for BMD estimation. In the case of birth weight, an extra risk of 5% is selected given that this level of response is typically used when modeling developmental responses from animal toxicology studies, and that low birthweight confers increased risk for adverse health effects throughout life, thus supporting a BMR lower than the standard BMR of 10% extra risk.

Therefore, given a background response and a BMR = 5% extra risk, the BMD would be the dose that results in 12.86% of the responses falling below the 2,500 g cutoff value:

$$Extra\ Risk(ER) = (P(d) - P(0)) \,/\, (1 - P(0)) \quad \text{(C-3)}$$

$$P(d) = ER(1 - P(0)) + P(0) = 0.05(1 - 0.0827) + 0.0827 = 0.1286 \quad \text{(C-4)}$$

On the basis of the mean birth weight for all births in the United States of 3,261.6 g with a standard deviation of 590.7 g, EPA calculated the mean response that would be associated with the 12.86th percentile of the distribution falling below 2,500 g. In this case, the mean birth weight would be 3,169.2 g. Given the median exposure among NHANES females as $\overline{x}$, the mean birth weight in the United States as $\overline{y}$, and the re-expressed β as m term, the intercept b can be estimated as:

$$b = \overline{y} - m\overline{x} = 3{,}261.6\ g - \left(-207.7\ g\left(\frac{ng}{mL}\right)^{-1}\right) 0.19\frac{ng}{mL} = 3{,}301.1\ g \quad \text{(C-5)}$$

The BMD was calculated by rearranging the equation $y = mx + b$ and solving for x, using 3,301.1 g for the b term and −207.7 for the m term. This resulted in a value of 0.63 ng/mL:

$$x = (y - b)/m = (3{,}169.2\ g - 3{,}301.1\ g)/(-207.7\ g\left(\frac{ng}{mL}\right)^{-1}) = 0.63\ ng/mL \quad \text{(C-6)}$$

To calculate the BMDL, the method is essentially the same except that the lower limit on the re-expressed β coefficient (−516.8 g per ng/mL) is used for the m term. However, (Valvi et al.,

[4]While the explicit application of the hybrid approach is not commonly used in IRIS dose/concentration/exposure-response analyses, the more commonly used SD definition of the BMR for continuous data is simply one specific application of the hybrid approach. The SD definition of the BMR assumes the cutoff for adversity is the 1.4th percentile of a normally distributed response and shifting the mean of that distribution by 1 standard deviation approximates an extra risk of 10%.

2017) reports a two-sided 95% confidence interval for the β coefficient, meaning that the lower limit of that confidence interval corresponds to a 97.5% one-sided lower limit. The BMDL is defined as the 95% lower limit of the BMD (i.e., corresponds to a two-sided 90% confidence interval), so the corresponding lower limit on the re-expressed β coefficient needs to be calculated before calculating the BMDL. First, the standard error of the re-expressed β coefficient can be calculated as:

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{91.2\ g(\frac{ng}{mL})^{-1} - \left(-516.8\ g(\frac{ng}{mL})^{-1}\right)}{3.92} = 155.1\ g(\frac{ng}{mL})^{-1} \quad \text{(C-7)}$$

Then the corresponding 95% one-sided lower limit on the re-expressed β coefficient is calculated as:

$$95\%\ one-sided\ LL = \beta - 1.645(SE(\beta)) = -207.7\ g(\frac{ng}{mL})^{-1} - 1.645\left(155.1\ g(\frac{ng}{mL})^{-1}\right) = -462.9 g(\frac{ng}{mL})^{-1} \quad \text{(C-8)}$$

Using this value for the m term results in a BMDL value of 0.28 ng/mL maternal serum concentration.

Valvi et al. (2017) also reported a β coefficient of −44 g per $\log_2$(ng/mL) (95% CI: −133, 44 g per $\log_2$(ng/mL) for boys and −28 g per $\log_2$(ng/mL) (95% CI: −110, 54 g per $\log_2$(ng/mL)) for girls. The re-expressed β coefficients are −222.9 g per ng/mL (95% CI: −673.9, 222.9 g per ng/mL) and −141.9 g per ng/mL (95% CI: −557.3, 273.6 g per ng/mL), and the intercepts *b* are 3,304.0 g and 3,288.6 g for boys and girls, respectively. Using these sex-specific values, the estimated BMD values are 0.60 ng/mL for boys and 0.84 ng/mL for girls.

To calculate the BMDL, the same procedure as above is used to calculate the corresponding 95% one-sided lower limit for the re-expressed β coefficient from the re-expressed lower limit on the 95% two-sided confidence interval of −673.9 g per ng/mL for boys and −557.3 g per ng/mL for girls. Using the corresponding lower limit (−599.2 g per ng/mL for boys and −490.5 g per ng/mL for girls), the BMDLs of 0.22 ng/mL for boys and 0.24 ng/mL for girls are calculated.

Division of Environmental Epidemiology et al. (2016) reported a β coefficient of −43.9 g per ln(ng/mL) (95% CI: −104.8, 17.0 g per ln(ng/mL) for the association between birth weight and maternal PFDA serum concentrations in a multicountry cohort. Given the reported study-specific geometric mean (0.25) and standard deviation of ln-transformed exposure (0.70), EPA estimated the mean (−1.41) and standard deviation (0.70) of the log normally distributed exposure. The re-expressed β coefficient is −122.2 g (95% CI: −291.5, 47.2) per ng/mL, and the intercept b is 3,284.8 g. The 95% one-sided lower limits for the re-expressed β coefficient are −264.3 g per ng/mL. The values of the BMD and BMDL are 0.95 ng/mL and 0.44 ng/mL, respectively.

Luo et al. (2021) reported a β coefficient of −96.8 g per ln(ng/mL) (95% CI: −178.0, −15.5 g per ln(ng/mL)) for the association between birth weight and maternal PFDA serum concentrations in a China cohort. Given the reported study-specific median (0.48 ng/mL) and IQR (0.34–0.70 ng/mL) of the exposure, EPA estimated the mean (−0.73) and standard deviation (0.54) of the

log normally distributed exposure. The re-expressed β coefficient is −195.8 g per ng/mL (95% CI: −360.2, −31.4 g per ng/mL), and the intercept *b* is 3,298.8 g. The 95% one-sided lower limits for the re-expressed β coefficient are −333.8 g per ng/mL. The values of the BMD and BMDL are 0.66 ng/mL and 0.39 ng/mL, respectively.

Wikström et al. (2020) reported a β coefficient of −58.0 g per ln(ng/mL) (95% CI: −103.0, −13.0 g per ln(ng/mL)) for the association between birth weight and maternal PFDA serum concentrations in a Swedish cohort. Given the reported study-specific median (0.26 ng/mL) and IQR (0.19–0.34 ng/mL) of the exposure, EPA estimated the mean (−1.35) and standard deviation (0.43) of the log normally distributed exposure. The re-expressed β coefficient is −218.9 g per ng/mL (95% CI: −388.7, −49.1 g per ng/mL) and the intercept *b* is 3,303.2 g. The 95% one-sided lower limits for the re-expressed β coefficient are −361.4 g per ng/mL. The values of the BMD and BMDL are 0.61 ng/mL and 0.37 ng/mL, respectively.

Wikström et al. (2020) also reported β coefficients of −47 g per ln(ng/mL) (95% CI: −112, 17 g per ln(ng/mL)) for boys and −69 g per ln(ng/mL) (95% CI: −133, −6 g per ln(ng/mL)) for girls. The re-expressed β coefficients are −177.4 g per (95% CI: −422.7, 64.2 g per ng/mL) and −260.4 g per (95% CI: −501.9, −22.6 g per ng/mL), and the intercepts *b* are 3,295.3 g and 3,311.1 g for boys and girls, respectively. Using these sex-specific values, the estimated BMD values are 0.71 ng/mL for boys and 0.54 ng/mL for girls. The corresponding 95% one-sided lower limits for the re-expressed β coefficient are −381.6 g per and −461.5 g per for boys and girls, respectively. The BMDL values are 0.33 ng/mL for boys and 0.31 ng/mL for girls.

Yao et al. (2021) reported a β coefficient of −46.3 g per ln(ng/mL) (95% CI: −131.1, 38.5 g per ln(ng/mL)) for the association between birth weight and maternal PFDA serum concentrations in a China cohort. Given the reported study-specific median (0.55 ng/mL) and IQR (0.37–0.74 ng/mL) of the exposure, EPA estimated the mean (−0.60) and standard deviation (0.51) of the log normally distributed exposure. The re-expressed β coefficient is −82.0 g per (95% CI: −232.1, 68.1 g per ng/mL) and the intercept *b* is 3,277.2 g. The 95% one-sided lower limits for the re-expressed β coefficient are −208.0 g per ng/mL. The values of the BMD and BMDL are 1.32 ng/mL and 0.52 ng/mL, respectively.

For all the above calculations, EPA used the exact percentage (8.27%) of live births in the United States 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 ($P(0)$). However, this exact percentage of 8.27% was calculated without accounting for the existence of background PFDA exposure in the U.S. population (i.e., 8.27% is not the tail probability of extreme response at zero dose). Thus, EPA considers an alternative control-group response distribution ($N(\mu_c, \sigma_c)$), using the study-specific intercept *b* obtained through equation (C-5) (representing the baseline value of birth weight in an unexposed population) as μ_c and the standard deviation of the U.S. population as σ_c, to estimate the tail probability that fell below the cutoff of 2,500 g. EPA estimated the study-specific tail probability of live births falling below the public health definition of low birth weight (2,500 g) as:

$$P(0) = \frac{1}{\sigma_c\sqrt{2\pi}}\int_{-\infty}^{2500} e^{\left(-\frac{(x-b)^2}{2\sigma_c^2}\right)}\,dx = \frac{1}{590.7\sqrt{2\pi}}\int_{-\infty}^{2500} e^{\left(-\frac{(x-b)^2}{2*590.7^2}\right)}\,dx \quad \text{(C-9)}$$

$$b = \overline{y} - m\overline{x} = 3{,}261.6 - \left(\beta_{re-expressed} * 0.19\frac{ng}{mL}\right) \quad \text{(C-10)}$$

In this alternative approach, $P(0)$ is 9.86% if there is no background exposure ($\overline{x} = 0$). By using the median serum PFDA concentrations (0.19 ng/mL) from NHANES females in 2011–2012 as background exposure ($\overline{x}$), the tail probabilities using this alternative approach were study specific and ranged from 8.48% to 9.41%. As such, the results from this alternative approach, presented under the column of "Alternative Tail Probability" in Table C-10, are very similar to the main results, presented under the column of "Exact Percentage" in Table C-10, when background exposure was not accounted for when estimating the tail probability.

The SAS code and results are provided by (Ru and Davis, 2024). Table C-10 presents the BMDs and BMDLs for all studies considered for POD derivation, with and without accounting for background exposure when estimating the percentage of the population falling below the cutoff value from (Ru and Davis, 2024). The BMDLs across the studies and methods ranged from 0.22 ng/mL to 0.66 ng/mL.

Table C-10. BMDs and BMDLs for effect of PFDA on decreased birth weight, using percentage (8.27%) of live births falling below the public health definition of low birth weight, or alternative study-specific tail probability

Study	Exposure median (IQR) or GM (SD)	Exposure distribution (μ, σ)	Reported β (95% CI)	Re-expressed β (95% CI) g/ng/mL	Intercept b	SE of β	95% one-sided LL of β	Exact percentage ($p(0) = (0) =$ 8.27%)		Alternative tail probability		
								BMD (ng/mL)	BMDL (ng/mL)	$P(0)$	BMD (ng/mL)	BMDL (ng/mL)
Valvi et al. (2017)	0.28 (0.22–0.38)	(−1.27, 0.41)	−41.0 (−102.0, 18.0) g/log$_2$(ng/mL)	−207.7 (−516.8, 91.2)	3,301.1	155.11	−462.9	0.63	0.28	8.75%	0.70	0.31
Valvi et al. (2017) Boys	0.28 (0.22–0.38)	(−1.27, 0.41)	−44.0 (−133.0, 44.0) g/log$_2$(ng/mL)	−222.9 (−673.9, 222.9)	3,304.0	228.78	−599.2	0.60	0.22[b]	8.67%	0.65	0.24
Valvi et al. (2017) Girls	0.28 (0.22–0.38)	(−1.27, 0.41)	−28.0 (−110.0, 54.0) g/log$_2$(ng/mL)	−141.9 (−557.3, 273.6)	3,288.6	211.98	−490.5	0.84	0.24	9.09%	0.99	0.29
Division of Environmental Epidemiology et al. (2016)	0.25 (0.70)[c]	(−1.41, 0.70)	−43.9 (−104.8, 17.0) g/ln(ng/mL)	−122.2 (−291.5, 47.2)	3,284.8	86.40	−264.3	0.95	0.44	9.20%	1.14	0.53
Luo et al. (2021)	0.48 (0.34–0.70)	(−0.73, 0.54)	−96.8 (−178.0, −15.5) g/ln(ng/mL)	−195.8 (−360.2, −31.4)	3,298.8	83.88	−333.8	0.66	0.39	8.81%	0.73	0.43
Wikström et al. (2020)	0.26 (0.19–0.34)	(−1.35, 0.43)	−58.0 (−103.0, −13.0) g/ln(ng/mL)	−218.9 (−388.7, −49.1)	3,303.2	86.64	−361.4	0.61	0.37	8.69%	0.66	0.40
Wikström et al. (2020) Boys	0.26 (0.19–0.34)	(−1.35, 0.43)	−47.0 (−112.0, 17.0) g/ln(ng/mL)	−177.4 (−422.7, 64.2)	3,295.3	124.19	−381.6	0.71	0.33	8.91%	0.80	0.37
Wikström et al. (2020) Girls	0.26 (0.19–0.34)	(−1.35, 0.43)	−69.0 (−133.0, −6.0) g/ln(ng/mL)	−260.4 (−501.9, −22.6)	3,311.1	122.26	−461.5	0.54	0.31	8.48%	0.57	0.32
Yao et al. (2021)	0.55 (0.37–0.74)	(−0.60, 0.51)	−46.3 (−131.1, 38.5) g/ln(ng/mL)	−82.0 (−232.1, 68.1)	3,277.2	76.58	−208.0	1.32	0.52	9.41%	1.68	0.66

[a]The alternative study-specific tail probability of live births falling below the public health definition of low birth weight is based on a normal distribution with intercept b as the mean and a standard deviation of 590.7 based on the U.S. population.

[b]Smallest BMDL using the five individual studies.

[c]Division of Environmental Epidemiology et al. (2016) reports Geometric Mean (GM) and standard deviation (SD) of ln-transformed concentrations.

C.2. BENCHMARK DOSE MODELING RESULTS FROM ANIMAL STUDIES

C.2.1. Benchmark Dose Modeling Approaches

The endpoints selected for benchmark dose (BMD) modeling are listed in Table C-11. The animal doses in the study were used in the BMD modeling and then converted to human equivalent doses (HEDs) using data-derived extrapolation factors (DDEFs) described in Section 3.1.7 of the main document; the modeling results are presented in this appendix and (Ru and Davis, 2024).

Modeling Procedure for Dichotomous Noncancer Data

BMD modeling of dichotomous noncancer data was conducted using EPA's Benchmark Dose Software (BMDS, version 3.2). For these data, the Gamma, Logistic, Log-Logistic, Log-Probit, Multistage, Probit, Weibull, and Dichotomous Hill models available within the software were fit using a benchmark response (BMR) of 10% extra risk (see Toxicological Review, Section 5.2.1 for justification of selected BMRs). The Multistage model is run for all polynomial degrees up to $n - 2$, where n is the number of dose groups including control. Adequacy of model fit was judged on the basis of χ^2 goodness-of-fit p-value ($p > 0.1$), scaled residuals at the data point (except the control) closest to the predefined benchmark response (absolute value < 2.0), and visual inspection of the model fit. In the cases for which no best model was found to fit to the data, a reduced dataset without the high-dose group was used for modeling and the result presented with that of the full dataset. In cases for which a model with several parameters equal to the number of dose groups was fit to the dataset, all parameters were estimated, and no p-value was calculated, that model was not considered for estimating a point of departure (POD) *unless* no other model provided adequate fit. Among all models providing adequate fit, the benchmark dose lower confidence limit (BMDL) from the model with the lowest Akaike's information criterion (AIC) was selected as a potential POD when BMDL values were sufficiently close (within threefold). Otherwise, the lowest BMDL was selected as a potential POD.

Modeling Procedure for Continuous Noncancer Data

BMD modeling of continuous noncancer data was conducted using EPA's Benchmark Dose Software (BMDS, version 3.2). For these data, the Exponential, Hill, Polynomial, and Power models available within the software are fit using a BMR of 1 standard deviation (SD) when no toxicological information was available to determine an adverse level of response. When toxicological information was available, the BMR was based on relative deviation, as outlined in the Benchmark Dose Technical Guidance (U.S. EPA, 2012) (see Toxicological Review, Section 5.2.1 justification for using BMRs); when a BMR based on relative deviation was used, modeling results using BMRs based on SD are included for reference. An adequate fit is judged on the basis of χ^2 goodness-of-fit p-value ($p > 0.1$), scaled residuals at the data point (except the control) closest to the predefined benchmark response (absolute value <2.0), and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination is made on whether the

variance across dose groups is homogeneous. If a homogeneous variance model is deemed appropriate on the basis of the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated from a homogeneous variance model. If the test for homogeneity of variance is rejected ($p < 0.05$), the model is run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model does not adequately fit the data (i.e., Test 3; $p < 0.05$), alternative approaches are assessed on a case-by-case basis. For example, in cases for which neither variance model fit, or constant variance did not fit (with adequate Test-4 *p*-value) and nonconstant variance did fit (with inadequate Test-4 *p*-value), the log-normal distribution was attempted.

In cases for which a model with several parameters equal to the number of dose groups was fit to the dataset, all parameters were estimated, and no *p*-value was calculated, that model was not considered for estimating a POD *unless* no other model provided adequate fit. Among all models providing adequate fit, the BMDL from the model with the lowest AIC was selected as a potential POD when BMDL estimates differed by less than threefold. When BMDL estimates differed by greater than threefold, the model with the lowest BMDL was selected to account for model uncertainty.

Modeling Procedure for Continuous Noncancer Developmental Toxicity Data

For continuous developmental toxicity data, data for individual animals were requested from the study authors when possible. The use of individual animal data allows for the correct measure of variance to be calculated. When a biological rationale for selecting a benchmark response level is lacking, a BMR equal to 0.5 SD was used. The use of 1 SD for the BMR for continuous endpoints is based on the observation that shifting the distribution of the control group by 1 SD results in ~10% of the animal data points falling beyond an adversity cutoff defined at the ~1.5 percentile (Crump, 1995). This approximates the 10% extra risk commonly used as the BMR for dichotomous endpoints. Thus, the use of 0.5 SD for continuous developmental toxicity endpoints approximates the extra risk commonly used for dichotomous developmental toxicity endpoints.

Data Used for Modeling

The source of the data used for modeling endpoints from animal studies is provided in Table C-11. These data also are included in full in the tables below.

Table C-11. Sources of data used in benchmark dose modeling of PFDA endpoints from animal studies

Endpoint/Reference	Reference	HAWC link
↑ AST – M	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506861/
↑ AST – F	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506957/
↑ ALP – F	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506956/
↑ Relative liver weight – M	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506814/
↑ Relative liver weight – F	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506920/
↑ Relative liver weight – F (Histopathology cohort)	Frawley et al. (2018)	https://hawcprd.epa.gov/ani/endpoint/100506676/
↑ Relative liver weight – F (MPS cohort)	Frawley et al. (2018)	https://hawcprd.epa.gov/ani/endpoint/100506669/
↑ Relative liver weight – F (TDAR to SRBC cohort)	Frawley et al. (2018)	https://hawcprd.epa.gov/ani/endpoint/100506677/
↓ Fetal body weight (GD 6–15)	Harris and Birnbaum (1989)	https://hawcprd.epa.gov/ani/endpoint/100506643/
↓ Caudal epididymis sperm count	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506879/
↓ Absolute testis weight	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506820/
↓ Absolute cauda epididymis weight	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506878/
↓ Absolute whole epididymis weight	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506877/
↓ Estrus time	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100524936/
↑ Diestrus time	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100524930/
↓ Relative uterus weight	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506941/
↓ Absolute uterus weight	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100506940/

C.2.2. Increased AST – Male Rats (NTP, 2018)[5]

Table C-12. Dose-response data for increased AST in male rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	10	65.3	10.18
0.156	10	74	9.55

[5]Throughout this section, in the benchmark dose results tables, the "Restriction" column denotes the restriction status of applied models, and the "Classification" column denotes whether a model can be considered for model selection purposes. See BMDS User Guide: https://www.epa.gov/bmds. If a model was selected as appropriately fitting the modeled data, that model's entries in the tables are in green shaded cells and the text is bolded.

Dose (mg/kg-d)	n	Mean	SD
0.312	10	77.3	16.98
0.625	10	81.3	9.84
1.25	10	87.5	14.61
2.5	9	92.67	8.04

Table C-13. Benchmark dose results for increased AST in male rats – constant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	1.3924	1.0640	0.1386	467.4755	Viable – Alternate	
Exponential 3 (CV – normal)	Restricted	1.3924	1.0640	0.1386	467.4755	Viable – Alternate	
Exponential 4 (CV – normal)	Restricted	0.3933	0.1723	0.8692	463.2441	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	0.3949	0.1723	0.8692	463.2441	Viable – Alternate	
Hill (CV – normal)	**Restricted**	**0.3266**	**0.1227**	**0.9560**	**462.8481**	**Viable – Recommended**	**Lowest BMDL**
Polynomial (5 degree) (CV – normal)	Restricted	1.2558	0.9260	0.1910	466.6376	Viable – Alternate	
Polynomial (4 degree) (CV – normal)	Restricted	1.2558	0.9260	0.1910	466.6376	Viable – Alternate	
Polynomial (3 degree) (CV – normal)	Restricted	1.2558	0.9260	0.1910	466.6376	Viable – Alternate	
Polynomial (2 degree) (CV – normal)	Restricted	1.2558	0.9260	0.1910	466.6376	Viable – Alternate	
Power (CV – normal)	Restricted	1.2558	0.9260	0.1910	466.6376	Viable – Alternate	
Linear (CV – normal)	Unrestricted	1.2558	0.9260	0.1910	466.6376	Viable – Alternate	

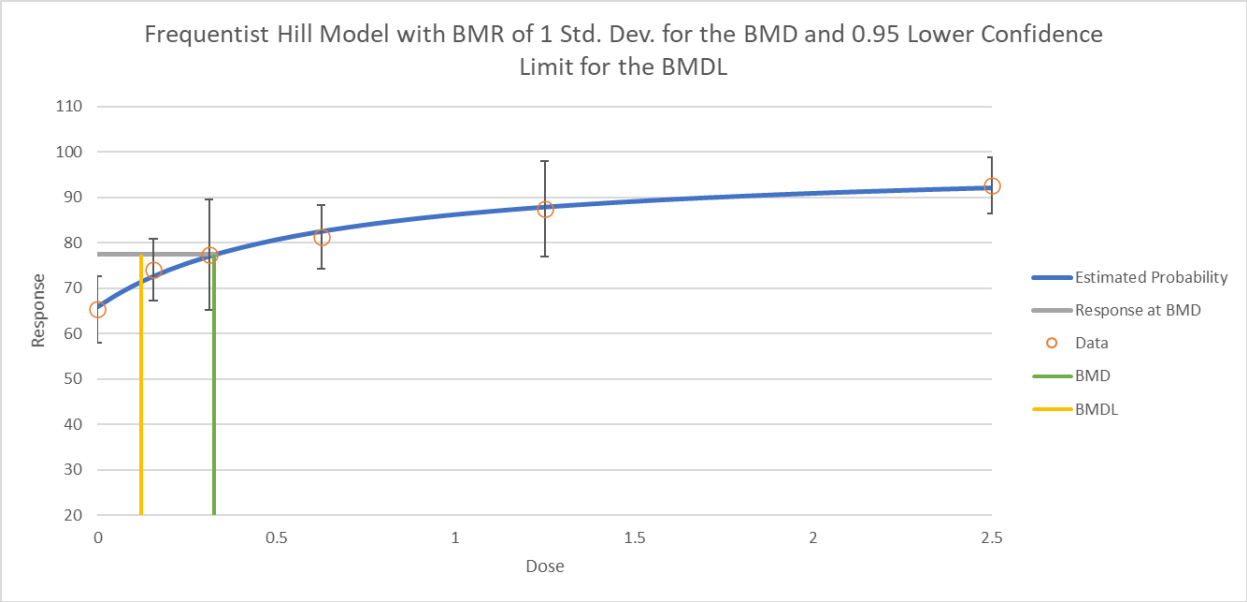


Figure C-3. Dose-response curve for the Hill model fit to increased AST in male rats (NTP, 2018).

User Input

Info	
Model	frequentist Hill v1.1
Dataset Name	AST_M_NTP
User notes	[Add user notes here]
Dose-Response Model	M[dose] = g + v*dose^n/(k^n + dose^n)
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Std. Dev.
BMRF	1
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	6
Adverse Direction	Automatic

Figure C-4. User Input for dose-response modeling of increased AST in male rats (NTP, 2018).

Model Results

Benchmark Dose	
BMD	0.32659537
BMDL	0.122653237
BMDU	0.926151614
AIC	462.8480778
Test 4 P-value	0.956041631
D.O.F.	3

Model Parameters	
# of Parameters	5
Variable	Estimate
g	65.96003464
v	32.30491688
k	0.59693749
n	Bounded
alpha	130.5126471

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	65.96003464	65.3	65.3	11.4242132	10.18	10.18	-0.182700792
0.156	10	72.65324238	74	74	11.4242132	9.55	9.55	0.372789045
0.312	10	77.0489535	77.3	77.3	11.4242132	16.98	16.98	0.06949089
0.625	10	82.48344375	81.3	81.3	11.4242132	9.84	9.84	-0.327582975
1.25	10	87.82387482	87.5	87.5	11.4242132	14.61	14.61	-0.089650123
2.5	9	92.03814971	92.67	92.67	11.4242132	8.04	8.04	0.165923977

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-227.2635646	7	468.527129
A2	-223.0848415	12	470.169683
A3	-227.2635646	7	468.527129
fitted	-227.4240389	4	462.848078
R	-241.1426777	2	486.285355

* Includes additive constant of -54.21737. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	36.11567239	10	<0.0001
2	8.357446131	5	0.13760531
3	8.357446131	5	0.13760531
4	0.320948692	3	0.95604163

Figure C-5. Model Results for increased AST in male rats (NTP, 2018).

C.2.3. Increased AST – Female Rats (NTP, 2018)

Table C-14. Dose-response data for increased AST in female rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	10	62.6	10.75
0.156	9	60.44	6.51
0.312	10	57.9	4.11
0.625	10	63.3	5
1.25	10	81.9	8.29
2.5	7	112.57	22.54

Table C-15. Benchmark dose results for increased AST in female rats – constant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.6219	0.5312	0.1426	427.8867	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Exponential 3 (CV – normal)	Restricted	0.8024	0.5551	0.1375	428.5314	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Exponential 4 (CV – normal)	Restricted	0.5006	0.0000	0.0153	433.4316	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 5 (CV – normal)	Restricted	0.1055	0.1048	<0.0001	553.6193	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. > |1.5| actual response std. dev.
Hill (CV – normal)	Restricted	0.9445	0.6992	0.5341	426.2660	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Polynomial (5 degree) (CV – normal)	Restricted	0.8055	0.5285	0.1331	428.6052	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Polynomial (4 degree) (CV – normal)	Restricted	0.8055	0.5285	0.1331	428.6052	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Polynomial (3 degree) (CV – normal)	Restricted	0.8055	0.5285	0.1331	428.6052	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Polynomial (2 degree) (CV – normal)	Restricted	0.8055	0.5285	0.1331	428.6052	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Power (CV – normal)	Restricted	0.8126	0.5686	0.2122	427.5127	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Linear (CV – normal)	Unrestricted	0.5006	0.4134	0.0339	431.4316	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2

Table C-16. Benchmark dose results for increased AST in female rats – nonconstant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV – normal)	Restricted	0.4683	0.3822	0.0006	417.7886	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 3 (NCV – normal)	Restricted	0.7433	0.5327	0.0048	413.2499	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Exponential 4 (NCV – normal)	Restricted	0.4044	0.3201	<0.0001	425.5227	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 5 (NCV – normal)	Restricted	0.9173	0.6965	0.0484	408.4035	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Hill (NCV – normal)	Restricted	1.1570	0.6738	0.0375	408.9143	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Polynomial (5 degree) (NCV – normal)	Restricted	0.8488	0.5738	0.0172	410.3710	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (4 degree) (NCV – normal)	Restricted	0.8488	0.5738	0.0172	410.3710	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (3 degree) (NCV – normal)	Restricted	0.8488	0.5738	0.0172	410.3710	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (NCV – normal)	Restricted	0.8488	0.5738	0.0172	410.3710	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Power (NCV – normal)	Restricted	0.7553	0.5621	0.0104	411.6066	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Linear (NCV – normal)	Unrestricted	0.4052	0.3203	<0.0001	423.4964	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2

Table C-17. Benchmark dose results for increased AST in female rats – log-normal, constant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – log-normal)	Restricted	0.4981	0.4114	0.0353	410.1569	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Exponential 3 (CV – log-normal)	Restricted	0.7017	0.4707	0.0518	409.5663	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 4 (CV – log-normal)	Restricted	0.4173	0.0000	0.0061	414.2361	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Exponential 5 (CV – log-normal)	Restricted	–9,999.0000	0.0000	<0.0001	482.3726	Unusable	BMD computation failed; lower limit includes zero BMD not estimated BMDL not estimated Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Hill (CV – log-normal)	Restricted	0.8526	0.6413	0.4051	405.6388	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) |Residual at control| > 2
Polynomial (5 degree) (CV – log-normal)	Restricted	0.7220	0.4645	0.0501	409.6412	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Polynomial (4 degree) (CV – log-normal)	Restricted	0.7220	0.4645	0.0501	409.6412	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Polynomial (3 degree) (CV – log-normal)	Restricted	0.7220	0.4645	0.0501	409.6412	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Polynomial (2 degree) (CV – log-normal)	Restricted	0.7220	0.4645	0.0501	409.6412	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Power (CV – log-normal)	Restricted	0.7158	0.5034	0.0953	408.1933	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Linear (CV – log-normal)	Unrestricted	0.4170	0.3303	0.0061	414.2360	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2

C.2.4. Increased ALP – Female Rat (NTP, 2018)

Table C-18. Dose-response data for increased ALP in female rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	9	136.4	18.6
0.156	9	156.1	24
0.312	10	182.8	36.68
0.625	10	184.2	33.2
1.25	10	281.1	72.42
2.5	7	262.4	60.06

Table C-19. Benchmark dose results for increased ALP in female rats— BMR = constant variance, 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	1.2058	0.9747	<0.0001	598.0449	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 Modeled control response std. dev. > |1.5| actual response std. dev.

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 3 (CV – normal)	Restricted	1.2058	0.9747	<0.0001	598.0449	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 4 (CV – normal)	Restricted	0.3043	0.1894	0.0206	585.6900	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 5 (CV – normal)	Restricted	0.6977	0.3389	0.0530	583.7962	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Hill (CV – normal)	Restricted	0.6547	0.6162	0.1011	582.1450	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (5 degree) (CV – normal)	Restricted	0.9018	0.6940	0.0005	594.1122	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (4 degree) (CV – normal)	Restricted	0.9018	0.6940	0.0005	594.1122	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (3 degree) (CV – normal)	Restricted	0.9018	0.6940	0.0005	594.1122	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (2 degree) (CV – normal)	Restricted	0.9018	0.6940	0.0005	594.1122	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Power (CV – normal)	Restricted	0.9018	0.6941	0.0005	594.1122	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Linear (CV – normal)	Unrestricted	0.9018	0.6940	0.0005	594.1122	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.

Table C-20. Benchmark dose results for increased ALP in female rats—nonconstant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV – normal)	Restricted	0.3761	0.2620	<0.0001	578.1584	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 3 (NCV – normal)	Restricted	0.3761	0.2620	<0.0001	578.1584	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 4 (NCV – normal)	Restricted	0.1191	0.0720	0.0174	565.0835	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 5 (NCV – normal)	Restricted	0.1556	0.0758	0.0083	566.5363	Questionable	Goodness-of-fit *p*-value < 0.1
Hill (NCV – normal)	Restricted	0.1501	0.0700	0.0056	567.3018	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (5 degree) (NCV – normal)	Restricted	0.2457	0.1655	0.0012	570.9484	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (4 degree) (NCV – normal)	Restricted	0.2457	0.1655	0.0012	570.9484	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (3 degree) (NCV – normal)	Restricted	0.2457	0.1655	0.0012	570.9484	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (NCV – normal)	Restricted	0.2457	0.1655	0.0012	570.9484	Questionable	Goodness-of-fit *p*-value < 0.1
Power (NCV – normal)	Restricted	0.2457	0.1655	0.0012	570.9484	Questionable	Goodness-of-fit *p*-value < 0.1

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Linear (NCV – normal)	Unrestricted	0.2457	0.1655	0.0012	570.9484	Questionable	Goodness-of-fit *p*-value < 0.1

Table C-21. Benchmark dose results for increased ALP in female rats—log-normal, constant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – log-normal)	Restricted	0.8447	0.6570	0.0001	575.0495	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Exponential 3 (CV – log-normal)	Restricted	0.8447	0.6570	0.0001	575.0495	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Exponential 4 (CV – log-normal)	Restricted	0.2215	0.1355	0.0337	563.1028	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Exponential 5 (CV – log-normal)	Restricted	0.3331	0.1470	0.0200	564.2382	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Hill (CV – log-normal)	Restricted	0.2860	0.1283	0.0121	565.2461	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Polynomial (5 degree) (CV – log-normal)	Restricted	0.5606	0.4106	0.0017	569.7238	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Polynomial (4 degree) (CV – log-normal)	Restricted	0.5606	0.4106	0.0017	569.7238	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Polynomial (3 degree) (CV – log-normal)	Restricted	0.5606	0.4106	0.0017	569.7238	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Polynomial (2 degree) (CV – log-normal)	Restricted	0.5606	0.4106	0.0017	569.7238	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Power (CV – log-normal)	Restricted	0.5606	0.4107	0.0017	569.7238	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Linear (CV – log-normal)	Unrestricted	0.5606	0.4106	0.0017	569.7238	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2

C.2.5. Increased Relative Liver Weight – Male Rat (NTP, 2018)

Table C-22. Dose-response data for increased relative liver weight in male rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	10	35.5	3.07
0.156	10	39.32	1.68
0.312	10	42.61	1.77
0.625	10	45.56	2.66
1.25	10	54.77	2.15
2.5	10	67.9	3.76

Table C-23. Benchmark dose results for increased relative liver weight in male rats—constant variance, BMR = 10% relative deviation (NTP, 2018)

Models	Restriction	10% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.4081	0.3852	<0.0001	314.8501	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 3 (CV – normal)	Restricted	0.4081	0.3852	<0.0001	314.8501	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 4 (CV – normal)	Restricted	0.2116	0.1764	0.2654	291.5391	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	0.2112	0.1764	0.2653	291.5398	Viable – Alternate	
Hill (CV – normal)	**Restricted**	**0.2078**	**0.1710**	**0.2774**	**291.4313**	**Viable Recommended**	**Lowest AIC**
Polynomial (5 degree) (CV – normal)	Restricted	0.2978	0.2836	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (4 degree) (CV – normal)	Restricted	0.2978	0.2778	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (3 degree) (CV – normal)	Restricted	0.2978	0.2775	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (2 degree) (CV – normal)	Restricted	0.2978	0.2775	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Power (CV – normal)	Restricted	0.2978	0.2775	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Linear (CV – normal)	Unrestricted	0.2978	0.2775	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2

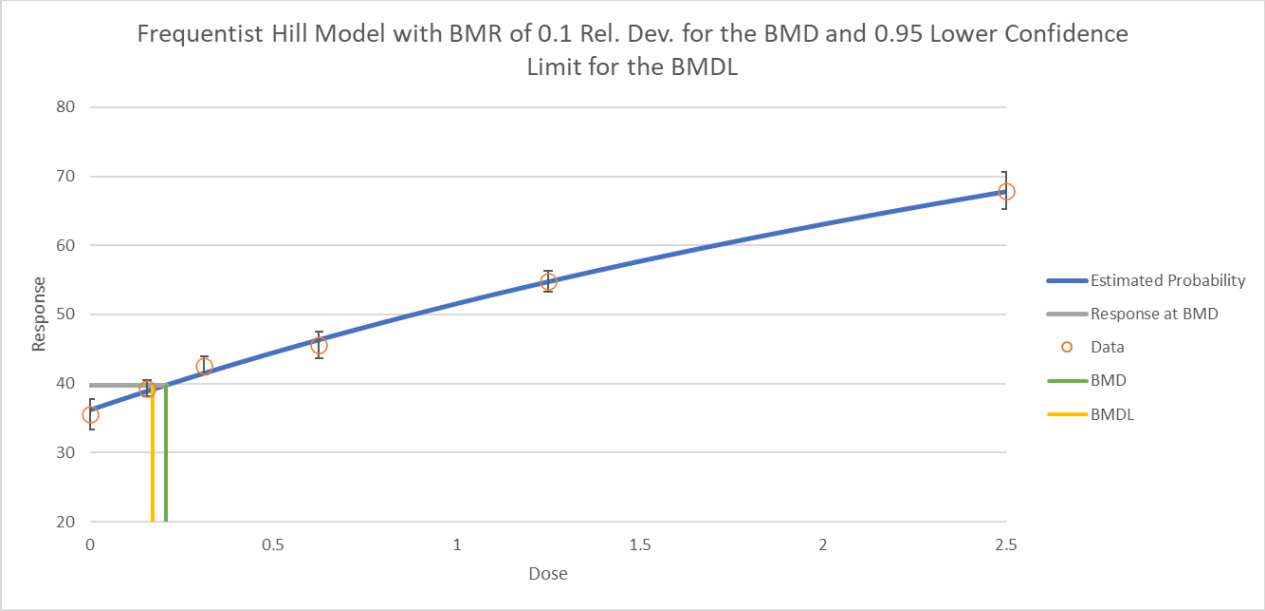


Figure C-6. Dose-response curve for the Hill model fit to increased relative liver weight in male rats (NTP, 2018).

User Input

Info	
Model	frequentist Hill v1.1
Dataset Name	LiverWt_Rel_M_NTP
User notes	[Add user notes here]
Dose-Response Model	M[dose] = g + v*dose^n/(k^n + dose^n)
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Rel. Dev.
BMRF	0.1
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	6
Adverse Direction	Automatic

Figure C-7. User input for dose-response modeling of increased relative liver weight in male rats (NTP, 2018).

Model Results

Benchmark Dose	
BMD	0.207847359
BMDL	0.170963922
BMDU	0.269772648
AIC	291.4312778
Test 4 P-value	0.277392913
D.O.F.	3

Model Parameters	
# of Parameters	5
Variable	Estimate
g	36.19093843
v	106.3618737
k	5.900597337
n	Bounded
alpha	6.592795984

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	36.19093843	35.5	35.5	2.56764405	3.07	3.07	-0.850950961
0.156	10	38.93050512	39.32	39.32	2.56764405	1.68	1.68	0.479696924
0.312	10	41.53248929	42.61	42.61	2.56764405	1.77	1.77	1.32704845
0.625	10	46.37792479	45.56	45.56	2.56764405	2.66	2.66	-1.007345743
1.25	10	54.78411826	54.77	54.77	2.56764405	2.15	2.15	-0.017387866
2.5	10	67.84400709	67.9	67.9	2.56764405	3.76	3.76	0.068960153

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-139.7874356	7	293.574871
A2	-134.7721348	12	293.54427
A3	-139.7874356	7	293.574871
fitted	-141.7156389	4	291.431278
R	-229.7698577	2	463.539715

* Includes additive constant of -55.13631. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	189.9954459	10	<0.0001
2	10.03060162	5	0.07437279
3	10.03060162	5	0.07437279
4	3.856406652	3	0.27739291

Figure C-8. Model results for increased relative liver weight in male rats (NTP, 2018).

Table C-24. Benchmark dose results for increased relative liver weight in male rats—constant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	10% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.3381	0.2930	<0.0001	314.8501	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 3 (CV – normal)	Restricted	0.3381	0.2930	<0.0001	314.8501	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 4 (CV – normal)	Restricted	0.1486	0.1209	0.2654	291.5391	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	0.1485	0.1209	0.2653	291.5398	Viable – Alternate	
Hill (CV – normal)	**Restricted**	**0.1460**	**0.1169**	**0.2774**	**291.4313**	**Viable – Recommended**	**Lowest AIC**
Polynomial (5 degree) (CV – normal)	Restricted	0.2202	0.1909	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (4 degree) (CV – normal)	Restricted	0.2202	0.1976	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (3 degree) (CV – normal)	Restricted	0.2202	0.1894	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (2 degree) (CV – normal)	Restricted	0.2202	0.1894	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Power (CV – normal)	Restricted	0.2202	0.1894	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Linear (CV – normal)	Unrestricted	0.2202	0.1894	0.0115	298.5321	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2

C.2.6. Increased Relative Liver Weight – Female Rat (NTP, 2018)

Table C-25. Dose-response data for increased relative liver weight in female rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	10	33.52	2.37
0.156	10	37.66	2.81
0.312	10	40.08	1.77
0.625	10	44.25	2.59
1.25	10	50.84	2.12
2.5	10	67.75	2.85

Table C-26. Benchmark dose results for increased relative liver weight in female rats – BMR = constant variance, 10% relative deviation (NTP, 2018)

Models	Restriction	10% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.3761	0.3585	0.0005	297.3583	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 3 (CV – normal)	Restricted	0.3761	0.3585	0.0005	297.3583	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 4 (CV – normal)	Restricted	0.2457	0.2042	0.0512	287.1715	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 5 (CV – normal)	Restricted	0.2456	0.2042	0.0512	287.1717	Questionable	Goodness-of-fit *p*-value < 0.1
Hill (CV – normal)	Restricted	0.2446	0.2018	0.0518	287.1453	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (5 degree) (CV – normal)	Restricted	0.2688	0.2545	0.0764	285.8573	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (4 degree) (CV – normal)	Restricted	0.2688	0.2528	0.0764	285.8573	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (3 degree) (CV – normal)	Restricted	0.2688	0.2524	0.0764	285.8573	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (2 degree) (CV – normal)	Restricted	0.2688	0.2524	0.0764	285.8573	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Power (CV – normal)	Restricted	0.2688	0.2524	0.0764	285.8573	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Linear (CV – normal)	Unrestricted	0.2688	0.2524	0.0764	285.8573	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2

Table C-27. Benchmark dose results for increased relative liver weight in female rats – nonconstant variance, BMR = 10% relative deviation (NTP, 2018)

Models	Restriction	10% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV – normal)	Restricted	0.3779	0.3586	0.0005	299.1741	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 3 (NCV – normal)	Restricted	0.3779	0.3586	0.0005	299.1741	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 4 (NCV – normal)	Restricted	0.2443	0.2017	0.0468	289.1376	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 5 (NCV – normal)	Restricted	0.2464	0.2016	0.0466	289.1432	Questionable	Goodness-of-fit *p*-value < 0.1
Hill (NCV – normal)	Restricted	0.2431	0.1997	0.0474	289.1075	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (5 degree) (NCV – normal)	Restricted	0.2688	0.2519	0.0695	287.8570	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (4 degree) (NCV – normal)	Restricted	0.2688	0.2519	0.0695	287.8570	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (3 degree) (NCV – normal)	Restricted	0.2688	0.2521	0.0695	287.8570	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (2 degree) (NCV – normal)	Restricted	0.2688	0.2521	0.0695	287.8570	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Power (NCV – normal)	Restricted	0.2688	0.2521	0.0695	287.8570	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Linear (NCV – normal)	Unrestricted	0.2688	0.2521	0.0695	287.8570	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2

Table C-28. Benchmark dose results for increased relative liver weight in female rats – log-normal, constant variance, BMR = 10% relative deviation (NTP, 2018)

Models	Restriction	10% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – log-normal)	Restricted	0.3617	0.3404	<0.0001	304.9243	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Exponential 3 (CV – log-normal)	Restricted	0.3617	0.3404	<0.0001	304.9243	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Exponential 4 (CV – log-normal)	Restricted	0.2228	0.1850	<0.0001	291.5746	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Exponential 5 (CV – log-normal)	Restricted	0.2228	0.1850	<0.0001	291.5746	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Hill (CV – g-normal)	Restricted	0.2200	0.1800	<0.0001	291.4503	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Polynomial (5 degree) (CV – log-normal)	Restricted	0.2622	0.2441	<0.0001	291.8437	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Polynomial (4 degree) (CV – log-normal)	Restricted	0.2622	0.2454	<0.0001	291.8437	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2

Models	Restriction	10% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Polynomial (3 degree) (CV – log-normal)	Restricted	0.2622	0.2433	<0.0001	291.8437	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Polynomial (2 degree) (CV – log-normal)	Restricted	0.2622	0.2433	<0.0001	291.8437	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Power (CV – log-normal)	Restricted	0.2622	0.2433	<0.0001	291.8437	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2
Linear (CV – log-normal)	Unrestricted	0.2622	0.2433	<0.0001	291.8437	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 |Residual at control| > 2

Table C-29. Benchmark dose results for increased relative liver weight in female rats, high dose dropped – BMR = constant variance, 10% relative deviation (NTP, 2018)

Models	Restriction	10% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.3195	0.2902	0.0031	242.3745	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 3 (CV – normal)	Restricted	0.3195	0.2902	0.0031	242.3745	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 4 (CV – normal)	Restricted	0.1611	0.1214	0.5849	231.5654	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	0.1610	0.1214	0.5849	231.5654	Viable – Alternate	
Hill (CV – normal)	**Restricted**	**0.1544**	**0.1117**	**0.6566**	**231.3342**	**Viable – Recommended**	**Lowest AIC**

Models	Restriction	10% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Polynomial (5 degree) (CV – normal)	Restricted	0.2659	0.2374	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (4 degree) (CV – normal)	Restricted	0.2659	0.2374	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (3 degree) (CV – normal)	Restricted	0.2659	0.2374	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (2 degree) (CV – normal)	Restricted	0.2659	0.2374	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Power (CV – normal)	Restricted	0.2659	0.2374	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Linear (CV – normal)	Unrestricted	0.3195	0.2902	0.0031	242.3745	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2

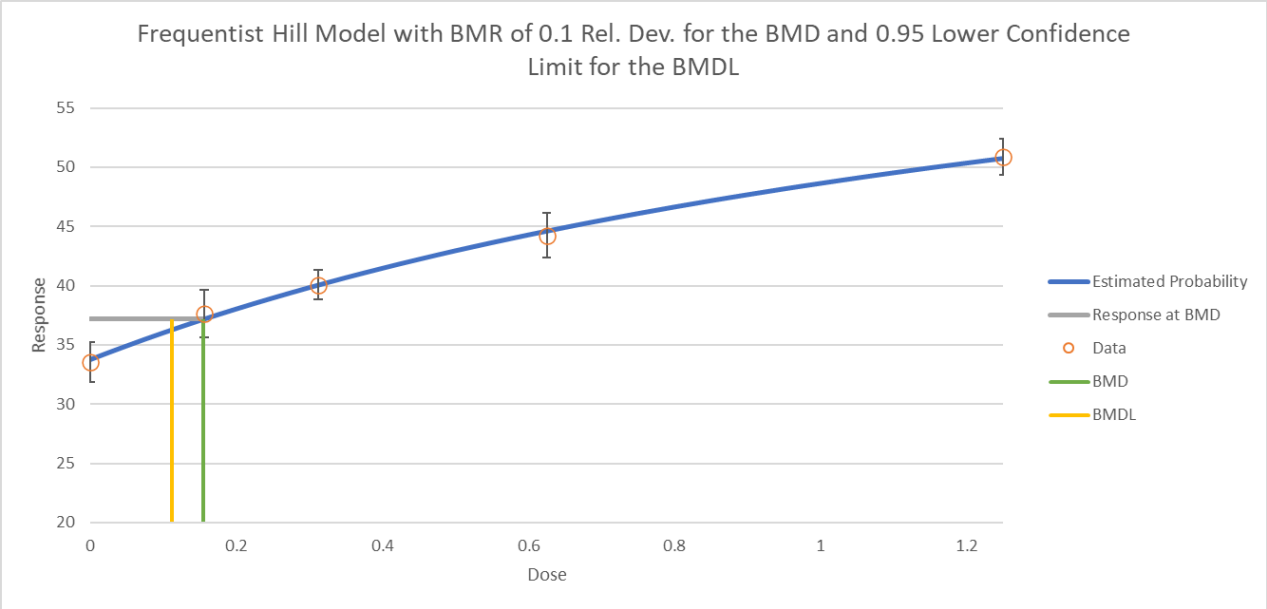
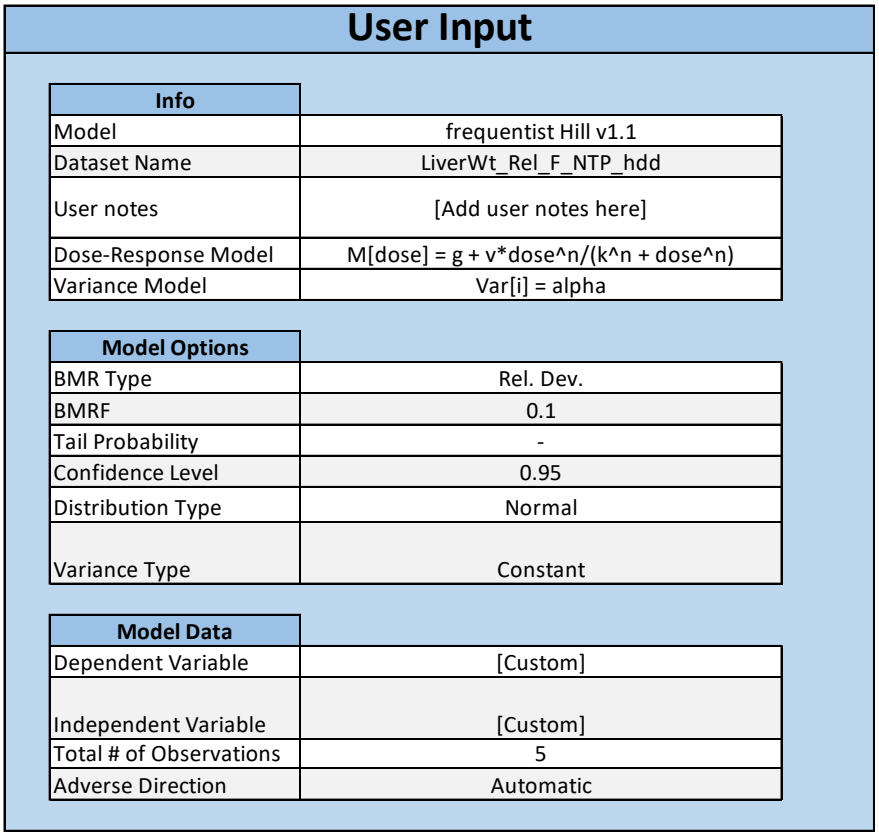


Figure C-9. Dose-response curve for the Hill model fit to increased relative liver weight in female rats with the highest dose dropped (NTP, 2018).



User Input

Info	
Model	frequentist Hill v1.1
Dataset Name	LiverWt_Rel_F_NTP_hdd
User notes	[Add user notes here]
Dose-Response Model	M[dose] = g + v*dose^n/(k^n + dose^n)
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Rel. Dev.
BMRF	0.1
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	[Custom]
Independent Variable	[Custom]
Total # of Observations	5
Adverse Direction	Automatic

Figure C-10. User input for dose-response modeling of increased relative liver weight in female rats with highest dose dropped (NTP, 2018).

Model Results

Benchmark Dose	
BMD	0.154369377
BMDL	0.111740633
BMDU	0.218901711
AIC	231.3341743
Test 4 P-value	0.656565161
D.O.F.	2

Model Parameters	
# of Parameters	5
Variable	Estimate
g	33.78210999
v	38.98056451
k	1.626870887
n	Bounded
alpha	5.097775081

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	33.78210999	33.52	33.52	2.2578253	2.37	2.37	-0.367107486
0.156	10	37.19288309	37.66	37.66	2.2578253	2.81	2.81	0.654237225
0.312	10	40.05480005	40.08	40.08	2.2578253	1.77	1.77	0.035294693
0.625	10	44.60104858	44.25	44.25	2.2578253	2.59	2.59	-0.491673589
1.25	10	50.71915985	50.84	50.84	2.2578253	2.12	2.12	0.169246978

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-111.2463538	6	234.492708
A2	-110.0141933	10	240.028387
A3	-111.2463538	6	234.492708
fitted	-111.6670871	4	231.334174
R	-163.1738575	2	330.347715

* Includes additive constant of -45.94693. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	106.3193285	8	<0.0001
2	2.464321029	4	0.65103586
3	2.464321029	4	0.65103586
4	0.84146667	2	0.65656516

Figure C-11. Model results for increased relative liver weight in female rats with highest dose dropped (NTP, 2018).

Table C-30. Benchmark dose results for increased relative liver weight in female rats, high dose dropped – constant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	10% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.2341	0.1980	0.0031	242.3745	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 3 (CV – normal)	Restricted	0.2341	0.1980	0.0031	242.3745	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Exponential 4 (CV – normal)	Restricted	0.1050	0.0785	0.5849	231.5654	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	0.1049	0.0785	0.5849	231.5654	Viable – Alternate	
Hill (CV – normal)	**Restricted**	**0.1000**	**0.0722**	**0.6566**	**231.3342**	**Viable – Recommended**	**Lowest AIC**
Polynomial (5 degree) (CV – normal)	Restricted	0.1854	0.1675	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (4 degree) (CV – normal)	Restricted	0.1854	0.1553	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (3 degree) (CV – normal)	Restricted	0.1854	0.1553	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Polynomial (2 degree) (CV – normal)	Restricted	0.1854	0.1553	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Power (CV – normal)	Restricted	0.1854	0.1553	0.0308	237.3809	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2
Linear (CV – normal)	Unrestricted	0.2341	0.1980	0.0031	242.3745	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| > 2

C.2.7. Increased Relative Liver Weight (Histopathology cohort) – Female Rats (Frawley et al., 2018)

Table C-31. Dose-response data for increased relative liver weight (Histopathology cohort) in female rats (Frawley et al., 2018)

Dose (mg/kg-d)	n	Mean	SD
0	8	4.02	0.28
0.125	8	4.06	0.28
0.25	8	4.35	0.28
0.5	8	4.68	0.34

Table C-32. Benchmark dose results for increased relative liver weight (Histopathology cohort) in female rats – constant variance, BMR = 10% relative deviation (Frawley et al., 2018)

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	**Restricted**	**0.2929**	**0.2224**	**0.6024**	**15.6701**	**Viable – Recommended**	**Lowest AIC**
Exponential 3 (CV – normal)	Restricted	0.3215	0.2240	0.3551	17.5116	Viable – Alternate	
Exponential 4 (CV – normal)	Restricted	0.2823	0.1647	0.2944	17.7557	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	0.2729	0.1840	NA	18.6564	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Hill (CV – normal)	Restricted	0.2777	0.1901	NA	18.6564	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (CV – normal)	Restricted	0.3170	0.2099	0.3338	17.5904	Viable – Alternate	
Polynomial (2 degree) (CV – normal)	Restricted	0.3170	0.2099	0.3338	17.5904	Viable – Alternate	
Power (CV – normal)	Restricted	0.3195	0.2113	0.3675	17.4686	Viable – Alternate	
Linear (CV – normal)	Unrestricted	0.2824	0.2081	0.5775	15.7543	Viable – Alternate	

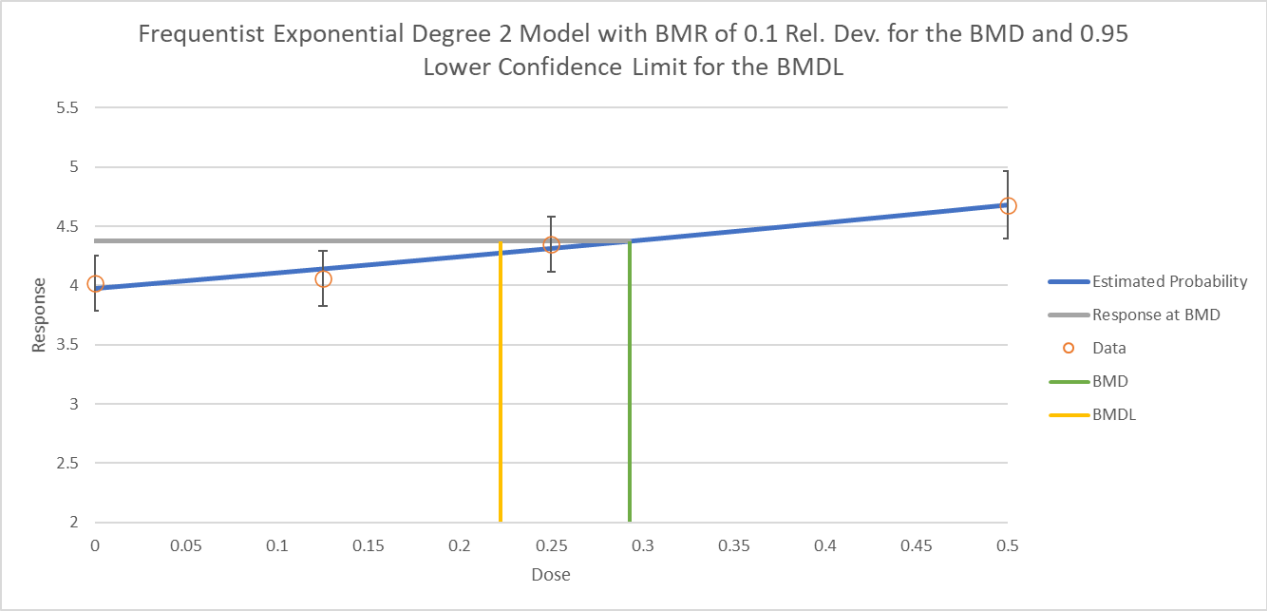


Figure C-12. Dose-response curve for the Exponential 2 model fit to increased relative liver weight (Histopathology cohort) in female rats (Frawley et al., 2018).

User Input

Info	
Model	frequentist Exponential degree 2 v1.1
Dataset Name	LiverWt_Rel_Frawley_Histo
User notes	[Add user notes here]
Dose-Response Model	M[dose] = a * exp(±1 * b * dose)
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Rel. Dev.
BMRF	0.1
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	4
Adverse Direction	Automatic

Figure C-13. User input for dose-response modeling of increased relative liver weight (Histo) in female rats (Frawley et al., 2018).

Model Results

Benchmark Dose	
BMD	0.292874336
BMDL	0.222375421
BMDU	0.429901615
AIC	15.67013988
Test 4 P-value	0.602376128
D.O.F.	2

Model Parameters	
# of Parameters	3
Variable	Estimate
a	3.97629556
b	0.325430373
log-alpha	-2.536765652

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	8	3.97629556	4.02	4.02	0.28128614	0.28	0.28	0.439462902
0.125	8	4.141381462	4.06	4.06	0.28128614	0.28	0.28	-0.818318074
0.25	8	4.31332132	4.35	4.35	0.28128614	0.28	0.28	0.368816506
0.5	8	4.678912956	4.68	4.68	0.28128614	0.34	0.34	0.01093059

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-4.328196707	5	18.6563934
A2	-4.087877276	8	24.1757546
A3	-4.328196707	5	18.6563934
fitted	-4.835069939	3	15.6701399
R	-14.72410737	2	33.4482147

* Includes additive constant of -29.40603. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	21.2724602	6	0.00163883
2	0.480638862	3	0.92312391
3	0.480638862	3	0.92312391
4	1.013746464	2	0.60237613

Figure C-14. Model results for increased relative liver weight (Histopathology cohort) in female rats (Frawley et al., 2018).

Table C-33. Benchmark dose results for increased relative liver weight (Histopathology cohort) in female rats – constant variance, BMR = 1 standard deviation (Frawley et al., 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	**Restricted**	**0.2100**	**0.1561**	**0.6024**	**15.6701**	**Viable – Recommended**	**Lowest AIC**
Exponential 3 (CV – normal)	Restricted	0.2405	0.1572	0.3551	17.5116	Viable – Alternate	
Exponential 4 (CV – normal)	Restricted	0.2003	0.1453	0.2944	17.7557	Viable – Alternate	

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 5 (CV – normal)	Restricted	0.2332	0.1314	NA	18.6564	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Hill (CV – normal)	Restricted	0.2310	0.1312	NA	18.6564	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (CV – normal)	Restricted	0.2343	0.1467	0.3338	17.5904	Viable – Alternate	
Polynomial (2 degree) (CV – normal)	Restricted	0.2343	0.1467	0.3338	17.5904	Viable – Alternate	
Power (CV – normal)	Restricted	0.2394	0.1476	0.3675	17.4686	Viable – Alternate	
Linear (CV – normal)	Unrestricted	0.2005	0.1455	0.5775	15.7543	Viable – Alternate	

C.2.8. Increased Relative Liver Weight (MPS cohort) – Female Rats (Frawley et al., 2018)

Table C-34. Dose-response data for increased relative liver weight (MPS cohort) in female rats (Frawley et al., 2018)

Dose (mg/kg-d)	n	Mean	SD
0	8	3.42	0.26
0.125	8	3.77	0.28
0.25	8	3.86	0.26
0.5	8	4.19	0.17

Table C-35. Benchmark dose results for increased relative liver weight (Histopathology cohort) in female rats – constant variance, BMR = 10% relative deviation (Frawley et al., 2018)

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.2575	0.2036	0.2714	5.4499	Viable – Alternate	
Exponential 3 (CV – normal)	Restricted	0.2575	0.2044	0.2714	5.4499	Viable – Alternate	
Exponential 4 (CV – normal)	Restricted	0.1644	0.0852	0.3121	5.8634	Viable – Alternate	

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Exponential 5 (CV – normal)	Restricted	0.1646	0.0851	0.3121	5.8634	Viable – Alternate	
Hill (CV – normal)	Restricted	0.1587	0.0730	0.3336	5.7766	Viable – Alternate	
Polynomial (3 degree) (CV – normal)	Restricted	0.2419	0.1864	0.3283	5.0691	Viable – Alternate	
Polynomial (2 degree) (CV – normal)	Restricted	0.2419	0.1864	0.3283	5.0691	Viable – Alternate	
Power (CV – normal)	Restricted	0.2419	0.1864	0.3283	5.0691	Viable – Alternate	
Linear (CV – normal)	**Unrestricted**	**0.2419**	**0.1864**	**0.3283**	**5.0691**	**Viable – Recommended**	**Lowest AIC**

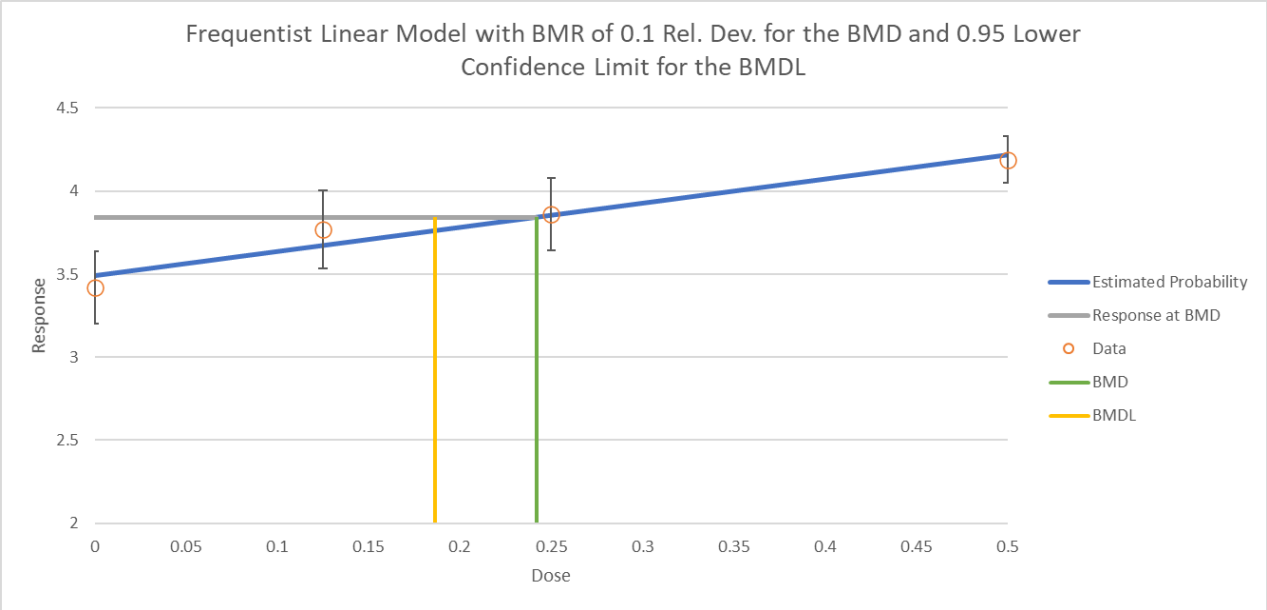
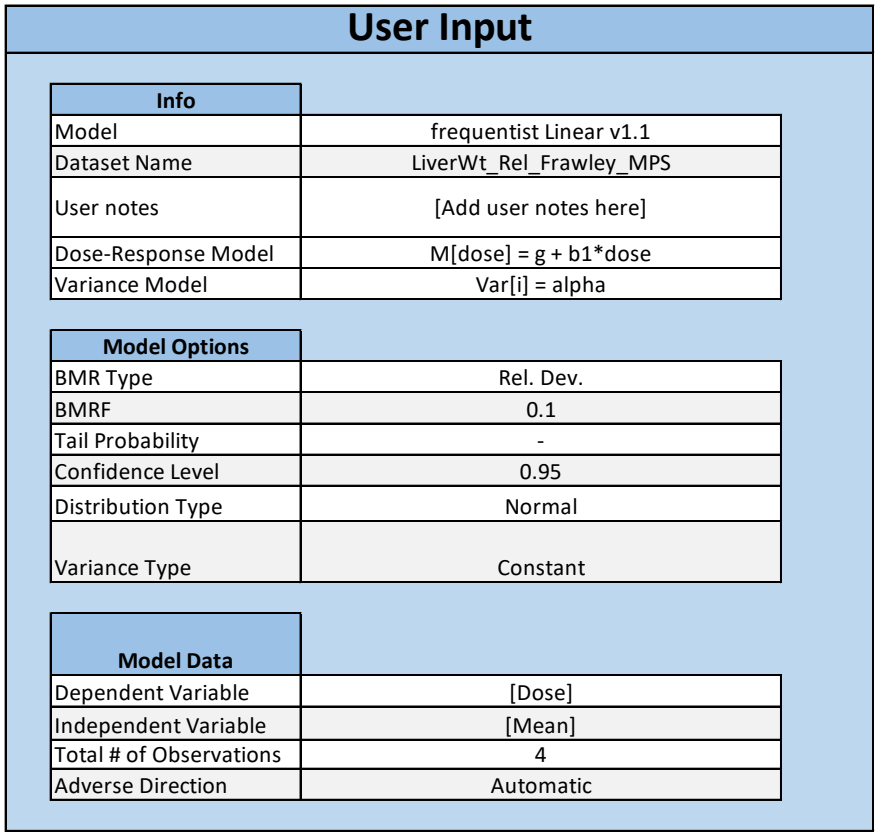


Figure C-15. Dose-response curve for the Linear model fit to increased relative liver weight (MPS) in female rats (Frawley et al., 2018).



User Input

Info	
Model	frequentist Linear v1.1
Dataset Name	LiverWt_Rel_Frawley_MPS
User notes	[Add user notes here]
Dose-Response Model	M[dose] = g + b1*dose
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Rel. Dev.
BMRF	0.1
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	4
Adverse Direction	Automatic

Figure C-16. User input for dose-response modeling of increased relative liver weight (MPS cohort) in female rats (Frawley et al., 2018).

Model Results

Benchmark Dose	
BMD	0.24187088
BMDL	0.186409723
BMDU	0.337253407
AIC	5.069125072
Test 4 P-value	0.328309463
D.O.F.	2

Model Parameters	
# of Parameters	3
Variable	Estimate
g	3.49399996
beta1	1.444571613
alpha	0.05687116

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	8	3.49399996	3.42	3.42	0.23847675	0.26	0.26	-0.877668349
0.125	8	3.674571412	3.77	3.77	0.23847675	0.28	0.28	1.13182022
0.25	8	3.855142863	3.86	3.86	0.23847675	0.26	0.26	0.057607531
0.5	8	4.216285767	4.19	4.19	0.23847675	0.17	0.17	-0.31175943

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	1.579236095	5	6.84152781
A2	2.643027712	8	10.7139446
A3	1.579236095	5	6.84152781
fitted	0.465437464	3	5.06912507
R	-12.53902329	2	29.0780466

* Includes additive constant of -29.40603. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	30.364102	6	<0.0001
2	2.127583234	3	0.54635267
3	2.127583234	3	0.54635267
4	2.227597262	2	0.32830946

Figure C-17. Model results for increased relative liver weight (MPS cohort) in female rats (Frawley et al., 2018).

Table C-36. Benchmark dose results for increased relative liver weight (MPS cohort) in female rats – constant variance, BMR = 1 standard deviation (Frawley et al., 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.1788	0.1367	0.2714	5.4499	Viable – Alternate	
Exponential 3 (CV – normal)	Restricted	0.1788	0.1367	0.2714	5.4499	Viable – Alternate	

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 4 (CV – normal)	Restricted	0.1046	0.0549	0.3121	5.8634	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	0.1048	0.0549	0.3121	5.8634	Viable – Alternate	
Hill (CV – normal)	**Restricted**	**0.0994**	**0.0450**	**0.3336**	**5.7766**	**Viable – Recommended**	**Lowest BMDL**
Polynomial (3 degree) (CV – normal)	Restricted	0.1651	0.1238	0.3283	5.0691	Viable – Alternate	
Polynomial (2 degree) (CV – normal)	Restricted	0.1651	0.1238	0.3283	5.0691	Viable – Alternate	
Power (CV – normal)	Restricted	0.1651	0.1238	0.3283	5.0691	Viable – Alternate	
Linear (CV – normal)	Unrestricted	0.1651	0.1238	0.3283	5.0691	Viable – Alternate	

C.2.9. Increased Relative Liver Weight (TDAR to SRBC cohort) – Female Rats (Frawley et al., 2018)

Table C-37. Dose-response data for increased relative liver weight (TDAR to SRBC cohort) in female rats (Frawley et al., 2018)

Dose (mg/kg-d)	n	Mean	SD
0	8	3.85	0.14
0.125	8	3.94	0.11
0.25	8	4.6	0.37
0.5	8	5.21	0.28

Table C-38. Benchmark dose results for increased relative liver weight (TDAR to SRBC cohort) in female rats – constant variance, BMR = 10% relative deviation (Frawley et al., 2018)

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.1478	0.1295	0.0284	10.5539	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 3 (CV – normal)	Restricted	0.1541	0.1297	0.0077	12.5248	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 4 (CV – normal)	Restricted	0.1294	0.0935	0.0073	12.6257	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 5 (CV – normal)	Restricted	0.1951	0.1458	NA	7.4299	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev. d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Hill (CV – normal)	Restricted	0.1904	0.1497	NA	7.4299	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev. d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (CV – normal)	Restricted	0.1419	0.1108	0.0079	12.4766	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (2 degree) (CV – normal)	Restricted	0.1419	0.1108	0.0079	12.4766	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Power (CV – normal)	Restricted	0.1556	0.1124	0.0103	12.0114	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Linear (CV – normal)	Unrestricted	0.1295	0.1103	0.0274	10.6256	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2 Modeled control response std. dev. > |1.5| actual response std. dev.

Table C-39. Benchmark dose results for increased relative liver weight (TDAR to SRBC cohort) in female rats – nonconstant variance, BMR = 10% relative deviation (Frawley et al., 2018)

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV – normal)	Restricted	0.1478	0.1284	0.0012	10.0543	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2
Exponential 3 (NCV – normal)	Restricted	0.1607	0.1292	0.0003	11.8202	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2
Exponential 4 (NCV – normal)	Restricted	0.1333	0.1030	0.0002	12.4411	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2
Exponential 5 (NCV – normal)	Restricted	0.1937	0.1654	NA	0.5572	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Hill (NCV – normal)	Restricted	0.1880	0.1653	NA	0.5577	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (NCV – normal)	Restricted	0.1507	0.1144	0.0002	11.9784	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2
Polynomial (2 degree) (NCV – normal)	Restricted	0.1507	0.1144	0.0002	11.9784	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2
Power (NCV – normal)	Restricted	0.1628	0.1183	0.0004	11.0771	Questionable	Goodness-of-fit *p*-value < 0.1
Linear (NCV – normal)	Unrestricted	0.1334	0.1127	0.0010	10.4397	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2

Table C-40. Benchmark dose results for increased relative liver weight (TDAR to SRBC cohort) in female rats – log-normal, constant variance, BMR = 10% relative deviation (Frawley et al., 2018)

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Log-normal Constant variance							
Exponential 2 (CV – log-normal)	Restricted	0.1478	0.1295	0.0172	7.4633	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 3 (CV – log-normal)	Restricted	0.1639	0.1304	0.0050	9.2051	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 4 (CV – log-normal)	Restricted	0.1315	0.1026	0.0033	9.9692	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 5 (CV – log-normal)	Restricted	0.1644	0.1111	NA	10.6210	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev. d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Hill (CV – log-normal)	Restricted	0.1918	0.1692	NA	3.3425	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev. d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (CV – log-normal)	Restricted	0.1541	0.1143	0.0046	9.3729	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Log-normal Constant variance							
Polynomial (2 degree) (CV – log-normal)	Restricted	0.1541	0.1143	0.0046	9.3729	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Power (CV – log-normal)	Restricted	0.1649	0.1176	0.0070	8.6207	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Linear (CV – log-normal)	Unrestricted	0.1315	0.1122	0.0134	7.9687	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.

C.2.10. Decreased Fetal Weight – Male and Female Rats (Harris and Birnbaum, 1989)

Table C-41. Dose-response data for decreased fetal weight in male and female rats (Harris and Birnbaum, 1989)

Dose (mg/kg-d)	n	Mean	SD
0	86.4	1.17	0.09
0.03	85.8	1.16	0.02
0.1	94.8	1.13	0.2
0.3	102	1.16	0.3
1	103.6	1.12	0.2
3	87.6	1.1	0.09
6.4	75.4	0.9	0.26
12.8	32.2	0.59	0.11

Table C-42. Benchmark dose results for decreased fetal weight in male and female rats – constant variance, BMR = 5% relative deviation (Harris and Birnbaum, 1989)

Models	Restriction	5% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	1.1862	1.0702	0.0010	−303.6182	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 3 (CV – normal)	Restricted	2.4486	1.8922	0.3529	−318.5263	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 4 (CV – normal)	Restricted	1.1862	1.0702	0.0010	−303.6182	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 5 (CV – normal)	Restricted	3.0401	2.0145	0.3470	−317.6098	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Hill (CV – normal)	Restricted	3.0451	2.0215	0.3383	−317.5367	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (7 degree) (CV – normal)	Restricted	1.9190	1.4664	0.1942	−316.6978	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (6 degree) (CV – normal)	Restricted	1.9190	1.4668	0.1942	−316.6978	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (5 degree) (CV – normal)	Restricted	1.9190	1.4667	0.1942	−316.6978	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.

Models	Restriction	5% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Polynomial (4 degree) (CV – normal)	Restricted	1.9190	1.4667	0.1942	−316.6978	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (3 degree) (CV – normal)	Restricted	1.9190	1.4681	0.1942	−316.6978	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (2 degree) (CV – normal)	Restricted	1.9190	1.4884	0.1942	−316.6978	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Power (CV – normal)	Restricted	2.1795	1.6300	0.2568	−317.5277	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Linear (CV – normal)	Unrestricted	1.3815	1.2741	0.0441	−313.1368	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.

Table C-43. Benchmark dose results for decreased fetal weight in male and female rats – nonconstant variance, BMR = 5% relative deviation (Harris and Birnbaum, 1989)

Models	Restriction	5% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV – normal)	Restricted	1.2032	1.0775	0.0012	−302.0911	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 3 (NCV – normal)	Restricted	2.4989	1.9388	0.4468	−317.3295	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.

Models	Restriction	5% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 4 (NCV – normal)	Restricted	1.2031	1.0775	0.0012	−302.0911	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 5 (NCV – normal)	Restricted	2.4942	1.9392	0.3140	−315.3322	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Hill (NCV – normal)	Restricted	2.9282	1.9155	0.3696	−315.8031	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (7 degree) (NCV – normal)	Restricted	1.9751	1.6128	0.2753	−315.7500	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (6 degree) (NCV – normal)	Restricted	1.9716	1.4955	0.2749	−315.7461	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (5 degree) (NCV – normal)	Restricted	1.9712	1.4921	0.2749	−315.7460	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (4 degree) (NCV – normal)	Restricted	1.9751	1.4965	0.2753	−315.7500	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (3 degree) (NCV – normal)	Restricted	1.9751	1.4973	0.2753	−315.7500	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Polynomial (2 degree) (NCV – normal)	Restricted	1.9751	1.5263	0.2753	−315.7500	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.

Models	Restriction	5% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Power (NCV – normal)	Restricted	2.2422	1.6842	0.3562	−316.5655	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Linear (NCV – normal)	Unrestricted	1.3772	1.2719	0.0450	−311.2042	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.

Table C-44. Benchmark dose results for decreased fetal weight in male and female rats – log-normal, constant variance, BMR = 5% relative deviation (Harris and Birnbaum, 1989)

Models	Restriction	5% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Log-normal, constant variance							
Exponential 2 (CV – log-normal)	Restricted	1.0479	0.9755	<0.0001	−307.8546	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 3 (CV – log-normal)	Restricted	2.1631	1.7042	0.0286	−326.0092	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 4 (CV – log-normal)	Restricted	1.0479	0.9755	<0.0001	−307.8546	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 5 (CV – log-normal)	Restricted	3.4280	2.4438	0.1216	−329.2234	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.

Models	Restriction	5% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Log-normal, constant variance							
Hill (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (7 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (6 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (5 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (4 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (3 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (2 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Power (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Linear (CV – log-normal)	Unrestricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution

C.2.11. Decreased Sperm Count – Male Rats (NTP, 2018)

Table C-45. Dose-response data for decreased sperm counts in male rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	10	136.3	32.26
0.625	10	120.8	17.39
1.25	10	112.9	23.09
2.5	10	95.7	36.37

Table C-46. Benchmark dose results for decreased sperm counts in male rats, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	**Restricted**	**1.5928**	**0.9634**	**0.9331**	**382.8116**	**Viable – Recommended**	**Lowest AIC**
Exponential 3 (CV – normal)	Restricted	1.5928	0.9634	0.9331	382.8116	Viable – Recommended	Lowest AIC
Exponential 4 (CV – normal)	Restricted	1.4241	0.5083	0.8023	384.7359	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	1.4241	0.5083	0.8023	384.7359	Viable – Alternate	
Hill (CV – normal)	Restricted	1.4208	0.4347	0.8120	384.7298	Viable – Alternate	
Polynomial (3 degree) (CV – normal)	Restricted	1.7202	1.1328	0.8756	382.9388	Viable – Alternate	
Polynomial (2 degree) (CV – normal)	Restricted	1.7202	1.1328	0.8756	382.9388	Viable – Alternate	
Power (CV – normal)	Restricted	1.7202	1.1329	0.8756	382.9388	Viable – Alternate	
Linear (CV – normal)	Unrestricted	1.7202	1.1328	0.8756	382.9388	Viable – Alternate	

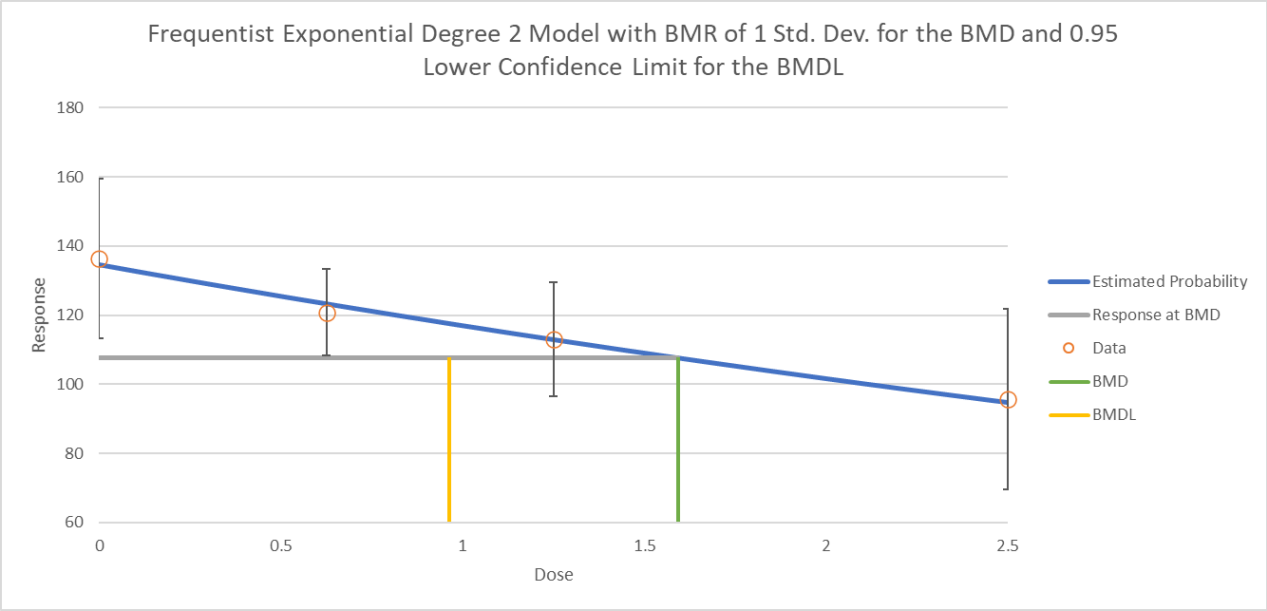


Figure C-18. Dose-response curve for the Exponential 2 model fit to decreased sperm counts in male rats (NTP, 2018).

User Input

Info	
Model	frequentist Exponential degree 2 v1.1
Dataset Name	Sperm_Count_NTP
User notes	[Add user notes here]
Dose-Response Model	M[dose] = a * exp(±1 * b * dose)
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Std. Dev.
BMRF	1
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	4
Adverse Direction	Automatic

Figure C-19. User input for dose-response modeling of decreased sperm counts in male rats (NTP, 2018).

Model Results

Benchmark Dose	
BMD	1.592768431
BMDL	0.963412903
BMDU	3.624046063
AIC	382.8116246
Test 4 P-value	0.933123027
D.O.F.	2

Model Parameters	
# of Parameters	3
Variable	Estimate
a	134.5572517
b	0.139886976
log-alpha	6.582413542

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	134.5572517	136.3	136.3	26.8752764	32.26	32.26	0.205060364
0.625	10	123.2926024	120.8	120.8	26.8752764	17.39	17.39	-0.293291902
1.25	10	112.9709891	112.9	112.9	26.8752764	23.09	23.09	-0.008352922
2.5	10	94.84768903	95.7	95.7	26.8752764	36.37	36.37	0.100287116

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-188.3365941	5	386.673188
A2	-185.2790038	8	386.558008
A3	-188.3365941	5	386.673188
fitted	-188.4058123	3	382.811625
R	-193.5430425	2	391.086085

* Includes additive constant of -36.75754. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	16.52807739	6	0.01118344
2	6.115180405	3	0.10613895
3	6.115180405	3	0.10613895
4	0.138436451	2	0.93312303

Figure C-20. Model results for decreased sperm counts in male rats (NTP, 2018).

C.2.12. Decreased Absolute Testis Weight – Male Rats (NTP, 2018)

Table C-47. Dose-response data for decreased absolute testis weight in male rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	9	1.777	0.17
0.156	10	1.797	0.15
0.312	10	1.742	0.12
0.625	10	1.74	0.1
1.25	10	1.695	0.11
2.5	10	1.553	0.2

Table C-48. Benchmark dose results for decreased absolute testis weight in male rats – constant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	1.4763	1.0220	0.9324	−59.4936	Viable – Alternate	
Exponential 3 (CV – normal)	Restricted	1.7052	1.0373	0.8973	−57.7417	Viable – Alternate	
Exponential 4 (CV – normal)	Restricted	1.4763	1.0220	0.9324	−59.4936	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	1.7049	0.8202	0.7420	−55.7409	Viable – Alternate	
Hill (CV – normal)	Restricted	1.7088	0.8010	0.7448	−55.7486	Viable – Alternate	
Polynomial (5 degree) (CV – normal)	Restricted	1.7976	1.0880	0.9114	−57.8041	Viable – Alternate	
Polynomial (4 degree) (CV – normal)	Restricted	1.7750	1.0878	0.9107	−57.8008	Viable – Alternate	
Polynomial (3 degree) (CV – normal)	Restricted	1.7482	1.0873	0.9089	−57.7926	Viable – Alternate	
Polynomial (2 degree) (CV – normal)	Restricted	1.7214	1.0861	0.9046	−57.7738	Viable – Alternate	
Power (CV – normal)	Restricted	1.7089	1.0848	0.8995	−57.7514	Viable – Alternate	
Linear (CV – normal)	**Unrestricted**	**1.5110**	**1.0742**	**0.9430**	**−59.5723**	**Viable – Recommended**	**Lowest AIC**

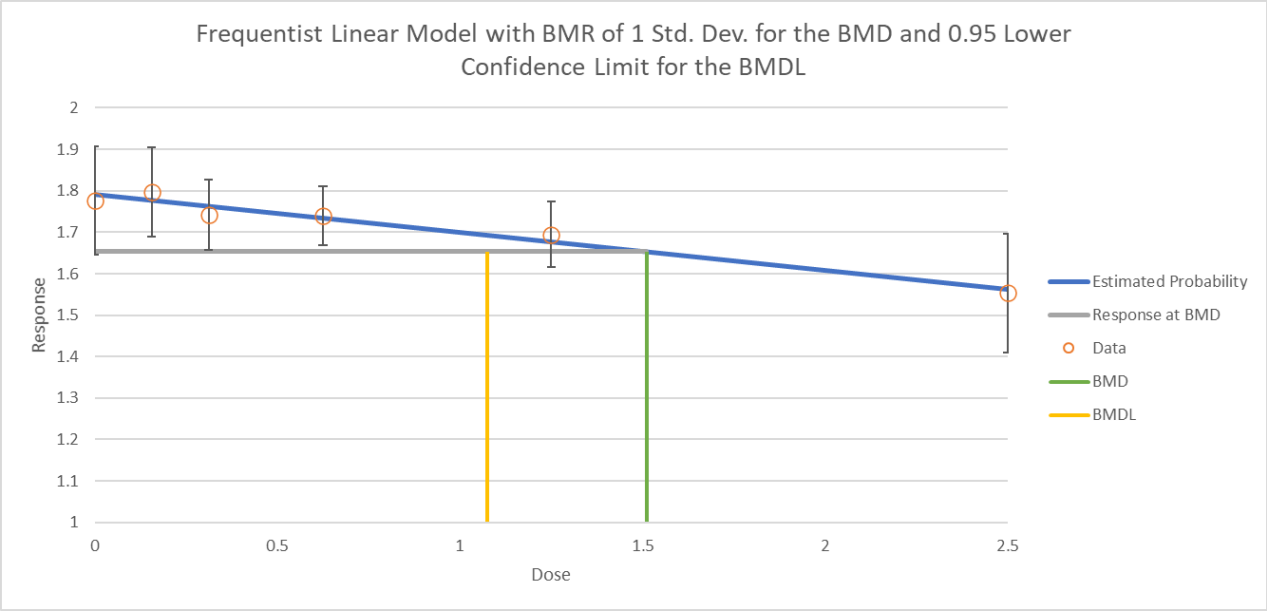


Figure C-21. Dose-response curve for the Linear model fit to decreased absolute testis weight in male rats (NTP, 2018).

User Input

Info	
Model	frequentist Linear v1.1
Dataset Name	TestisWt_Abs_NTP
User notes	[Add user notes here]
Dose-Response Model	M[dose] = g + b1*dose
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Std. Dev.
BMRF	1
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	6
Adverse Direction	Automatic

Figure C-22. User input for dose-response modeling of decreased absolute testis weight in male rats (NTP, 2018).

Model Results

Benchmark Dose	
BMD	1.511042118
BMDL	1.074196873
BMDU	2.542202182
AIC	-59.57226688
Test 4 P-value	0.943009409
D.O.F.	4

Model Parameters	
# of Parameters	3
Variable	Estimate
g	1.791729181
beta1	-0.091864992
alpha	0.019268735

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	9	1.791729181	1.777	1.777	0.13881187	0.17	0.17	-0.318326837
0.156	10	1.777398242	1.797	1.797	0.13881187	0.15	0.15	0.446548271
0.312	10	1.763067304	1.742	1.742	0.13881187	0.12	0.12	-0.479934917
0.625	10	1.734313561	1.74	1.74	0.13881187	0.1	0.1	0.129542952
1.25	10	1.676897941	1.695	1.695	0.13881187	0.11	0.11	0.412383595
2.5	10	1.5620667	1.553	1.553	0.13881187	0.2	0.2	-0.206548786

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	33.16889532	7	-52.3377906
A2	36.76108906	12	-49.5221781
A3	33.16889532	7	-52.3377906
fitted	32.78613344	3	-59.5722669
R	24.53190731	2	-45.0638146

* Includes additive constant of -54.21737. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	24.4583635	10	0.0064724
2	7.184387472	5	0.20728439
3	7.184387472	5	0.20728439
4	0.765523758	4	0.94300941

Figure C-23. Model results for decreased absolute testis weight in male rats (NTP, 2018).

C.2.13. Decreased Absolute Caudal Epididymis Weight – Male Rats (NTP, 2018)

Table C-49. Dose-response data for decreased absolute caudal epididymis weight in male rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	10	0.184	0.02
0.625	10	0.178	0.01
1.25	10	0.164	0.02
2.5	10	0.138	0.03

Table C-50. Benchmark dose results for decreased absolute caudal epididymis weight in male rats – constant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.9906	0.7014	0.6614	−192.1231	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Exponential 3 (CV – normal)	Restricted	1.2840	0.7347	0.7934	−190.8813	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Exponential 4 (CV – normal)	Restricted	0.9906	0.7014	0.6614	−192.1231	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Exponential 5 (CV – normal)	Restricted	1.2550	0.6841	NA	−188.9499	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Hill (CV – normal)	Restricted	1.2551	0.6802	NA	−188.9499	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (CV – normal)	Restricted	1.2961	0.8004	0.6972	−190.7984	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Polynomial (2 degree) (CV – normal)	Restricted	1.2961	0.8004	0.6972	−190.7984	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Power (CV – normal)	Restricted	1.2924	0.8027	0.7563	−190.8535	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Linear (CV – normal)	Unrestricted	1.0647	0.7868	0.7835	−192.4618	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)

Table C-51. Benchmark dose results for decreased absolute caudal epididymis weight in male rats – nonconstant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (NCV – normal)	Restricted	0.7898	0.5327	0.3071	−193.9474	Viable – Alternate	
Exponential 3 (NCV – normal)	Restricted	1.1440	0.6331	0.5123	−193.8789	Viable – Alternate	
Exponential 4 (NCV – normal)	Restricted	0.7902	0.5326	0.3070	−193.9463	Viable – Alternate	
Exponential 5 (NCV – normal)	Restricted	1.1558	0.6708	NA	−192.3083	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculatedd)
Hill (NCV – normal)	Restricted	1.1495	0.6702	NA	−192.3080	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (NCV – normal)	Restricted	1.1618	0.6304	0.4150	−193.6438	Viable – Alternate	
Polynomial (2 degree) (NCV – normal)	Restricted	1.1618	0.6304	0.4150	−193.6438	Viable – Alternate	
Power (NCV – normal)	Restricted	1.1497	0.6390	0.4771	−193.8028	Viable – Alternate	
Linear (NCV – normal)	**Unrestricted**	**0.8363**	**0.5824**	**0.4086**	**−194.5183**	**Viable – Recommended**	**Lowest AIC**

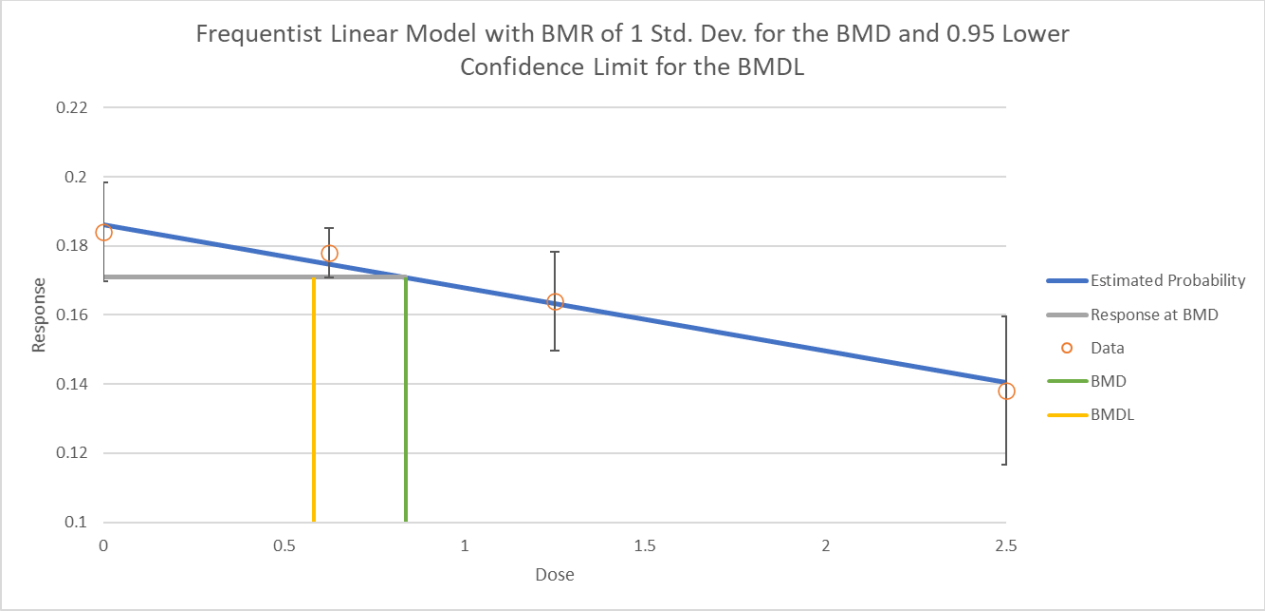


Figure C-24. Dose-response curve for the Linear model fit to decreased absolute caudal epididymis weight in male rats (NTP, 2018).

User Input

Info	
Model	frequentist Linear v1.1
Dataset Name	CaudaEpiWt_Abs_NTP
User notes	[Add user notes here]
Dose-Response Model	M[dose] = g + b1*dose
Variance Model	Var[i] = alpha * mean[i] ^ rho

Model Options	
BMR Type	Std. Dev.
BMRF	1
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Non-Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	4
Adverse Direction	Automatic

Figure C-25. User Input for dose-response modeling of decreased caudal epididymis weight in male rats (NTP, 2018).

Model Results

Benchmark Dose	
BMD	0.836267471
BMDL	0.582449886
BMDU	1.345231202
AIC	-194.5182635
Test 4 P-value	0.408601663
D.O.F.	2

Model Parameters	
# of Parameters	4
Variable	Estimate
g	0.186188825
beta1	-0.018332295
rho	-3.81191884
alpha	-14.76361024

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	0.186188825	0.184	0.184	0.01533068	0.02	0.02	-0.451491469
0.625	10	0.174731141	0.178	0.178	0.01730351	0.01	0.01	0.59739552
1.25	10	0.163273457	0.164	0.164	0.01969127	0.02	0.02	0.116677641
2.5	10	0.140358089	0.138	0.138	0.02626961	0.03	0.03	-0.283861514

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	99.47492849	5	-188.949857
A2	104.7074099	8	-193.41482
A3	102.1541463	6	-192.308293
fitted	101.2591317	4	-194.518263
R	87.99544268	2	-171.990885

* Includes additive constant of -36.75754. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	33.42393448	6	<0.0001
2	10.46496286	3	0.01500047
3	5.106527298	2	0.07782725
4	1.790029051	2	0.40860166

Figure C-26. Model results for decreased caudal epididymis weight in male rats (NTP, 2018).

C.2.14. Decreased Absolute Whole Epididymis Weight – Male Rats (NTP, 2018)

Table C-52. Dose-response data for decreased absolute whole epididymis weight in male rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	10	0.528	0.05
0.625	10	0.508	0.03
1.25	10	0.474	0.04
2.5	10	0.407	0.08

Table C-53. Benchmark dose results for decreased whole caudal epididymis weight in male rats – constant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.9572	0.6866	0.7614	−118.5715	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Exponential 3 (CV – normal)	Restricted	1.2024	0.7076	0.8891	−117.0973	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Exponential 4 (CV – normal)	Restricted	0.9572	0.6866	0.7614	−118.5715	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Exponential 5 (CV – normal)	Restricted	1.2024	0.7076	0.8891	−117.0973	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Hill (CV – normal)	Restricted	1.1911	0.6254	NA	−115.1168	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (CV – normal)	Restricted	1.2061	0.7720	0.7980	−117.0513	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Polynomial (2 degree) (CV – normal)	Restricted	1.2061	0.7720	0.7980	−117.0513	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Power (CV – normal)	Restricted	1.2076	0.7732	0.8530	−117.0825	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Linear (CV – normal)	Unrestricted	1.0266	0.7639	0.8678	−118.8333	Questionable	Constant variance test failed (Test 2 p-value < 0.05)

Table C-54. Benchmark dose results for decreased absolute whole epididymis weight in male rats – nonconstant variance, BMR = 1 standard deviation (NTP, 2018)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (NCV – normal)	Restricted	0.7358	0.5033	0.3609	−121.3235	Viable – Alternate	
Exponential 3 (NCV – normal)	Restricted	1.0959	0.5980	0.7979	−121.2963	Viable – Alternate	
Exponential 4 (NCV – normal)	Restricted	0.7360	0.5033	0.3609	−121.3235	Viable – Alternate	

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 5 (NCV – normal)	Restricted	1.0960	0.5986	NA	−119.2989	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Hill (NCV – normal)	Restricted	1.1035	0.6011	NA	−119.3619	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (NCV – normal)	Restricted	1.1012	0.5975	0.6702	−121.1805	Viable – Alternate	
Polynomial (2 degree) (NCV – normal)	Restricted	1.1012	0.5974	0.6702	−121.1805	Viable – Alternate	
Power (NCV – normal)	Restricted	1.0965	0.6018	0.7557	−121.2651	Viable – Alternate	
Linear (NCV – normal)	**Unrestricted**	**0.7766**	**0.5458**	**0.4809**	**−121.8975**	**Viable – Recommended**	**Lowest AIC**

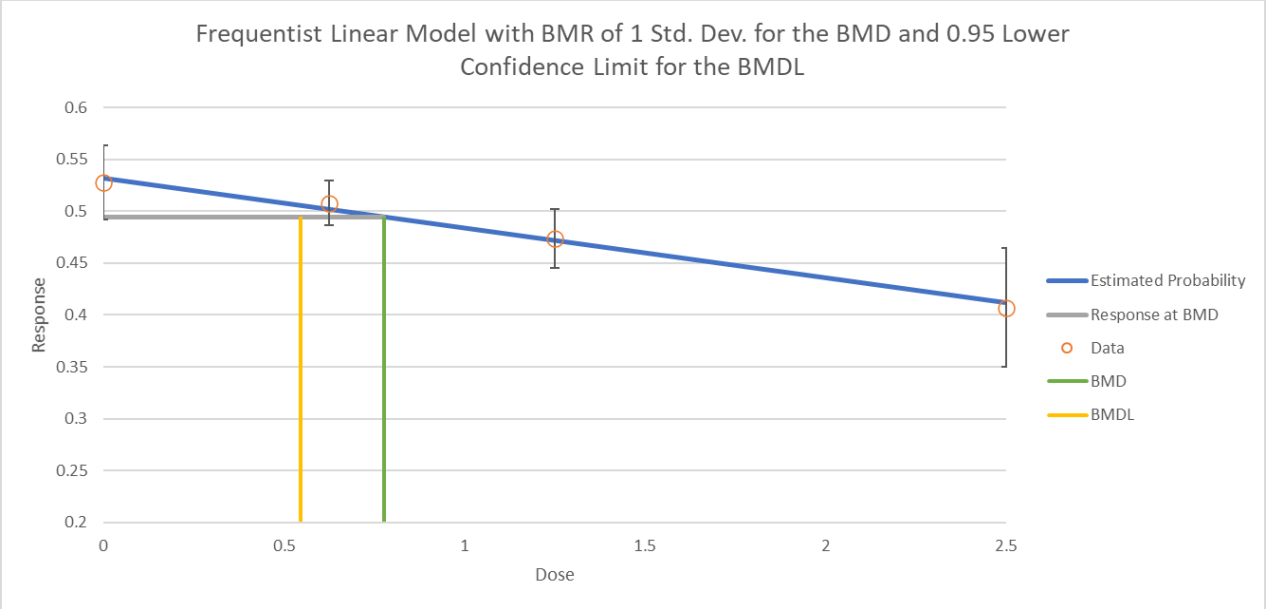
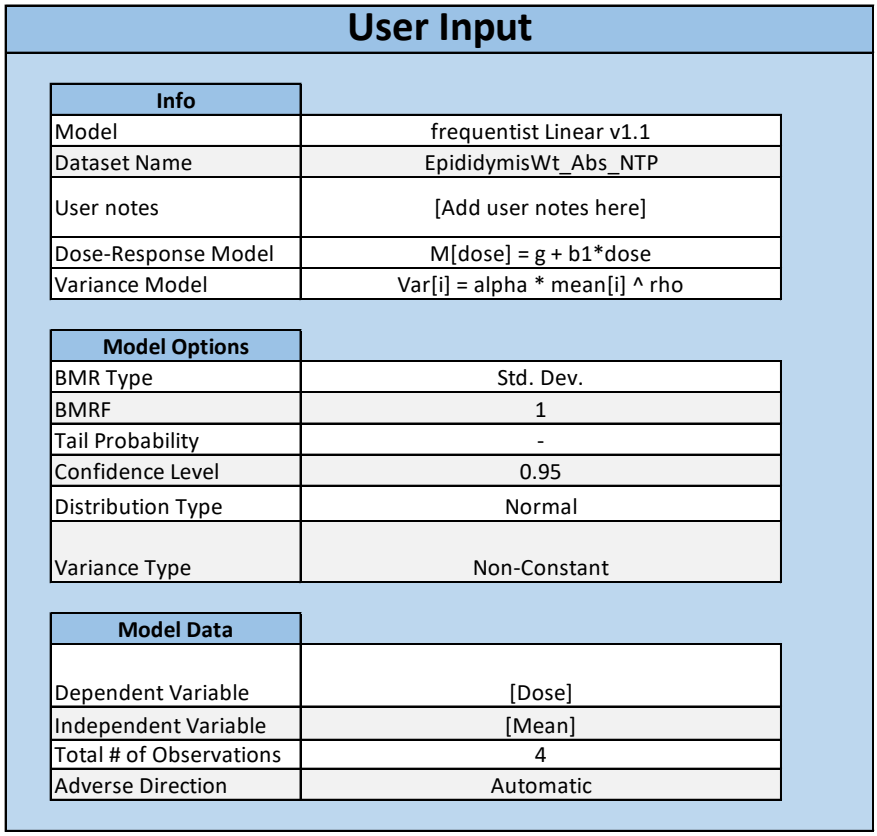


Figure C-27. Dose-response curve for the Linear model fit to decreased absolute whole epididymis weight in male rats (NTP, 2018).



User Input

Info	
Model	frequentist Linear v1.1
Dataset Name	EpididymisWt_Abs_NTP
User notes	[Add user notes here]
Dose-Response Model	M[dose] = g + b1*dose
Variance Model	Var[i] = alpha * mean[i] ^ rho

Model Options	
BMR Type	Std. Dev.
BMRF	1
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Non-Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	4
Adverse Direction	Automatic

Figure C-28. User input for dose-response modeling of decreased absolute whole epididymis weight in male rats (NTP, 2018).

Model Results

Benchmark Dose	
BMD	0.776560307
BMDL	0.545815255
BMDU	1.227214732
AIC	-121.8975001
Test 4 P-value	0.48085367
D.O.F.	2

Model Parameters	
# of Parameters	4
Variable	Estimate
g	0.532146909
beta1	-0.048115367
rho	-4.500456294
alpha	-9.413118476

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	0.532146909	0.528	0.528	0.03736447	0.05	0.05	-0.350966522
0.625	10	0.502074805	0.508	0.508	0.0425899	0.03	0.03	0.439942633
1.25	10	0.472002701	0.474	0.474	0.0489403	0.04	0.04	0.129055502
2.5	10	0.411858493	0.407	0.407	0.06650773	0.08	0.08	-0.231009273

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	62.55839468	5	-115.116789
A2	67.81861539	8	-119.637231
A3	65.68094232	6	-119.361885
fitted	64.94875004	4	-121.8975
R	50.54148697	2	-97.0829739

* Includes additive constant of -36.75754. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	34.55425682	6	<0.0001
2	10.52044141	3	0.01462287
3	4.275346136	2	0.11792894
4	1.46438455	2	0.48085367

Figure C-29. Model results for decreased absolute whole epididymis weight in male rats (NTP, 2018).

C.2.15. Decreased Days in Estrus – Female Rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Table C-55. Dose-response data for decreased days in estrus in female rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Dose (mg/kg-d)	n	Mean	SD
0	10	5.5	1.5092
0.625	10	4.3	2.0575
1.25	10	3.2	1.8136
2.5	10	0.9	0.9944

Table C-56. Benchmark dose results for decreased days in estrus in female rats – constant variance, BMR = 5% relative deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	5% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.0923	0.0687	0.3592	157.0377	Viable – Alternate	BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose
Exponential 3 (CV – normal)	Restricted	0.2611	0.0778	0.6119	157.2473	Viable – Alternate	BMDL 3× lower than lowest nonzero dose
Exponential 4 (CV – normal)	Restricted	0.0923	0.0687	0.3592	157.0377	Viable – Alternate	BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose
Exponential 5 (CV – normal)	Restricted	0.2608	0.0776	0.6119	157.2473	Viable – Alternate	BMDL 3× lower than lowest nonzero dose
Hill (CV – normal)	Restricted	0.1487	0.0739	NA	158.9967	Questionable	BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose d.f. = 0, saturated model (Goodness-of-fit p value cannot be calculated)
Polynomial (3 degree) (CV – normal)	Restricted	0.1495	0.1283	0.9965	154.9969	Viable – Alternate	BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose
Polynomial (2 degree) (CV – normal)	**Restricted**	**0.1495**	**0.1283**	**0.9965**	**154.9969**	**Viable – Recommended**	**Lowest AIC BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose**
Power (CV – normal)	Restricted	0.1495	0.1283	0.9965	154.9969	Viable – Alternate	BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose
Linear (CV – normal)	Unrestricted	0.1495	0.1283	0.9965	154.9969	Viable – Alternate	BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose

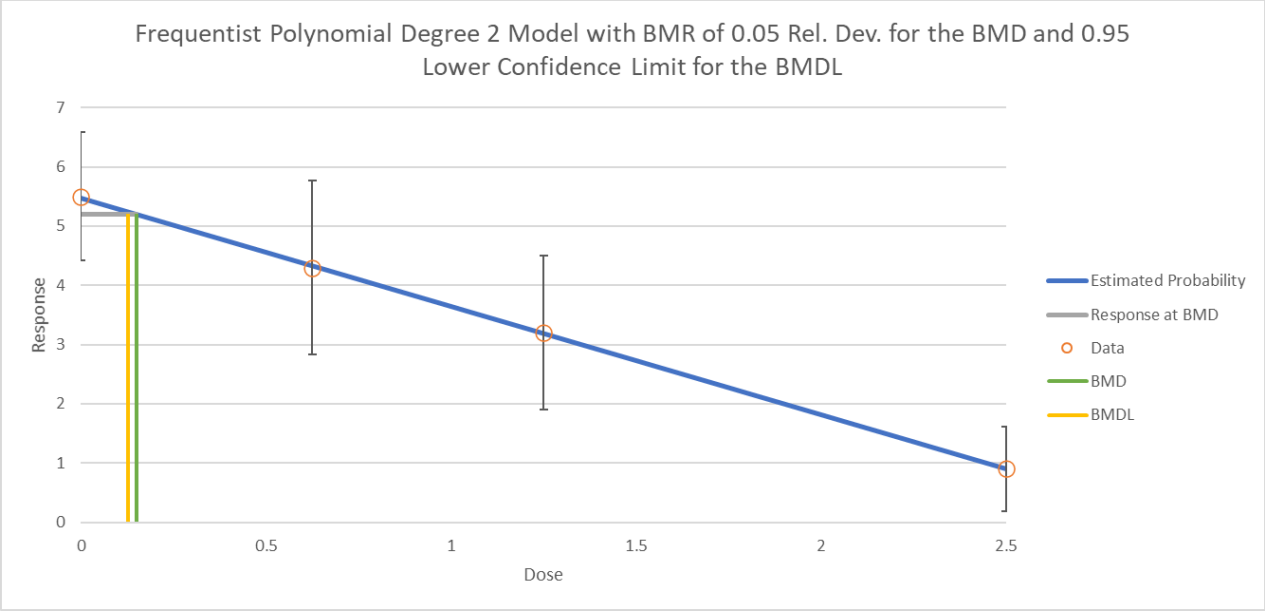


Figure C-30. Dose-response curve for the Polynomial 2 model fit to decreased days in estrus in female rats (Butenhoff et al., 2012; van Otterdijk, 2007).

User Input

Info	
Model	frequentist Polynomial degree 2 v1.1
Dataset Name	Estrus_Days_NTP
User notes	[Add user notes here]
Dose-Response Model	M[dose] = g + b1*dose + b2*dose^2 + ...
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	4
Adverse Direction	Automatic

Figure C-31. User input for dose-response modeling of decreased days in estrus in female rats (NTP, 2018).

Model Results

Benchmark Dose	
BMD	0.149469972
BMDL	0.128321644
BMDU	0.470941256
AIC	154.9969111
Test 4 P-value	0.996475595
D.O.F.	2

Model Parameters	
# of Parameters	4
Variable	Estimate
g	5.479999986
beta1	-1.833142847
beta2	Bounded
alpha	2.427946195

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	5.479999986	5.5	5.5	1.55818683	1.5092	1.5092	0.040589227
0.625	10	4.334285706	4.3	4.3	1.55818683	2.0575	2.0575	-0.069581465
1.25	10	3.188571426	3.2	3.2	1.55818683	1.8136	1.8136	0.023193832
2.5	10	0.897142867	0.9	0.9	1.55818683	0.9944	0.9944	0.005798436

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-74.49492494	5	158.98985
A2	-71.87802546	8	159.756051
A3	-74.49492494	5	158.98985
fitted	-74.49845557	3	154.996911
R	-90.10938562	2	184.218771

* Includes additive constant of -36.75754. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	36.46272031	6	<0.0001
2	5.233798961	3	0.15545622
3	5.233798961	3	0.15545622
4	0.007061261	2	0.9964756

Figure C-32. Model results for decreased days in estrus in female rats (NTP, 2018).

Table C-57. Benchmark dose results for decreased days in estrus in female rats – constant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.5895	0.3889	0.3592	157.0377	Viable – Alternate	
Exponential 3 (CV – normal)	Restricted	0.8806	0.4576	0.6119	157.2473	Viable – Alternate	
Exponential 4 (CV – normal)	Restricted	0.5895	0.3889	0.3592	157.0377	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	0.8804	0.4576	0.6119	157.2473	Viable – Alternate	
Hill (CV – normal)	Restricted	0.8393	0.4491	NA	158.9967	Questionable	d.f. = 0, saturated model (Goodness-of-fit p value cannot be calculated)
Polynomial (3 degree) (CV – normal)	Restricted	0.8500	0.6520	0.9965	154.9969	Viable – Alternate	
Polynomial (2 degree) (CV – normal)	**Restricted**	**0.8500**	**0.6520**	**0.9965**	**154.9969**	**Viable – Recommended**	**Lowest AIC**
Power (CV – normal)	Restricted	0.8500	0.6520	0.9965	154.9969	Viable – Alternate	
Linear (CV – normal)	Unrestricted	0.8500	0.6520	0.9965	154.9969	Viable – Alternate	

C.2.16. Increased Days in Diestrus – Female Rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Table C-58. Dose-response data for increased days in diestrus in female rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Dose (mg/kg-d)	n	Mean	SD
0	10	9.2	1.874
0.625	10	10.1	2.1833
1.25	10	11.7	2.2632
2.5	10	15	1.0541

Table C-59. Benchmark dose results for increased days in diestrus in female rats – constant variance, BMR = 5% relative deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	5% relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	**Restricted**	**0.2430**	**0.2000**	**0.9231**	**167.0076**	**Viable – Recommended**	**Lowest AIC BMDL 3× lower than lowest nonzero dose**
Exponential 3 (CV – normal)	Restricted	0.2891	0.2006	0.7433	168.9548	Viable – Alternate	BMDL 3× lower than lowest nonzero dose
Exponential 4 (CV – normal)	Restricted	0.1870	0.1136	0.4064	169.5368	Viable – Alternate	BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose
Exponential 5 (CV – normal)	Restricted	0.4063	0.1241	NA	170.8476	Questionable	BMDL 3× lower than lowest nonzero dose d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Hill (CV – normal)	Restricted	0.4079	0.1226	NA	170.8476	Questionable	BMDL 3× lower than lowest nonzero dose d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (CV – normal)	Restricted	0.2770	0.1470	0.7388	168.9588	Viable – Alternate	BMDL 3× lower than lowest nonzero dose
Polynomial (2 degree) (CV – normal)	Restricted	0.2770	0.1470	0.7388	168.9588	Viable – Alternate	BMDL 3× lower than lowest nonzero dose
Power (CV – normal)	Restricted	0.3283	0.1475	0.8200	168.8993	Viable – Alternate	BMDL 3× lower than lowest nonzero dose
Linear (CV – normal)	Unrestricted	0.1872	0.1427	0.7099	167.5330	Viable – Alternate	BMD 3× lower than lowest nonzero dose

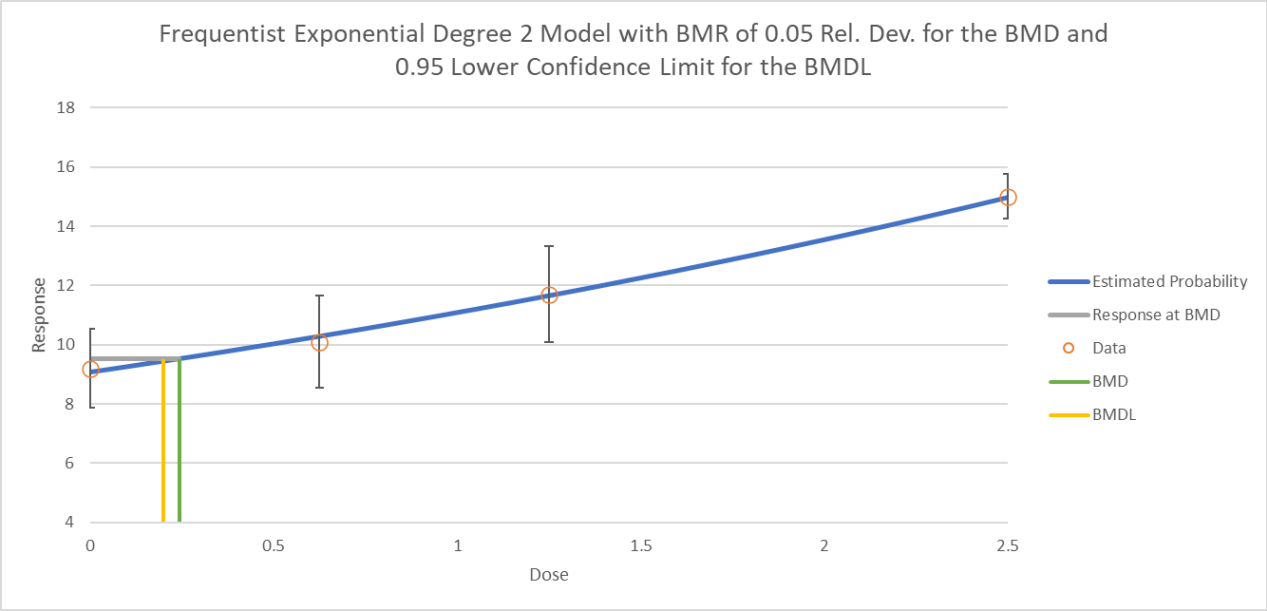


Figure C-33. Dose-response curve for the Exponential 2 model fit to increased days in diestrus in female rats (Butenhoff et al., 2012; van Otterdijk, 2007).

User Input

Info	
Model	frequentist Exponential degree 2 v1.1
Dataset Name	Diestrus_Days_NTP
User notes	[Add user notes here]
Dose-Response Model	M[dose] = a * exp(±1 * b * dose)
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Mean]
Total # of Observations	4
Adverse Direction	Automatic

Figure C-34. User input for dose-response modeling of increased days in diestrus in female rats (NTP, 2018).

Model Results

Benchmark Dose	
BMD	0.242986679
BMDL	0.200009167
BMDU	0.309079273
AIC	167.0076126
Test 4 P-value	0.923101914
D.O.F.	2

Model Parameters	
# of Parameters	3
Variable	Estimate
a	9.070650097
b	0.200793313
log-alpha	1.187317248

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	9.070650097	9.2	9.2	1.81060062	1.874	1.874	0.225914154
0.625	10	10.28349063	10.1	10.1	1.81060062	2.1833	2.1833	-0.320472836
1.25	10	11.65850059	11.7	11.7	1.81060062	2.2632	2.2632	0.072480181
2.5	10	14.98466312	15	15	1.81060062	1.0541	1.0541	0.026786401

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-80.42379068	5	170.847581
A2	-77.43412842	8	170.868257
A3	-80.42379068	5	170.847581
fitted	-80.50380632	3	167.007613
R	-98.71832217	2	201.436644

* Includes additive constant of -36.75754. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	42.56838749	6	<0.0001
2	5.97932452	3	0.11262048
3	5.97932452	3	0.11262048
4	0.160031269	2	0.92310191

Figure C-35. Model results for increased days in diestrus in female rats (NTP, 2018).

Table C-60. Benchmark dose results for increased days in diestrus in female rats – constant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	**Restricted**	**0.9064**	**0.7377**	**0.9231**	**167.0076**	**Viable – Recommended**	**Lowest AIC**
Exponential 3 (CV – normal)	Restricted	0.9766	0.7391	0.7433	168.9548	Viable – Alternate	
Exponential 4 (CV – normal)	Restricted	0.7661	0.5970	0.4064	169.5368	Viable – Alternate	
Exponential 5 (CV – normal)	Restricted	0.9947	0.5599	NA	170.8476	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Hill (CV – normal)	Restricted	0.9936	0.5580	NA	170.8476	Questionable	d.f. = 0, saturated model (Goodness-of-fit *p* value cannot be calculated)
Polynomial (3 degree) (CV – normal)	Restricted	0.9687	0.6117	0.7388	168.9588	Viable – Alternate	
Polynomial (2 degree) (CV – normal)	Restricted	0.9687	0.6117	0.7388	168.9588	Viable – Alternate	
Power (CV – normal)	Restricted	0.9805	0.6134	0.8200	168.8993	Viable – Alternate	
Linear (CV – normal)	Unrestricted	0.7667	0.5963	0.7099	167.5330	Viable – Alternate	

C.2.17. Decreased Relative Uterine Weight – Female Rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Table C-61. Dose-response data for decreased relative uterine weight in female rats (Butenhoff et al., 2012; van Otterdijk, 2007))

Dose (mg/kg-d)	n	Mean	SD
0	10	3.26	1.3
0.156	10	2.73	0.41
0.312	10	2.94	0.79
0.625	10	3.65	1.68
1.25	10	2.05	0.61
2.5	10	1.81	0.32

Table C-62. Benchmark dose results for decreased relative uterine weight in female rats – BMR = constant variance, 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS Classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	1.6357	0.9728	0.0296	178.4420	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Exponential 3 (CV – normal)	Restricted	1.8431	1.0220	0.0170	179.8915	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Exponential 4 (CV – normal)	Restricted	1.6357	0.9728	0.0296	178.4420	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Exponential 5 (CV – normal)	Restricted	1.2312	0.7036	0.1496	175.0232	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Hill (CV – normal)	Restricted	1.2139	0.7285	0.1496	175.0233	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Polynomial (5 degree) (CV – normal)	Restricted	1.8244	1.2032	0.0147	180.2109	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (4 degree) (CV – normal)	Restricted	1.8244	1.2032	0.0147	180.2109	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (3 degree) (CV – normal)	Restricted	1.8244	1.2032	0.0147	180.2109	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (CV – normal)	Restricted	1.8244	1.2032	0.0147	180.2109	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Power (CV – normal)	Restricted	1.8813	1.2094	0.0153	180.1247	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Linear (CV – normal)	Unrestricted	1.7547	1.2018	0.0324	178.2308	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1

Table C-63. Benchmark dose results for decreased relative uterine weight in female rats – nonconstant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV – normal)	Restricted	2.3599	1.4658	<0.0001	168.8763	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 3 (NCV – normal)	Restricted	2.4946	1.8929	<0.0001	167.1138	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 4 (NCV – normal)	Restricted	2.3592	1.4658	<0.0001	168.8763	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 5 (NCV – normal)	Restricted	1.2787	1.1724	0.0011	157.4375	Questionable	Goodness-of-fit *p*-value < 0.1
Hill (NCV – normal)	Restricted	1.3094	1.1258	0.0011	157.4376	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (5 degree) (NCV – normal)	Restricted	2.5118	1.9996	<0.0001	165.4887	Questionable	Goodness-of-fit *p*-value < 0.1 BMD higher than maximum dose
Polynomial (4 degree) (NCV – normal)	Restricted	2.5118	1.9997	<0.0001	165.4887	Questionable	Goodness-of-fit *p*-value < 0.1 BMD higher than maximum dose
Polynomial (3 degree) (NCV – normal)	Restricted	2.5118	1.9997	<0.0001	165.4887	Questionable	Goodness-of-fit *p*-value < 0.1 BMD higher than maximum dose
Polynomial (2 degree) (NCV – normal)	Restricted	2.5118	1.9997	<0.0001	165.4887	Questionable	Goodness-of-fit *p*-value < 0.1 BMD higher than maximum dose
Power (NCV – normal)	Restricted	2.5092	1.9643	<0.0001	167.4725	Questionable	Goodness-of-fit *p*-value < 0.1 BMD higher than maximum dose
Linear (NCV – normal)	Unrestricted	2.4008	1.7105	<0.0001	167.5269	Questionable	Goodness-of-fit *p*-value < 0.1

Table C-64. Benchmark dose results for decreased relative uterine weight in female rats – log-normal, constant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – log-normal)	Restricted	1.9961	0.9991	0.0518	147.6232	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Exponential 3 (CV – log-normal)	Restricted	2.0457	1.0012	0.0249	149.5811	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Exponential 4 (CV – log-normal)	Restricted	1.9491	0.6763	0.0246	149.6001	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Exponential 5 (CV – log-normal)	Restricted	1.2532	0.6896	0.2457	145.0275	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Hill (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (5 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (4 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (3 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (2 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Power (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Linear (CV – log-normal)	Unrestricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution

C.2.18. Decreased Absolute Uterine Weight – Female Rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Table C-65. Dose-response data for decreased absolute uterine weight in female rats (NTP, 2018)

Dose (mg/kg-d)	n	Mean	SD
0	10	0.731	0.27
0.156	10	0.646	0.09
0.312	10	0.691	0.18
0.625	10	0.818	0.35
1.25	10	0.409	0.13
2.5	10	0.26	0.03

Table C-66. Benchmark dose results for decreased absolute uterine weight in female rats – BMR = constant variance, 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – normal)	Restricted	0.8877	0.5920	0.0083	−6.1338	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2
Exponential 3 (CV – normal)	Restricted	1.2592	0.7971	0.0140	−7.2318	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 4 (CV – normal)	Restricted	0.8877	0.5920	0.0083	−6.1338	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2
Exponential 5 (CV – normal)	Restricted	1.2039	0.9713	0.2538	−13.7789	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Hill (CV – normal)	Restricted	1.1828	0.8675	0.1306	−11.7788	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05)
Polynomial (5 degree) (CV – normal)	Restricted	1.2569	0.8354	0.0076	−5.9234	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (4 degree) (CV – normal)	Restricted	1.2569	0.8354	0.0076	−5.9234	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (3 degree) (CV – normal)	Restricted	1.2569	0.8354	0.0076	−5.9234	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (CV – normal)	Restricted	1.2569	0.8354	0.0076	−5.9234	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Power (CV – normal)	Restricted	1.3086	0.8477	0.0088	−6.2298	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Linear (CV – normal)	Unrestricted	1.0823	0.8275	0.0163	−7.7099	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1

Table C-67. Benchmark dose results for decreased absolute uterine weight in female rats – nonconstant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV – normal)	Restricted	1.3500	0.9186	<0.0001	−25.2943	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Exponential 3 (NCV – normal)	Restricted	1.8175	1.3964	<0.0001	−33.2616	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2
Exponential 4 (NCV – normal)	Restricted	1.3502	0.9186	<0.0001	−25.2943	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Exponential 5 (NCV – normal)	Restricted	1.2424	1.1367	0.0036	−42.1526	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Hill (NCV – normal)	Restricted	1.2387	1.1069	0.0103	−44.1525	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (5 degree) (NCV – normal)	Restricted	2.0088	1.5693	0.0001	−33.9754	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (4 degree) (NCV – normal)	Restricted	2.0088	1.5692	0.0001	−33.9754	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (3 degree) (NCV – normal)	Restricted	2.0088	1.5692	0.0001	−33.9754	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (NCV – normal)	Restricted	2.0088	1.5692	0.0001	−33.9754	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1
Power (NCV – normal)	Restricted	1.9555	1.5188	<0.0001	−32.0845	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Linear (NCV – normal)	Unrestricted	1.6526	1.2761	<0.0001	−30.8879	Questionable	Nonconstant variance test failed (Test 3 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 |Residual for dose group near BMD| > 2

Table C-68. Benchmark dose results for decreased absolute uterine weight in female rats – log-normal, constant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV – log-normal)	Restricted	1.0282	0.5795	0.0129	−43.7584	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 3 (CV – log-normal)	Restricted	1.2617	0.6141	0.0101	−43.1248	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 4 (CV – log-normal)	Restricted	1.0282	1.0189	0.0129	−43.7584	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response std. dev. > |1.5| actual response std. dev.
Exponential 5 (CV – log-normal)	Restricted	1.2149	0.9197	0.3929	−50.5863	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response std. dev. > |1.5| actual response std. dev.
Hill (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution

Models	Restriction	1 standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Polynomial (5 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (4 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (3 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Polynomial (2 degree) (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Power (CV – log-normal)	Restricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution
Linear (CV – log-normal)	Unrestricted	-	-	-	-	Unusable	BMD computation failed Model was not run. Adverse direction "down" not compatible with log-normal distribution

APPENDIX D. ADVERSE OUTCOME PATHWAY/ MODE OF ACTION (AOP/MOA)-BASED APPROACH FOR EVALUATING PERFLUORODECANOIC ACID (PFDA)-INDUCED MECHANISM OF HEPATOXITY

D.1. OBJECTIVE AND METHODOLOGY

The goal of the qualitative analysis described here is to evaluate the available mechanistic evidence for perfluorodecanoic acid (PFDA)-induced liver effects to assess the biological plausibility of effects observed in animal models and identify mechanistic pathways that are conserved across species and strains of animals and liver cell culture models and are therefore more relevant to human health. The available mechanistic and toxicological evidence was organized and evaluated in concordance with the frameworks used for mode-of-action (MOA) analysis for noncancer effects and development of adverse outcome pathways (AOP)[6] (Edwards et al., 2016; Boobis et al., 2008; IPCS, 2007). PFDA-induced hepatic effects reported in in vivo and cell culture studies were organized according to the following levels of biological organization: molecular interactions, cellular effects, organ effects, and organism effects. The analysis described here was focused on the concordance of key events and adverse responses across species to obtain clarification on the relevance of animal studies to human health.

In addition to analyzing the available evidence published in the peer-reviewed literature, EPA also considered mechanistic evidence from in vitro high-throughput screening (HTS) assays on PFDA available from EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard) ((U.S. EPA, 2022a); data retrieved on November 03, 2022). Bioactivity data from the ToxCast and Tox21 collaborative projects were also considered at the same levels of biological organization described below. A more detailed description of the HTS analysis and results is provided in Appendix E.

[6]Although the World Health Organization (WHO)-International Programme on Chemical Safety (IPCS)-MOA and the Organization for Economic Co-operation and Development (OECD)-AOP frameworks are similar in the identification and analysis of key events following modified Bradford-Hill criteria (Meek et al., 2014), AOPs are chemically agnostic, whereas MOA analyses are intended to inform health assessments of individual (or groups of) chemical(s) (Edwards et al., 2016).

D.2. PROPOSED MOA/AOP APPROACH FOR EVALUATING PFAS-INDUCED LIVER TOXICITY

The proposed MOA displayed in Figure D1 is based on molecular initiating events, key events, and adverse outcomes identified in previous mechanistic evaluations and reviews of perfluorooctane sulfonic acid (PFOS) and perfluorinated alkylated substances (PFOA) (U.S. EPA, 2024a, b; ATSDR, 2018; Li et al., 2017a; U.S. EPA, 2016a, b), which are structurally related to PFDA and among the most well-studied PFAS. Additional reviews on biological pathways associated with chemical-induced cancer and noncancer liver effects were also consulted (see citations below). A summary of the MOA is presented below.

At the molecular level, experimental studies using in vivo and cell culture models have shown that perfluorinated compounds such as PFOS and PFOA can activate several nuclear receptor pathways including the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), the farnesoid X receptor (FXR), the peroxisome proliferator-activated receptor alpha (PPARα) and gamma (PPARγ), estrogen receptor alpha (ERα) and other receptor-independent cell signaling pathways (e.g., phosphatidylinositol 3-kinase-serine/threonine protein kinase (PI3K-Akt) signal transduction pathway, and the nuclear factor kappa B pathway [NFκB]) (ATSDR, 2018; Li et al., 2017a; U.S. EPA, 2016a, b). PFOS- and PFOA-induced activation of PPARα is associated with hepatocellular hypertrophy caused by peroxisome proliferation, increased peroxisomal fatty acid β oxidation and cytochrome P450 4A (CYP4A) expression and activity (U.S. EPA, 2024a, b; ATSDR, 2018; U.S. EPA, 2016a, b), and altered cholesterol metabolism (Li et al., 2017a). Increased PPARα activity can lead to oxidative stress via induction of acyl-CoA oxidase expression and activity and to H_2O_2 production in peroxisomes (Hall et al., 2012). Several studies have used genetically modified animal and cell culture models and immortalized human cell lines to evaluate potential PFOS or PFOA activation of the human PPARα. COS-1 cells transfected with the murine or human PPARα were responsive to PFAS exposure (U.S. EPA, 2024a, b, 2016a, b), and F1 generation PPARα-humanized mice were responsive to PFOA-induced expression responsive genes on GD 18, but unlike wild-type animals this response was not apparent on postnatal day 20 (U.S. EPA, 2016b; Takacs and Abbott, 2007). Studies using human liver cell lines or humanized animal models suggest humans are less sensitive to PPARα activation by the perfluorinated compounds PFOS and PFOA (reviewed in Li et al. (2017a) and U.S. EPA (2016a)). PPARα has also been shown to be activated by exposure to several PFAS, including PFOS, PFOA, PFNA, and PFHxS (ATSDR, 2018; Li et al., 2017a). Although PPARα is not expressed in high levels in the liver, its activation by pharmaceuticals and xenobiotic compounds has been proposed to be associated with hepatic steatosis caused by lipid accumulation (Angrish et al., 2016; Mellor et al., 2016).

As described above, exposure to perfluorinated compounds such as PFOS and PFOA has also been shown to activate other nuclear receptor and cell signaling pathways including the CAR, PXR, FXR, ERα, NFκB, and the oxidative stress responsive nuclear factor erythroid 2 related factor 2 (Nrf2) (ATSDR, 2018; Li et al., 2017a; U.S. EPA, 2016a). Furthermore, experiments using null animal

models exposed to several PFAS suggest that activation of CAR/PXR occurs independently of PPARα (ATSDR, 2018; Li et al., 2017a). Previous analyses of chemical-induced hepatotoxicity suggest that activation of these cell signaling pathways in experimental models is associated with increased expression and activity of xenobiotic metabolizing enzymes (XMEs) (Joshi-Barve et al., 2015; Hall et al., 2012), formation of reactive metabolites, alterations in cellular lipid metabolism (Angrish et al., 2016), and endoplasmic reticulum damage (Joshi-Barve et al., 2015).

At the cellular level, exposure to PFAS such as PFOS and PFOA has been shown to increase reactive oxygen species production and oxidative damage to cellular macromolecules (ATSDR, 2018; Li et al., 2017a; U.S. EPA, 2016a); promote mitochondrial damage, inhibit mitochondrial function, activate mitochondrial-mediated cell death (Li et al., 2017a; U.S. EPA, 2016b); increase endoplasmic reticulum stress (U.S. EPA, 2016b); induce DNA damage (ATSDR, 2018; U.S. EPA, 2016b); disrupt intercellular gap junction communication (ATSDR, 2018); elevate production/levels of proinflammatory cytokines (U.S. EPA, 2016b); alter lipid and glucose metabolism and bile acid biosynthesis (U.S. EPA, 2024a, b, 2016a, b); and increase hepatocellular death (Li et al., 2017a; U.S. EPA, 2016a). These pathways/mechanisms are associated with toxicant-induced liver disease and can promote steatohepatitis and fibrosis (Angrish et al., 2016; Cao et al., 2016; Joshi-Barve et al., 2015; Wahlang et al., 2013).

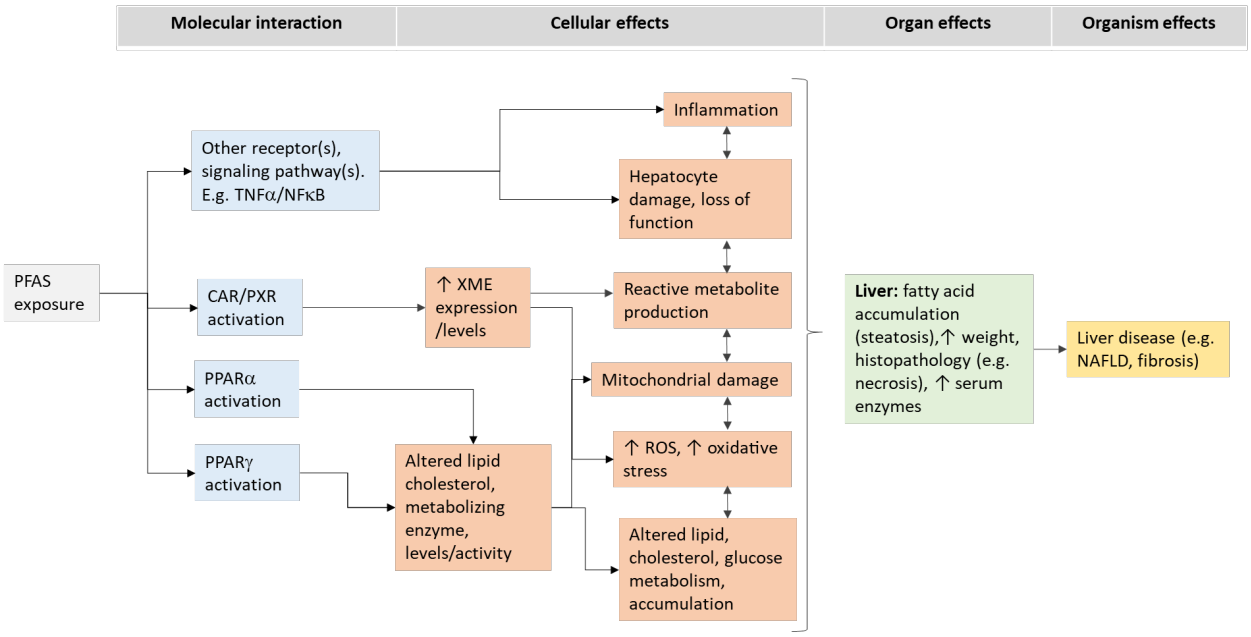


Figure D-1. This proposed MOA is based on previous analyses on PFAS-induced (e.g., PFOA/PFOS) liver toxicity and the role of nuclear receptor pathways in hepatotoxicity.

D.3. SYNTHESIS OF MECHANISTIC STUDIES AND SUPPLEMENTAL INFORMATION FOR PFDA

As mentioned previously, mechanistic evidence from peer-reviewed studies and HTS assays from EPA's ToxCast/Tox21 database were organized and evaluated according to the proposed MOA for the noncancer liver effects associated with exposure to PFAS (see Figure D-1). The evidence consists primarily of in vitro and in vivo studies conducted in liver tissues derived from human and animal models. When available, cell-free receptor binding studies and gene reporter assays profiling different key events in receptor signaling pathways in other cell tissue models (e.g., receptor dimerization, cofactor recruitment, DNA binding, and gene transactivation) were included in the analysis to provide additional information on the activation of nuclear receptor pathways and on potential species-specific differences in receptor sensitivity relevant to the mechanisms of liver toxicity for PFDA and other PFAS.

D.3.1. Molecular Initiating Events

As discussed below, the available studies have examined several nuclear receptor and cell signaling pathways associated with chemical-induced liver toxicity.

PPARα

PPARα is involved in a variety of processes, including nutrient metabolism, tissue development, cell differentiation, xenobiotic biotransformation, and inflammation (Li et al., 2017a). Induction of PPARα activity is primarily associated with increased CYP450 activity, peroxisomal proliferation, and hepatomegaly (liver enlargement) (Hall et al., 2012) and has been implicated in the mechanisms of hepatotoxicity of PFAS such as PFOS and PFOA (ATSDR, 2018; U.S. EPA, 2016a). Several experimental studies have evaluated PFDA-induced activation of PPARα in vivo in rat and mouse liver, and in human and rodent hepatocyte cell cultures. PFDA exposure was associated with increased hepatic expression of PPARα-responsive genes in Sprague-Dawley rats (NTP, 2018; Sterchele et al., 1996), C57BL/6J mice (Abe et al., 2017; Cheng and Klaassen, 2008a, b; Maher et al., 2008), and SV129 mice (Luo et al., 2017). PFDA treatment has also been shown to increase hepatic PPARα mRNA levels (Sterchele et al., 1996) and activity of the PPARα-responsive enzyme acyl-CoA oxidase in Sprague-Dawley rats (NTP, 2018). Chinje et al. (1994) exposed male Wistar rats and Harley guinea pigs to PFDA and reported increased CYP4A1 mRNA levels (indicative of PPARα activation) in rats, but no effects in guinea pigs. These findings are consistent with analyses that conclude guinea pigs, along with Syrian hamsters and nonhuman primates, are less responsive to PPARα activation than other rodent models (Corton et al., 2018; Hall et al., 2012).

Several cell culture and in vitro studies also report evidence considered supportive of the in vivo findings. PFDA exposure increased mRNA levels of PPARα and PPARα-responsive genes in rat hepatoma FaO cells (Sterchele et al., 1996). Two studies evaluated PFDA-induced effects on PPARα-responsive genes in human hepatic progenitor cells (HepaRG). One study was unable to

measure activation of PPARα or other nuclear receptors because of PFDA exposure associated with cytotoxicity (100 μM) but detected gene reported activity in nonhuman primate kidney cells transfected with the mouse PPARα (COS-1) (Abe et al., 2017). The other study, which tested a lower PFDA concentration (45 μM), confirmed PPARα activation (Lim et al., 2021). Rosen et al. (2013) analyzed gene expression changes in response to PFDA treatment and reported higher transcriptional activity in cultured primary human versus mouse hepatocytes, including the induction of PPARα-dependent and PPARα-independent genes. The lower-than-expected pattern of transcriptional activity for PFDA and other PFAS in cultured primary mouse hepatocytes compared with previous in vivo studies was attributed to cell culture conditions and the absence of hepatic nonparenchymal cells (Rosen et al., 2013). The authors also noted inconsistencies in the dose-response patterns of transcriptional activity in human hepatocytes across PFAS that could be due to interindividual variation in donor cells or inherent differences in the pattern of gene expression of tested chemicals (Rosen et al., 2013). PPARα-dependent reporter gene expression was also induced after PFDA treatment in human hepatoma HepG2 cells (Rosenmai et al., 2018) and human embryonic kidney HEK293 cells (Buhrke et al., 2013). HTS assays showed induction of PPARα transactivation in HepG2 cells but no activity in a binding reporter assay for the human PPARα (see Table E-2). However, a recent in vitro study in the peer-reviewed literature reported that PFDA can bind to the human PPARα ligand binding domain, albeit with lower affinity than in the Baikal seal PPARα (Ishibashi et al., 2019). Potential interspecies differences in PPARα activation were also described by Routti et al. (2019); Wolf et al. (2012); Wolf et al. (2008), showing induction of transcriptional activity of the mouse and polar PPARα isoforms but minimal or no activity toward the human PPARα in nonhuman primate kidney cells (COS-1 and COS-7) exposed to PFDA.

Overall, the available ***evidence suggests*** that PFDA can activate hepatic PPARα in rats and mice in vivo and in cell culture models. There are inconsistencies with respect to the activation of PPARα in in vitro human models possibly due to differences in experimental design and/or potential confounding with PFDA-induced cytotoxicity. However, some ***evidence indicates*** that PFDA interacts with the human PPARα in immortalized and primary cells derived from liver tissue. The data also suggest potential species differences in the binding affinity and activity of PPARα with the human isoform being potentially less sensitive compared with other mammalian species. In vivo studies with genetically modified animals in which the gene encoding PPARα is inactivated are needed to further characterize these differences.

Other PPARs (PPARγ and PPARβ/δ)

Two other PPAR subtypes have been characterized, PPARγ and PPAR beta/delta (β/δ), that play an essential role in energy homeostasis and metabolism. PPARγ is known to regulate adipogenesis, lipid and glucose metabolism, and inflammatory pathways, and its hepatic upregulation has been proposed as a key mechanism in the pathogenesis of nonalcoholic fatty liver disease (NAFLD) (Al Sharif et al., 2014). PFDA-induced transactivation of human PPARγ was observed in HEK263 (Buhrke et al., 2013) and HepG2 cells (Zhang et al., 2014) and HTS results

from EPA's ToxCast/Tox21 database, which are displayed in Table E-2. PFDA also showed affinity for the human PPARγ in receptor-ligand binding assays (see Zhang et al. (2014) and Table E-2) but displayed no activity in agonist/antagonist or cofactor recruitment assays related to this receptor conducted in HEK293T cells (see Table E-2). Further, PFDA upregulated the expression of the PPARγ gene in primary human hepatocytes (Rosen et al., 2013).

PPARβ/δ is involved in fatty acid metabolism and suppression of macrophage-derived inflammation (Barish et al., 2006). Studies examining potential interaction between PFAS and PPARβ/δ are limited. In vitro evidence showed that PFDA is capable of binding to the human PPARβ/δ and activating its transcriptional activity in HEK293 cells at noncytotoxic concentrations (<100 μM) (Li et al., 2019a). In contrast, PFDA was inactive in ToxCast/Tox21 assays (see Table E-2), evaluating human PPARβ/δ transactivation in HEK293 and HepG2 cells at concentrations up to 200 μM. Differences in experimental design (e.g., reporter system) could account for discrepancies in the results.

There is in vitro evidence that suggests potential activation of other human PPAR subtypes after PFDA treatment, primarily PPARγ and possibly PPARβ/δ. Experimental studies in animals and humanized models would be critical to confirming and better characterizing the potential role of these receptors in the mechanism(s) of hepatotoxicity from PFDA exposure.

CAR/PXR

Chemical-induced activation of CAR and PXR leads to increased expression and activity of XMEs (Li et al., 2017a; Hall et al., 2012) and drug transport proteins (Mackowiak et al., 2018). In addition to metabolism and excretion of xenobiotic compounds (and endogenous substrates such as steroids and fatty acids), CAR/PXR-induced xenobiotic enzyme activities have been proposed to promote formation of reactive metabolites (Wang et al., 2014; Li et al., 2012), alter drug interactions (Mackowiak et al., 2018), and increase oxidative stress, immune responses, and mitochondrial disfunction (Wang et al., 2014). CAR/PXR activation can also alter lipid homeostasis and promote hepatic steatosis (Mackowiak et al., 2018; Mellor et al., 2016).

Experimental studies have evaluated PFDA-mediated activation of CAR and PXR in rodents. PFDA exposure led to increased CAR mRNA levels, nuclear translocation of CAR, and increased mRNA and/or protein levels of CAR- and PXR-responsive genes, such as Cyp2B10 and Cyp3A11, in C57BL6/6J mice (Abe et al., 2017; Cheng and Klaassen, 2008b). NTP (2018) also reported increased mRNA levels of CAR-responsive genes, Cyp1B1 and Cyp1B2, in Sprague-Dawley rats. Further evaluation of the effects of PFDA on CYP450s in genetically modified mice devoid of function of specific nuclear receptors revealed that PFDA-mediated Cyp2B10 mRNA expression is regulated by CAR and independent of PPARα, PXR or FXR (Cheng and Klaassen, 2008b). PXR was also not required for the induction of Cyp3A11 mRNA after PFDA exposure (Cheng and Klaassen, 2008b).

Cell culture studies and HTS assays from the ToxCast/Tox21 database have also evaluated PFDA-induced activation of CAR and PXR. PFDA exposure resulted in increased mRNA and protein levels of PXR but did not affect the expression of the PXR target gene, Cyp3A23, in primary rat

hepatocytes (Ma et al., 2005). PXR-dependent CYP3A4 activation by PFDA was reported in HepG2 cells transfected with the human PXR (Zhang et al., 2017), and increased mRNA levels of CAR/PXR-responsive genes, CYP2B6 and CYP3A4, were detected in primary human hepatocytes after PFDA treatment (Rosen et al., 2013). In primary mouse hepatocytes, PFDA treatment had no effect on CAR-responsive genes, but according to the study authors this may have been caused by cell culture conditions and time in culture before and during exposure (Rosen et al., 2013). An additional study reported no effects on the induction of the mouse or human CAR in gene reporter assays using nonhuman primate kidney COS-1 cells but failed to assess PFDA-induced expression of CAR-responsive genes in HepaRG cells due to increased cytotoxicity after chemical exposure (100 μM) (Abe et al., 2017). Using a lower PFDA concentration (45 μM), Lim et al. (2021) showed upregulation of the CAR-target gene, CYP2B6. Gene reporter activity measured in HTS assays conducted in HepG2 cells revealed PFDA-induced activation of the human PXR in one of three assays but no activation of the human CAR across four assays (see Table E-2). PFDA also demonstrated binding activity for the human PXR (see Table E-2).

Overall, the available ***evidence suggests*** that PFDA exposure can activate the murine CAR resulting in altered levels of CYP450s in vivo and, although not all available experiments were clearly positive, PFDA appears to interact with PXR in in vitro rodent and human model systems. Future studies focusing on the potential involvement of these receptors in the mechanisms of PFDA-induced liver effects would be informative.

FXR

FXR is a key regulator of bile acid synthesis and lipid metabolism (Russell, 2003). Deletion of the mouse FXR gene (Nr1h4) leads to fatty liver and insulin resistance (Ma et al., 2006) and exacerbation of chemical-induced acute liver injury (Takahashi et al., 2017), while activation of FXR in response to liver injury and disease may have a protective role (Han, 2018). PFDA was evaluated in HTS from EPA's ToxCast/Tox21 database (see Appendix E for more details). No FXR activity was detected in assays related to receptor/cofactor interaction or agonist/antagonist transactivation in human embryonic kidney HEK293 cells (see Table E-2). Conversely, PFDA displayed agonist activity in a cell-free receptor-ligand binding assay and was active in one of two assays profiling transcriptional activity of this receptor in a human liver cell line (HepG2) (see Table E-2). Importantly, PFDA exhibited high potency for the human FXR compared with other nuclear receptors (e.g., PPARα/γ and CAR/PXR) on the basis of estimated effective concentrations (i.e., AC50 values) (see Figure E-3B). In summary, FXR appears to be a sensitive target of PFDA in HTS assays and thus, similar to CAR above, experiments specifically targeting the potential role of this receptor in the liver effects of PFDA would be informative.

Other Pathways

Additional cell signaling pathways have been evaluated in vivo and in liver cells in vitro. In Wistar rats and SV129 mice, PFDA exposure had no effects on mRNA levels of c-Jun/c-Fos (Luo et

al., 2017) (Oguro et al., 1998). Similarly, PFDA exposure had no significant effects on aryl hydrocarbon receptor (AHR)-inducible P450 activity in C57BL/6J mice (Brewster and Birnbaum, 1989) or mRNA expression of AHR-responsive genes (Cyp1A1/2) in C57BL/6J mice (Cheng and Klaassen, 2008b) and HepaRG cells (Lim et al., 2021). However, PFDA increased 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced AHR transactivation in an antagonist assay conducted in mouse hepatoma Hepa 1.12cR cells (Long et al., 2013). Effects on inflammatory and oxidative/cellular stress signaling involving the nuclear factor erythroid 2 related factor 2 (Nrf2), nuclear factor kappa B pathway (NFκB), tumor necrosis factor alpha (TNFα), c-Jun-N-terminal kinase (JNK) and activating transcription factor 2 (ATF-2) were reported following PFDA exposure in rodents (see syntheses on inflammation and cellular stress in Section D.3.2 for more details).

In vitro HTS assays from ToxCast/Tox21 showed induction of target gene pathways in HepG2 and HepaRG cells (measured as gene reporter activity) (see Table E-1), including several nuclear receptors discussed previously. According to estimated AC50 values (concentration at half maximal response), gene-specific activities occurred upstream but in some cases were closely associated with responses indicative of cellular stress/cytotoxicity (see Figure E-1). Specifically, PFDA was active in all three assays measuring Nrf2 transcriptional or agonist activity but was inactive in transactivation assays for NFκB and AHR in HepG2 and HepaRG cells (see Table E-1). Induction of transcriptional activity for JUN/FOS was demonstrated in HepaRG cells but not HepG2 cells with PFDA exposure (see Table E-1).

Overall, the available experimental studies suggest that in addition to activation of PPARα and CAR/PXR nuclear receptor pathways (and possibly PPARγ and FXR on the basis of limited in vitro studies in human cells), exposure to PFDA may also promote activation of other cell signaling pathways associated with inflammatory and oxidative/cellular stress responses (see syntheses on inflammation and cellular stress in Section D.3.2 for more details).

D.3.2. Cellular Effects

As discussed below, the available studies provide evidence on potential PFDA-induced alterations in hepatic expression and/or activity of XMEs, oxidative stress, cell and mitochondrial damage, inflammation, and alterations in liver metabolic functions.

Expression and Activity of XMEs

Several in vivo studies have evaluated PFDA-induced effects on the expression and activity of XMEs. In Wistar rats, PFDA exposure was associated with increased cytochrome P450 content and activity of NADPH-cytochrome c (P-450) reductase (Yamamoto and Kawashima, 1997) and decreased GST protein levels and activity (Oguro et al., 1998; Kawashima et al., 1995; Schramm et al., 1989). Furthermore, PFDA exposure altered bilirubin glucuronosyltransferase activities and bilirubin, morphine, testosterone, and naphthol glucuronidation (Arand et al., 1991). In Fischer rats, PFDA treatment resulted in decreased sulfotransferase protein levels (Witzmann et al., 1996) and microsomal carboxylesterase activity (Derbel et al., 1996). A study using SV129 mice found that

PFDA exposure decreased hepatic mRNA levels of CYP450s, and organic-anion-transporting polypeptides (OATPs) involved in the bile acid synthesis and uptake, while increasing mRNA levels of UDP-glucuronosyltransferases (UGT) enzymes (Luo et al., 2017). PPARα-null mice were mostly resistant to these effects (Luo et al., 2017). Similarly, Cheng and Klaassen (2008b) reported that PFDA-mediated downregulation of hepatic bile acid uptake transporters (OATPs and the Na+-taurocholate cotransporting peptide) is notably disrupted in PPARα-null mice but not in CAR-, PXR-, Nrf2- or FXR-null counterparts. As such, PPARα appears to be involved in the modulation of metabolizing enzymes and transport mechanisms important for bile acid homeostasis.

Several in vivo studies evaluated the effects of PFDA exposure on multidrug resistance proteins, which play important roles in hepatic metabolic and detoxifying functions, including bile acid excretion (Roth et al., 2019; Yang et al., 2014). In Sprague-Dawley rats, PFDA exposure was associated with decreased mRNA and protein levels of the hepatic multidrug resistance protein 2 (Mrp2), albeit effects were not statically significant (Johnson and Klaassen, 2002). A separate study reported that PFDA exposure significantly increased Mrp2 mRNA levels in SV129 mice and that PPARα-null animals were resistant to this effect (Luo et al., 2017). Two studies using wild-type and PPARα-null mice evaluated PFDA-induced changes in hepatic levels of Mrp3 and Mrp4 (Luo et al., 2017; Maher et al., 2008). Both studies report that PFDA treatment increased Mrp4 mRNA levels in wild-type SV129 or C57BL/6J mice, but the responses in PPARα-null animals differed: Maher et al. (2008) observed that elimination of PPARα ameliorated this effect, while Luo et al. (2017) reported that PPARα-nulls were as responsive as wild-type animals. Maher et al. (2008) observed that unlike wild-type mice, PPARα-null animals were resistant to PFDA induction of Mrp3, and Luo et al. (2017) reported no exposure-related effects on Mrp3 levels in either wild-type or null animals. Luo et al. (2017) and Maher et al. (2008) used a similar dose regimen (single i.p. injection of 80 mg/kg) but Luo et al. (2017) sampled animals on day 5 post exposure whereas Maher et al. (2008) sampled animals 48 hours post exposure and test mouse strain (SV129 and C57BL/6, respectively) differed between studies. These differences in experimental model and/or design features could account for the perceived discrepancies in the results. Maher et al. (2008) also reported that Nrf2-null mice were resistant to PFDA-induced expression of Mrp3 and Mrp4, and that pretreatment with gadolinium chloride ameliorated PFDA induction of Mrp4 mRNA levels but had no effect on Mrp3. Overall, the results suggest that PPARα and other signaling pathways (i.e., Nrf2 and Kupffer cell activation) participate in PFDA-mediated disruption of hepatic efflux Mrp transporters.

A study evaluating transcriptomic changes in HepaRG cells with exposure to PFDA and other long-chain PFAS observed enrichment of gene pathways involved in phase I and phase II metabolism, transporters, bile acid metabolism, amino acid metabolism and carbohydrate metabolism (Lim et al., 2021). An increase in transcriptomic response was reported with increasing carbon chain length with PFDA being the most potent PFAS tested. Specifically with respect to transporters, PFDA exposure was associated with the upregulation of xenobiotic efflux transporters (e.g., ABCA3, ABCC3/MRP3, ABCC10/MRP7, and ABCG2/BCRP) and amino acid transporters

involved in protein synthesis (e.g., SLC1A4, SLC1A5, SLC6A9, SLC7A1, SLC7A2, SLC7A5, SLC7A11, and SLC43A1), as well as the downregulation of bile acid or xenobiotic uptake transporters (e.g., SLC10A1/NTCP, SLCO2B1 and SLCO4C1). These observations are consistent with a potential compensatory mechanism against chemical-induced injury. The authors also noted that PFDA-mediated regulation of transporters appeared to be associated with the induction of Nrf2 rather than PPARα or CAR (Lim et al., 2021). Similarly, HTS ToxCast/Tox21 assays showed PFDA-mediated induction of gene pathways associated with xenobiotic metabolism and transport (i.e., CYP1A1, CYP2C19, CYP4A11, CYP4A22, ABCC3, and ABCG2,) in HepaRG cells (see Figure E-2 and Table E-1).

The findings described above suggest that exposure to PFDA results in increased XME levels and activity in animal models, which is supported by evidence on PFDA-induced activation of the CAR/PXR signaling pathways, two key regulators of XMEs. Furthermore, evidence from experiments using null animals suggests that PPARα is important for PFDA-induced regulation of a number of XMEs and transporters involved in bile acid homeostasis (e.g., CYP450, UGT OATP, and Mrp proteins). Additional mechanisms involving Nrf2 and Kupffer cell-mediated inflammatory responses appear to also play a role in regulating the expression of hepatic transporters in response to chemical-induced toxicity. The disruption of bile acid synthesis and transport mechanisms is consistent with the observed increases in markers of hepatobiliary function/injury in mice following PFDA exposure (see synthesis discussions on Cellular Stress and Metabolic Effects, below). Further studies are necessary to clarify inconsistencies in the results described above and to characterize the specific role of PPARα, Nrf2, and other cell signaling pathways (e.g., CAR/PXR) in modulating XME expression and activity and associated downstream effects that could contribute to the observed hepatic effects of PFDA exposure.

Oxidative Stress

Increased production of reactive oxygen species (ROS) can lead to hepatocellular toxicity, as it can result in cellular damage (e.g., increase lipid peroxidation, protein oxidation, and oxidative DNA damage) (Joshi-Barve et al., 2015; Wahlang et al., 2013) and activation of proinflammatory cell signaling cascades (Joshi-Barve et al., 2015).

Several in vivo and cell culture studies have evaluated PFDA-induced oxidative stress. In CD-1 mice, PFDA decreased the activity of antioxidant enzymes such as total superoxide dismutase (T-SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, while increasing the level of hepatic oxidative markers including ROS, thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) in hepatic tissue (Wang et al., 2020). Likewise, PFDA exposure increased hepatic expression of ROS-responsive genes (Maher et al., 2008; Permadi et al., 1993) and microsomal lipid peroxidation (Cai et al., 1995) in C57BL/6J mice. In Sprague-Dawley and Wistar rats, PFDA exposure consistently altered expression of ROS-sensitive proteins known to respond to increased ROS including, glutathione-S-transferase, catalase, and glutathione reductase (Chen et al., 2001; Kim et al., 1998; Glauert et al., 1992; Ikeda et al., 1985). These findings are supported by the

observation that PFDA exposure results in the activation of the ROS-sensitive transcription factor, Nrf2, in C57BL/6J mice (as indicated by the increase in the hepatic expression of the Nrf2 gene marker, Nqo1) (Maher et al., 2008). Studies in PPARα-null mice determined that PFDA-mediated activation of the mouse Nrf2 was independent of PPARα (Maher et al., 2008). Moreover, PFDA was associated with an increase in oxidative DNA damage in rat liver (Huang et al., 1994; Takagi et al., 1991) in studies with repeated-dose exposure up to 54 weeks, while no alterations in oxidative DNA damage (Kim et al., 1998), lipid peroxidation (Glauert et al., 1992), or changes in cellular antioxidant levels (Glauert et al., 1992) were reported in single exposure studies in rats. Notably, induction of microsomal lipid peroxidation in mice was also achieved after repeated-dose exposure to PFDA for 2 weeks (Cai et al., 1995).

PFDA exposure induced ROS levels (Ojo et al., 2021; Wielsøe et al., 2015) and reduced intracellular glutathione (GSH) (Ojo et al., 2021) in HepG2 cells but did not affect the total cellular antioxidant capacity (Wielsøe et al., 2015).

The available ***evidence suggests*** that PFDA exposure increases ROS production in animal models and in HepG2 cells and may also promote ROS-related cellular damage (e.g., DNA oxidation and lipid peroxidation) in rodent species after prolonged or repeated exposure. The specific involvement of Nrf2 and other cell signaling pathways in PFDA-induced ROS and potential effects on cellular antioxidant capacity and oxidative cellular and tissue damage with prolonged chemical exposure remains to be elucidated.

Mitochondrial Damage

Mitochondrial damage is a mechanism associated with toxicant-induced alterations in hepatocellular lipid balance (Angrish et al., 2016) and increased liver toxicity (Wahlang et al., 2013). Damage to mitochondria caused by oxidative stress, attenuation in mitochondrial transmembrane potential, and alterations in membrane permeability, electron transport, and calcium fluxes are considered stimuli that induce hepatic steatosis (Kaiser et al., 2012) and mitochondrial-mediated liver cell death (Li et al., 2017a; Cao et al., 2016).

Several in vivo studies using different animal species and strains have evaluated PFDA-induced responses in hepatic mitochondria. In Sprague-Dawley rats, exposure to PFDA led to reduced cytochrome c oxidase activity (Harrison et al., 1988) and increased mitochondrial swelling (Harrison et al., 1988), a response that can lead to disruption of the mitochondrial membrane (Jaeschke et al., 2012). Consistent with this, PFDA exposure led to increased swelling and structural alterations in liver mitochondria in CF-1 mice, Fischer rats, Syrian hamsters, and guinea pigs; responses varied across species with rats being most sensitive (Van Rafelghem et al., 1987). In C57BL/6J mice and Fischer rats, PFDA treatment caused alterations in mitochondrial protein content and increased mitochondrial enzyme activity (Permadi et al., 1993); (Witzmann and Parker, 1991; Kelling et al., 1987). In vitro studies reported that isolated rat liver mitochondria exposed to PFDA display uncoupling of electron transport and oxidative phosphorylation (Langley, 1990) and induction of mitochondrial permeability transition (Wallace et al., 2013). In primary

Sprague-Dawley rat hepatocytes, PFDA treatment resulted in decreased mitochondrial metabolic functions (Vanden Heuvel et al., 1991). In vitro HTS data showed changes in mitochondrial mass but no effects on mitochondrial membrane potential in HepG2 cells after PFDA exposure (see Table E-1).

Overall, in vivo and in vitro studies suggest that PFDA exposure disrupts hepatic mitochondrial proteins, integrity and function, and some of the observed effects appeared to be conserved across different species of animals, including Syrian hamsters and guinea pigs, known to be low PPARα responders compared with other rodent models (Corton et al., 2018; Hall et al., 2012). Additional studies assessing the potential mitochondrial effects of PFDA in human primary and immortalized liver cells would help clarify the potential human relevance and essentiality of the apparent PFDA-induced disruptions of mitochondrial pathways in PFDA-induced hepatotoxicity.

Inflammation

Hepatic inflammation is a mechanism associated with toxicant-induced liver injury (Angrish et al., 2016; Wahlang et al., 2013). Activated macrophages and Kupffer cells produce cytokines (e.g., TNFα, interleukin[IL]-6 and IL-10) that activate hepatic stellate cells and contribute toxicant-induced liver damage (Joshi-Barve et al., 2015; Malhi and Gores, 2008).

PFDA-induced markers of hepatic inflammation and related mechanisms were evaluated in studies using rodent models. PFDA increased hepatic and/or serum protein levels of the proinflammatory cytokine TNFα in C57BL/6J mice (Maher et al., 2008), CD-1 mice (Wang et al., 2020), and Fisher-344 rats (Adinehzadeh and Reo, 1998). Induction of hepatic TNF-α levels were accompanied by increases in other proinflammatory cytokines such as IL-1β, IL-18, and IL-6 and increases in Nod-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome activation markers such as NLRP3, adaptor apoptosis-associated speck-like protein (ASC) and caspase-1 in CD-1 mice (Wang et al., 2020). Maher et al. (2008) also reported that pretreatment with gadolinium chloride, an anti-inflammatory agent that suppresses Kupffer cell responses, ameliorated induction of TNFα levels in PFDA-exposed C57BL/6J mice. These results suggest that Kupffer cells may play a role in pro-inflammatory responses following PFDA exposure. Another study evaluated the involvement of PPARα on PFDA-induced responses related to hepatic inflammation. Luo et al. (2017) reported that exposure to PFDA-induced anti-inflammatory responses such as increased IL-10 mRNA levels and decreased phosphorylation of NFκB in SV129 mice and that these effects did not occur in exposed PPARα-null animals. Hepatic TNFα and IL-6 mRNA levels were unaffected by exposure regardless of the genetic background of the animals. Similarly, Li et al. (2022b) showed enrichment of gene pathways associated with anti-inflammatory responses in the liver of female C57BL/6J mice exposed to PFDA. Specifically, mRNA expression of cytokines IL-1β and IL-18, caspase-1, inflammasome-related genes (NLRP1, NLRP3, and NLRC4), and key regulators of inflammasome assembly (e.g., cellular inhibitor of apoptosis 2 [cIAP2]) were suppressed. The data

also showed inhibition of T helper cell type 1 (Th1) differentiation in mouse livers treated with PFDA.

The inconsistent responses on TNFα levels of Luo et al. (2017) versus Maher et al. (2008), Adinehzadeh and Reo (1998), and Wang et al. (2020) may have been due to differences in experimental design. Adinehzadeh and Reo (1998) and Maher et al. (2008) measured protein levels 24 and 48 hours, respectively, after a single dose of 50–80 mg/kg via i.p. injection, whereas Luo et al. (2017) measured transcription (i.e., mRNA levels) on day 5 after a single i.p. injection of 80 mg/kg. The negative response on TNFα in the Luo et al. (2017) study is consistent with the observed anti-inflammatory response (i.e., inhibition NFκB and IL-10) and may reflect a compensatory mechanism following initial acute hepatic injury (Luo et al., 2017). Furthermore, Wang et al. (2020) evaluated protein levels of TNFα after oral administration of PFDA (13 mg/kg) for 12 days, demonstrating induction of TNF-α and other pro-inflammatory markers with sustained PFDA exposure.

In summary, although uncertainties remain, PFDA exposure appears capable of promoting both pro- and anti-inflammatory responses in rodents, and PPARα may be involved in some of these effects.

Cellular Stress

Several in vivo studies have evaluated markers of cellular stress after exposure to PFDA. As described in the main document for liver effects in animals (see Section 3.2.1), short-term oral exposure to PFDA has been shown to promote degenerative changes such as necrosis (Frawley et al., 2018; NTP, 2018) and increase in serum biomarkers of hepatocyte damage in Sprague-Dawley rats (NTP, 2018) and CD-1 mice (Wang et al., 2020). Liver cell necrosis can promote steatohepatitis and fibrosis by exacerbating tissue damage via increased release of cellular contents that in turn trigger proinflammatory responses and death of neighboring hepatocytes (Cattley and Cullen, 2018; Joshi-Barve et al., 2015). One study using Wistar rats evaluated PFDA-induced effects on cytoskeletal proteins and reported no exposure-related alterations (Witzmann and Parker, 1991). Additional effects indicative of cell damage/stress include PFDA-induced disruptions to the endoplasmic reticulum in the livers of Fischer or Sprague-Dawley rats, CD-1 mice, Syrian hamsters, and Guinea pigs (Harrison et al., 1988; Van Rafelghem et al., 1987), and dysregulation in intercellular gap junctions in Fischer rat and WB-F344 liver epithelial cells (Sovadinova et al., 2015). Wang et al. (2020) also reported increased expression of proapoptotic protein markers, Bax and cleaved caspase-3, in the liver of CD-1 mice exposed to PFDA. Furthermore, PFDA exposure was associated with increases in serum markers of hepatocyte and biliary damage (ALT, AST, and ALP) in wild-type SV129 mice that corresponded with the activation of responses indicative of cellular stress signaling, including phosphorylation of JNK and its downstream target, ATF-2 (Luo et al., 2017). Notably, PPARα-null animals did not show these effects (Luo et al., 2017).

Cell viability and DNA damage were not affected in HepG2 cells exposed to PFDA concentrations of up to 100 μM across two studies (Rosenmai et al., 2018; Wielsøe et al., 2015) but three other studies reported that PFDA induced cytotoxicity in HepG2 cells in a concentration-dependent manner (effective concentrations causing 50% cytotoxicity [IC_{50}] were 14.10–15 μM) (Ojo et al., 2021; Ojo et al., 2020; Buhrke et al., 2013). Similarly, PFDA elevated markers of cellular stress and cytotoxicity in HTS assays conducted in HepG2 cells at higher concentrations (AC50 values ranging from 106.54 to 122.76 μM). PFDA-induced cytotoxicity was also reported in HepaRG cells (see (Abe et al., 2017) and Table E-1 of the ToxCast/Tox21 data summary), primary rat and human hepatocytes (Rosen et al., 2013), immortalized human fetal liver cells (HL-7702) (Hu et al., 2014).

Overall, the available ***evidence suggests*** that PFDA exposure increases hepatocyte cytotoxicity in in vitro and in vivo animal models, including species considered less sensitive to PPARα activation (i.e., Syrian hamsters and Guinea pigs). Studies using null animals suggest that stress responses related to disruption of bile acid homeostasis in mice may be mediated, at least in part, by PPARα. However, the potential involvement of other cellular signaling pathways in PFDA-induced liver cell stress has not been investigated.

Metabolic Effects

Toxicant-induced alterations in hepatocyte function can result in abnormal metabolism and accumulation of cholesterol, fatty acids and triglycerides, and exacerbate effects caused by steatosis (Angrish et al., 2016), which in turn may increase susceptibility to other insults or progress to steatohepatitis (Yang et al., 2014; Wahlang et al., 2013).

PFDA-induced effects on liver metabolic function have been evaluated in multiple rodent models. In Wistar, Fischer, and Sprague-Dawley rats PFDA exposure was associated with alterations in lipid composition (Adinehzadeh et al., 1999; Yamamoto and Kawashima, 1997; Olson and Andersen, 1983), fatty acid transport (Vanden Heuvel et al., 1993) and metabolism (Reo et al., 1994; Davis et al., 1991); and increased fatty acid and triglyceride accumulation (Kudo and Kawashima, 2003; Adinehzadeh and Reo, 1998; Kawashima et al., 1995; Sterchele et al., 1994; Harrison et al., 1988; Van Rafelghem et al., 1988). Rat studies have also reported increased hepatic levels of cholesterol (Kawashima et al., 1995), bilirubin, and bile acids (NTP, 2018); decreased microsomal electron transport (Kawashima et al., 1995; Van Rafelghem and Andersen, 1988); alterations in hepatic cholesterol metabolism (Davis et al., 1991); glucose transport (Goecke-Flora et al., 1995) and metabolism (Goecke et al., 1994); and decreased albumin levels (NTP, 2018; Witzmann and Parker, 1991). PFDA also increases peroxisomal proliferation (Van Rafelghem et al., 1987), activity of responsive enzymes such as acyl-CoA oxidases (NTP, 2018; Kim et al., 1998; Huang et al., 1994; Borges et al., 1993; Vanden Heuvel et al., 1993; Borges et al., 1992; Glauert et al., 1992; Intrasuksri and Feller, 1991; Kozuka et al., 1991a; Borges et al., 1990), and β-oxidation (Kudo and Kawashima, 2003; Kudo et al., 2000; Adinehzadeh et al., 1999; Kawashima et al., 1995; Kozuka

et al., 1991b), which are consistent with the evidence of PPARα activation in experimental animal models (see synthesis on Molecular Initiating Events above). As mentioned previously, PPARs, including PPARα, regulate genes involved in lipid and cholesterol metabolism and promote β-oxidation of fatty acids (Xu et al., 2005). The findings from in vivo studies are supported by cell culture studies using primary rat hepatocytes that report alterations in fatty acid metabolism (Vanden Heuvel et al., 1991) and increased peroxisomal β–oxidation (Kudo et al., 2000).

Mice exposed to PFDA also demonstrate alterations in hepatic metabolic functions. PFDA exposure increased activity of fatty acid metabolizing enzymes (Permadi et al., 1993) and increased hepatic lipid accumulation in C57BL/6J mice (Brewster and Birnbaum, 1989), an initial manifestation of fatty liver disease that may progress to fibrosis (Wahlang et al., 2013). PFDA exposure caused alterations in the levels of bile acid metabolizing enzymes and transporters and increased serum levels of several indicators of cholestasis (including bile acids and their components and bilirubin) in mice (Luo et al., 2017; Maher et al., 2008) but PPARα-null animals were resistant to these effects (Luo et al., 2017). Finally, Van Rafelghem et al. (1987) reported extensive hepatic lipid vacuolization in hamsters and guinea pigs (and to a lesser extent in rats or mice) after PFDA treatment.

Studies examining PFDA-mediated liver metabolic effects in human models are mostly lacking. A study by Zhang et al. (2013) showed binding affinity toward the human liver fatty acid protein by multiple PFAS, including PFDA, which may disrupt fatty acid uptake and transport. More recently, Wang et al. (2022) showed increases in triglyceride content and accumulation in HepG2 cells in a concentration-dependent manner after PFDA treatment. Further, the authors demonstrated that PFDA can promote adipogenesis in vitro by activating the NLRP3 inflammasome-mediated sterol regulatory element binding transcription factor 1 (SREBP1) pathway. Similar results were observed in 3-T3-Li murine white preadipocytes treated with PFDA. NLRP3 inflammasome induction has also been demonstrated in in vivo rodent models (see section on inflammation above for more details) with PFDA and has previously been linked to metabolic impairment in human and animal studies (Oh et al., 2021; Traba et al., 2015).

The available ***evidence suggests*** that PFDA exposure alters liver metabolic functions across multiple rodent species, and in limited human in vitro studies. Studies using genetically modified animals suggest that PFDA-induced disruption of bile acid homeostasis is at least partially mediated by PPARα. More studies are needed to understand the specific role that PPARα and other cell signaling pathways play in PFDA-induced alterations in liver metabolic functions involving bile acid, glucose, lipid, and cholesterol metabolism and under what conditions these alterations might lead to steatohepatitis and other liver pathologies in humans following prolonged chemical exposure.

D.3.3. Organ-Level Effects

Animal toxicity studies via the oral route have reported effects on histological and clinical markers and organ weight measures, which are indicative of adverse responses in the liver. These include changes in the incidence of hepatocellular necrosis, serum biomarkers of hepatobiliary and liver damage and increased liver weights (see synthesis of Animal studies in section 3.2.1 of the Toxicological Review). A study by (NTP, 2018) compared liver effects in rats after short-term exposure between PFDA (and other PFAS) and Wyeth-14,643, which was used as a positive control for PPARα activation. Much like PFDA, Wyeth-14,643 caused increases in liver weights, changes in liver biomarkers in the blood and hepatocyte hypertrophy; however, no evidence of necrosis or other degenerative lesions were associated with Wyeth-14,643 exposure. The findings provide support for the hypothesis that some PFDA-induced liver responses are mediated by mechanisms independent of PPARα.

Additional evidence of PFDA-induced liver weight changes from i.p. injection studies is described herein. Several studies using rats and mice support increases in liver weight following PFDA exposure (Abe et al., 2017; Luo et al., 2017; Maher et al., 2008; Kim et al., 1998; Chen et al., 1994; Chinje et al., 1994; Borges et al., 1993; Borges et al., 1992; Kozuka et al., 1991b; Borges et al., 1990; Brewster and Birnbaum, 1989; Schramm et al., 1989; Van Rafelghem and Andersen, 1988; Kelling et al., 1987; Van Rafelghem et al., 1987; Kelling et al., 1986; Powers and Aust, 1986; Ikeda et al., 1985; Olson and Andersen, 1983). One study in particular used wild-type and PPARα-null mice and reported that PFDA exposure led to increases in liver weight regardless of the genetic background of the exposed animals (Luo et al., 2017). Two other studies evaluated PFDA-induced effects in guinea pigs and Syrian hamsters. In guinea pigs, exposure to PFDA did not have a significant impact on relative liver weight (Chinje et al., 1994; Van Rafelghem et al., 1987), whereas in Syrian hamsters, treatment was associated with increased liver weight (Van Rafelghem et al., 1987). As described above, guinea pigs and Syrian hamsters are less responsive to PPARα activation when compared with other rodent models. However, the observation that PFDA exposure caused increases in liver weights in Syrian hamsters and PPARα-null mice suggests that other cell signaling pathways may be contributing to PFDA-induced hepatomegaly in hamsters.

Overall, the available evidence from in vivo studies reports that PFDA exposure results in organ-level effects, such as increases in liver weights that are consistently observed across multiple species and may be mediated, at least in part, by PPARα-independent mechanisms.

APPENDIX E. ANALYSIS OF RELEVANT HIGH-THROUGHPUT SCREENING ASSAYS FROM EPA'S CHEMICALS DASHBOARD

E.1. IN VITRO BIOACTIVITY DATA RELEVANT TO THE MECHANISMS OF PFDA-INDUCED LIVER EFFECTS

In vitro high-throughput screening (HTS) assays for perfluorodecanoic acid (PFDA) were downloaded from EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard) ((U.S. EPA, 2022a), accessed November 3, 2022), which provides bioactivity data from the ToxCast and Tox21 collaborative projects. Available information most pertinent to the analysis of the potential mechanisms of PFDA-induced liver effects was extracted to supplement and augment mechanistic findings from studies in the peer-reviewed literature previously described. Results (active/inactive, AC50 values, and scaled activity) from in vitro assays in human hepatoma HepG2 cells and metabolically competent human hepatic progenitor cells (HepaRG) cells were compiled. Background control assays were filtered out along with nonspecific responses from inducible reporter gene assays analyzed in the negative fitting direction relative to the control ("_dn"). Bioactivity data were analyzed on the basis of the type of biological response or gene target using the annotation structure within the ToxCast assay summary information ((U.S. EPA, 2022a), accessed November 3, 2022).

PFDA was active in 74 of 238 unique assay endpoints (~31%) in HepG2 and HepaRG cells, inducing a range of cell- and gene-specific changes (see Figure E-1 and Table E-1). PFDA was associated with cell cycle arrest and proliferation responses and induction of markers of oxidative stress and cell death (see Table E-1). Alterations in nuclear size and mitochondrial mass were also observed in HTS assays for PFDA with no apparent changes in microtubule conformation and mitochondrial membrane potential and respiration (see Table E-1). Further, PFDA caused upregulation of transcriptional activity that occurred generally at lower effective concentrations (i.e., AC50) compared with the cell-based responses (see Figure E-1). Specifically, PFDA induced the expression of CYP450 enzymes, growth factors, transporters, and transcriptional factors, including several xenobiotic-sensing nuclear receptors previously implicated in the mechanisms of liver toxicity of PFDA or other PFAS (i.e., PPARα/γ, PXR, and FXR) (see Figure E-2 and Table E-1).

In summary, PFDA elicited in vitro responses in HTS assays conducted in HepG2 and HepaRG cells most consistently for cellular stress and cytotoxicity. Additionally, induction of gene target pathways corresponding to several transcriptional factor/nuclear receptor activities occurred upstream of the cell-mediated responses, albeit at similar effective concentrations.

Nuclear receptor activities were investigated more closely to provide further insights into the putative interaction of PFDA with these receptor-mediated signaling pathways in ToxCast/Tox21 assays profiling multiple endpoints (e.g., receptor binding, coregulator recruitment, and gene transactivation) and cell types (see Table E-2). As mentioned above, PFDA induced activity of specific steroid/xenobiotic-sensing receptors, most notably FXR, PPAR, and PXR (see Figure E-3A). PFDA interacted with the human FXR in a receptor-ligand binding assay evaluating agonist activity and in one of two independent assays measuring transcriptional activity in HepG2 cells but was inactive in four FXR-related assays in human embryonic kidney cells (HEK293T), targeting receptor/cofactor recruitment and agonist/antagonist activities (see Table E-2). Upregulation of transcriptional activity for PPARα and PPARγ but not PPARβ/δ (PPARD) was demonstrated in HepG2 cells, and PFDA was found to interact with the human PPARγ (but not human PPARα) in a receptor-ligand binding assay (see Table E-2). No activity was detected in assays conducted in HEK293T cells profiling agonist/antagonist activities for PPARγ or PPARβ/δ or receptor/cofactor recruitment for PPARγ (see Table E-2). PFDA was active in two of four assays for PXR, showing transcriptional induction in HepG2 cells (one of two independent assays) and direct binding to the human PXR but no activity in an agonist assay using HepG2 cells (see Table E-2). HNF4A, NURR1, RAR, ROR, RXR, and VDR were also targets of PFDA in reporter gene assays using HepG2 cells, and antagonist activity toward ERR was reported in HEK293T cells (see Table E-2). PFDA targeted the ER and AR in in vitro HTS assays; however, overall activity for these receptors was low (see Appendix E.2 for additional details on the HTS results for the ER and AR). PFDA showed no appreciable activity in assays for GR, CAR, LXR, TR, and PR (see Figure E-3A). Comparison of AC50 values across the nuclear receptor assays indicate that PFDA exerts the highest potency toward the human FXR with the lowest AC50 of 0.52 μM in a cell-free receptor binding assay (see Figure E-3B), which is below the lower bound of the ToxCast cytotoxicity limit estimated for this chemical (7.108 μM) ((U.S. EPA, 2022a), accessed November 3, 2022).

Altogether, the results of the ToxCast/Tox21 HTS analysis provide some mechanistic support for the PFDA-induced liver effects. PFDA caused upregulation of transcriptional activity in human hepatoma HepG2 cells involving multiple nuclear receptor pathways previously implicated in the MOA for PFDA-induced liver toxicity, namely PXR, FXR, and PPARα/γ. These target gene responses were associated with the induction of cellular stress/cytotoxicity. PFDA also interacted directly with the human PXR, FXR, and PPARγ in receptor binding assays, demonstrating particular sensitivity for the human FXR at concentrations below those associated with cytotoxicity and suggesting that FXR may be an important target for this chemical.

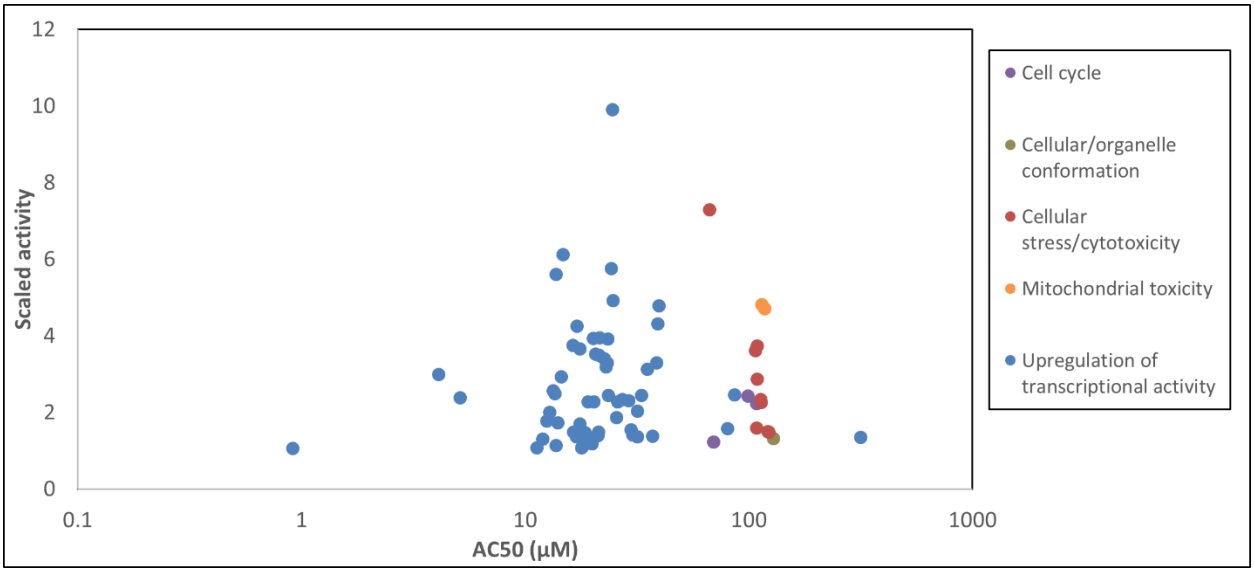


Figure E-1. Bioactivity data for PFDA from in vitro HTS ToxCast/Tox21 assays conducted in human liver cell lines (HepG2 and HepaRG cells).

Scatterplots show AC50 and scaled activity values from assays visualized according to the type of biological response. AC50 values refer to the concentration that elicits half maximal response and the scaled activity refers to the response value divided by the activity cutoff. Assays for which chemicals were inactive are not displayed. Additional information on all tested assays in HepG2 and HepaRG cells can be found in Table E-1.

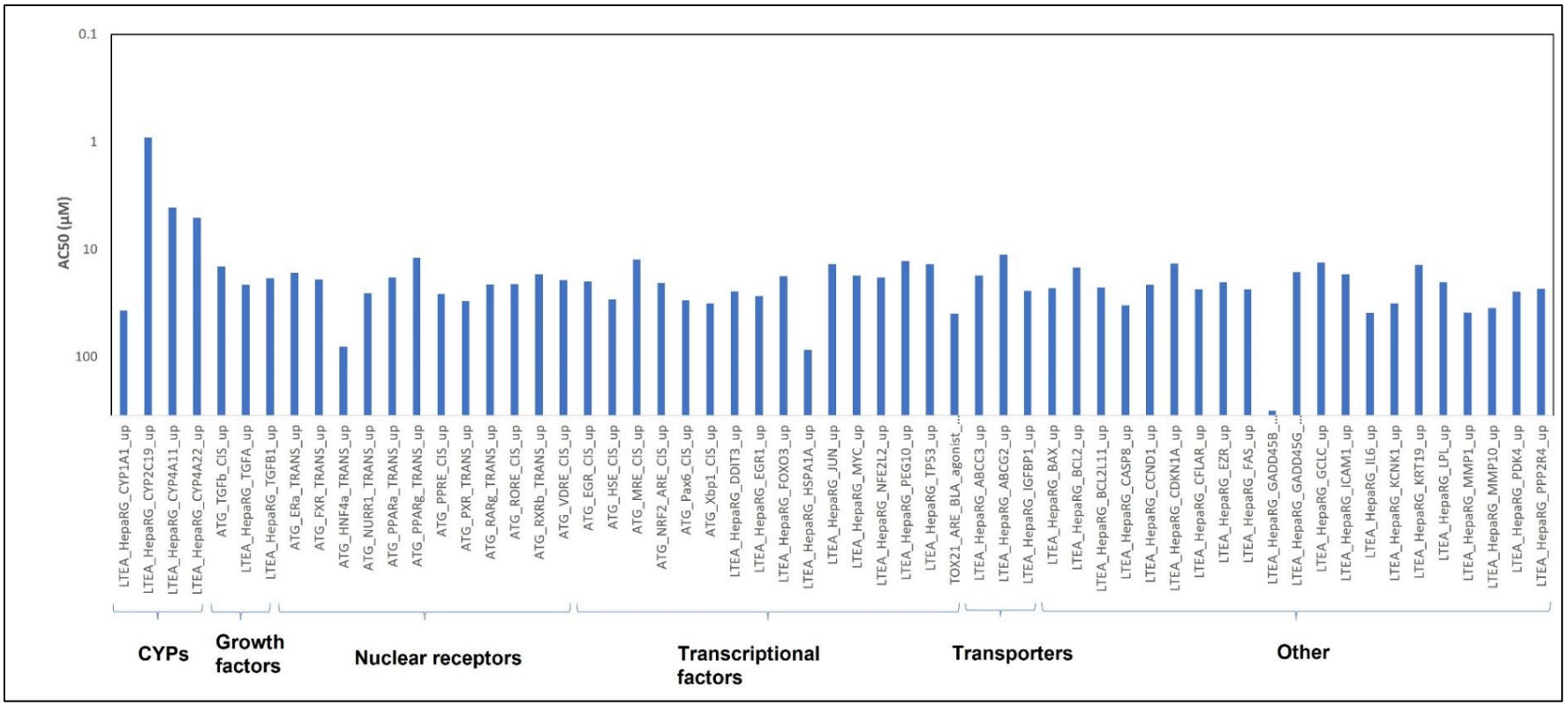


Figure E-2. Analysis of PFDA-induced upregulation of transcriptional activity in ToxCast/Tox21 assays conducted in human liver cell lines (HepG2 and HepaRG cells).

Bar graph compares AC50 values (concentration at half maximal response) for active assays. The scale for the AC50 values is shown in reverse order to visualize the most sensitive assays (the higher bar indicates a lower AC50 value). Additional information on the transcriptional activity assays can be found in Table E-1.

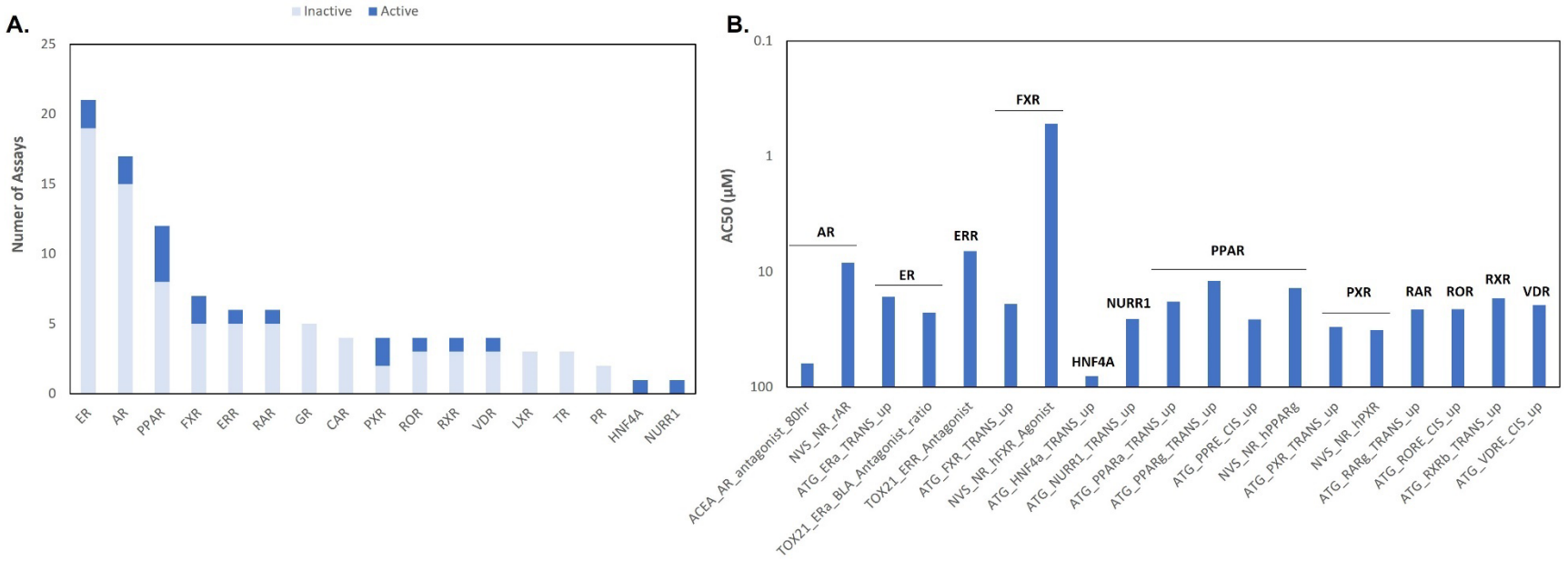


Figure E-3. Analysis of PFDA-induced nuclear receptor-related activities in ToxCast/Tox21 assays across multiple endpoints and cell types.

Panel A summarizes active/inactive calls from nuclear receptor assays mapped to specific target genes. Panel B compares AC50 values (concentration at half maximal response) for active assays. The scale for the AC50 values is shown in reverse order to visualize the most sensitive nuclear receptor activities (the higher bar indicates a lower AC50 value). Additional information on all tested nuclear receptor-related assays can be found in Table E-2.
AR = androgen receptor; CAR = constitutive androgen receptor; ER = estrogen receptor; ERR = estrogen-related receptor; FXR = farnesoid X receptor; GR = glucocorticoid receptor; HNF4A = hepatocyte nuclear factors 4 alpha; LXR = liver X receptor; NURR1 = nuclear receptor related-1 protein; PPAR = peroxisome proliferator-activated receptor; PXR = pregnane X receptor; RAR = retinoid acid receptor; ROR = RAR-related orphan receptor; RXR = retinoid X receptor; TR = thyroid hormone receptor; VDR = vitamin D receptor.

Table E-1. Bioactivity summary for PFDA from in vitro HTS assays from ToxCast/Tox21 conducted in human liver cell lines (HepG2 and HepaRG cells) and grouped by biological response/target[a,b]

Assay name	Activity call	Scaled activity	AC50 (μM)	Assay design	Cell line
Cell cycle					
APR_HepG2_CellCycleArrest_72h_dn	Active	1.23	69.51	Morphology reporter	HepG2
APR_HepG2_MitoticArrest_24h_up	Active	2.25	107.91	Morphology reporter	HepG2
APR_HepG2_MitoticArrest_72h_up	Active	2.44	98.57	Morphology reporter	HepG2
APR_HepG2_CellCycleArrest_24h_dn	Inactive	NA	NA	Morphology reporter	HepG2
APR_HepG2_CellCycleArrest_24h_up	Inactive	NA	NA	Morphology reporter	HepG2
APR_HepG2_CellCycleArrest_72h_up	Inactive	NA	NA	Morphology reporter	HepG2
APR_HepG2_MitoticArrest_24h_dn	Inactive	NA	NA	Morphology reporter	HepG2
APR_HepG2_MitoticArrest_72h_dn	Inactive	NA	NA	Morphology reporter	HepG2
Cellular/organelle conformation					
APR_HepG2_NuclearSize_24h_dn	Active	1.33	128.23	Morphology reporter	HepG2
APR_HepG2_NuclearSize_72h_dn	Active	1.51	121.20	Morphology reporter	HepG2
APR_HepG2_MicrotubuleCSK_24h_dn	Inactive	NA	NA	Conformation reporter	HepG2
APR_HepG2_MicrotubuleCSK_24h_up	Inactive	NA	NA	Conformation reporter	HepG2
APR_HepG2_MicrotubuleCSK_72h_dn	Inactive	NA	NA	Conformation reporter	HepG2
APR_HepG2_MicrotubuleCSK_72h_up	Inactive	NA	NA	Conformation reporter	HepG2
APR_HepG2_NuclearSize_24h_up	Inactive	NA	NA	Morphology reporter	HepG2
APR_HepG2_NuclearSize_72h_up	Inactive	NA	NA	Morphology reporter	HepG2
Cellular stress/cytotoxicity					
APR_HepG2_CellLoss_24h_dn	Active	3.75	108.88	Viability reporter	HepG2
APR_HepG2_CellLoss_72h_dn	Active	3.63	106.54	Viability reporter	HepG2
APR_HepG2_p53Act_24h_up	Active	1.61	107.89	Viability reporter	HepG2

Assay name	Activity call	Scaled activity	AC50 (μM)	Assay design	Cell line
APR_HepG2_p53Act_72h_up	Active	2.28	113.49	Viability reporter	HepG2
APR_HepG2_P-H2AX_24h_up	Active	2.35	112.97	Viability reporter	HepG2
APR_HepG2_P-H2AX_72h_up	Active	2.88	108.81	Viability reporter	HepG2
APR_HepG2_StressKinase_72h_up	Active	1.50	122.76	Enzyme reporter	HepG2
LTEA_HepaRG_LDH_cytotoxicity	Active	7.31	66.39	Viability reporter	HepaRG
APR_HepG2_CellLoss_24h_up	Inactive	NA	NA	Viability reporter	HepG2
APR_HepG2_CellLoss_72h_up	Inactive	NA	NA	Viability reporter	HepG2
APR_HepG2_p53Act_24h_dn	Inactive	NA	NA	Viability reporter	HepG2
APR_HepG2_p53Act_72h_dn	Inactive	NA	NA	Viability reporter	HepG2
APR_HepG2_P-H2AX_24h_dn	Inactive	NA	NA	Viability reporter	HepG2
APR_HepG2_P-H2AX_72h_dn	Inactive	NA	NA	Viability reporter	HepG2
APR_HepG2_StressKinase_24h_dn	Inactive	NA	NA	Enzyme reporter	HepG2
APR_HepG2_StressKinase_24h_up	Inactive	NA	NA	Enzyme reporter	HepG2
APR_HepG2_StressKinase_72h_dn	Inactive	NA	NA	Enzyme reporter	HepG2
ATG_XTT_Cytotoxicity_up	Inactive	NA	NA	Viability reporter	HepG2
CCTE_Simmons_MITO_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_AhR_LUC_Agonist_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_ARE_BLA_agonist_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_CAR_Agonist_viabillity	Inactive	NA	NA	Viability reporter	HepG2
TOX21_CAR_Antagonist_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_CASP3_HEPG2	Inactive	NA	NA	Inducible reporter	HepG2
TOX21_CASP3_HEPG2_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_MMP_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_PXR_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_FLO_00hr_ctrl_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_FLO_08hr_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_FLO_16hr_ctrl_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_FLO_24hr_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_FLO_32hr_ctrl_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_FLO_40hr_ctrl_viability	Inactive	NA	NA	Viability reporter	HepG2

Assay name	Activity call	Scaled activity	AC50 (μM)	Assay design	Cell line
TOX21_RT_HEPG2_GLO_00hr_ctrl_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_GLO_08hr_ctrl_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_GLO_16hr_ctrl_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_GLO_24hr_ctrl_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_GLO_32hr_ctrl_viability	Inactive	NA	NA	Viability reporter	HepG2
TOX21_RT_HEPG2_GLO_40hr_viability	Inactive	NA	NA	Viability reporter	HepG2
Mitochondrial toxicity					
APR_HepG2_MitoMass_24h_dn	Active	4.72	117.36	Morphology reporter	HepG2
APR_HepG2_MitoMass_72h_dn	Active	4.83	113.92	Morphology reporter	HepG2
APR_HepG2_MitoMass_24h_up	Inactive	NA	NA	Morphology reporter	HepG2
APR_HepG2_MitoMass_72h_up	Inactive	NA	NA	Morphology reporter	HepG2
APR_HepG2_MitoMembPot_24h_dn	Inactive	NA	NA	Membrane potential reporter	HepG2
APR_HepG2_MitoMembPot_24h_up	Inactive	NA	NA	Membrane potential reporter	HepG2
APR_HepG2_MitoMembPot_72h_dn	Inactive	NA	NA	Membrane potential reporter	HepG2
APR_HepG2_MitoMembPot_72h_up	Inactive	NA	NA	Membrane potential reporter	HepG2
CCTE_Simmons_MITO_basal_resp_rate_OCR_dn	Inactive	NA	NA	Respirometric reporter	HepG2
CCTE_Simmons_MITO_basal_resp_rate_OCR_up	Inactive	NA	NA	Respirometric reporter	HepG2
CCTE_Simmons_MITO_inhib_resp_rate_OCR_dn	Inactive	NA	NA	Respirometric reporter	HepG2
CCTE_Simmons_MITO_inhib_resp_rate_OCR_up	Inactive	NA	NA	Respirometric reporter	HepG2
CCTE_Simmons_MITO_max_resp_rate_OCR_dn	Inactive	NA	NA	Respirometric reporter	HepG2
CCTE_Simmons_MITO_max_resp_rate_OCR_up	Inactive	NA	NA	Respirometric reporter	HepG2
TOX21_MMP_ratio_down	Inactive	NA	NA	Membrane potential reporter	HepG2
TOX21_MMP_ratio_up	Inactive	NA	NA	Membrane potential reporter	HepG2
Upregulation of transcriptional activity					
ATG_EGR_CIS_up	Active	1.19	19.92377	Inducible reporter	HepG2

Assay name	Activity call	Scaled activity	AC50 (μM)	Assay design	Cell line
ATG_ERa_TRANS_up	Active	1.50	16.43561	Inducible reporter	HepG2
ATG_FXR_TRANS_up	Active	2.28	18.99931	Inducible reporter	HepG2
ATG_HNF4a_TRANS_up	Active	1.59	80.32058	Inducible reporter	HepG2
ATG_HSE_CIS_up	Active	2.31	28.98294	Inducible reporter	HepG2
ATG_MRE_CIS_up	Active	1.78	12.43083	Inducible reporter	HepG2
ATG_NRF2_ARE_CIS_up	Active	3.54	20.6361	Inducible reporter	HepG2
ATG_NURR1_TRANS_up	Active	1.87	25.56622	Inducible reporter	HepG2
ATG_Pax6_CIS_up	Active	1.56	29.70391	Inducible reporter	HepG2
ATG_PPARa_TRANS_up	Active	1.30	18.12921	Inducible reporter	HepG2
ATG_PPARg_TRANS_up	Active	1.31	11.97573	Inducible reporter	HepG2
ATG_PPRE_CIS_up	Active	2.29	25.89358	Inducible reporter	HepG2
ATG_PXR_TRANS_up	Active	1.42	30.14653	Inducible reporter	HepG2
ATG_RARg_TRANS_up	Active	1.50	21.20087	Inducible reporter	HepG2
ATG_RORE_CIS_up	Active	1.41	21.068	Inducible reporter	HepG2
ATG_RXRb_TRANS_up	Active	4.26	16.95397	Inducible reporter	HepG2
ATG_TGFb_CIS_up	Active	2.94	14.44227	Inducible reporter	HepG2
ATG_VDRE_CIS_up	Active	1.25	19.38327	Inducible reporter	HepG2
ATG_Xbp1_CIS_up	Active	2.05	31.73703	Inducible reporter	HepG2
LTEA_HepaRG_ABCC3_up	Active	1.71	17.53302	Inducible reporter	HepaRG
LTEA_HepaRG_ABCG2_up	Active	1.08	11.2217	Inducible reporter	HepaRG
LTEA_HepaRG_BAX_up	Active	3.20	22.88926	Inducible reporter	HepaRG
LTEA_HepaRG_BCL2_up	Active	6.13	14.76859	Inducible reporter	HepaRG
LTEA_HepaRG_BCL2L11_up	Active	3.41	22.55949	Inducible reporter	HepaRG
LTEA_HepaRG_CASP8_up	Active	2.45	33.09058	Inducible reporter	HepaRG
LTEA_HepaRG_CCND1_up	Active	3.50	21.35921	Inducible reporter	HepaRG
LTEA_HepaRG_CDKN1A_up	Active	2.49	13.57402	Inducible reporter	HepaRG
LTEA_HepaRG_CFLAR_up	Active	3.93	23.40259	Inducible reporter	HepaRG
LTEA_HepaRG_CYP1A1_up	Active	1.40	37.12706	Inducible reporter	HepaRG
LTEA_HepaRG_CYP2C19_up	Active	1.08	0.911362	Inducible reporter	HepaRG
LTEA_HepaRG_CYP4A11_up	Active	3.00	4.084149	Inducible reporter	HepaRG
LTEA_HepaRG_CYP4A22_up	Active	2.39	5.093503	Inducible reporter	HepaRG
LTEA_HepaRG_DDIT3_up	Active	9.91	24.56621	Inducible reporter	HepaRG
LTEA_HepaRG_EGR1_up	Active	2.35	27.13929	Inducible reporter	HepaRG
LTEA_HepaRG_EZR_up	Active	2.29	20.2641	Inducible reporter	HepaRG
LTEA_HepaRG_FAS_up	Active	2.46	23.51647	Inducible reporter	HepaRG
LTEA_HepaRG_FOXO3_up	Active	1.08	17.79771	Inducible reporter	HepaRG

Assay name	Activity call	Scaled activity	AC50 (μM)	Assay design	Cell line
LTEA_HepaRG_GADD45B_up	Active	1.37	316.2278	Inducible reporter	HepaRG
LTEA_HepaRG_GADD45G_up	Active	3.77	16.26879	Inducible reporter	HepaRG
LTEA_HepaRG_GCLC_up	Active	2.58	13.26529	Inducible reporter	HepaRG
LTEA_HepaRG_HSPA1A_up	Active	2.48	86.07431	Inducible reporter	HepaRG
LTEA_HepaRG_ICAM1_up	Active	1.37	16.93707	Inducible reporter	HepaRG
LTEA_HepaRG_IGFBP1_up	Active	5.77	24.20317	Inducible reporter	HepaRG
LTEA_HepaRG_IL6_up	Active	4.33	39.10404	Inducible reporter	HepaRG
LTEA_HepaRG_JUN_up	Active	1.15	13.67962	Inducible reporter	HepaRG
LTEA_HepaRG_KCNK1_up	Active	1.37	31.6189	Inducible reporter	HepaRG
LTEA_HepaRG_KRT19_up	Active	1.75	13.95732	Inducible reporter	HepaRG
LTEA_HepaRG_LPL_up	Active	3.94	20.11038	Inducible reporter	HepaRG
LTEA_HepaRG_MMP1_up	Active	3.30	38.55908	Inducible reporter	HepaRG
LTEA_HepaRG_MMP10_up	Active	3.14	35.00735	Inducible reporter	HepaRG
LTEA_HepaRG_MYC_up	Active	3.67	17.50487	Inducible reporter	HepaRG
LTEA_HepaRG_NFE2L2_up	Active	1.16	18.16403	Inducible reporter	HepaRG
LTEA_HepaRG_PDK4_up	Active	4.93	24.64551	Inducible reporter	HepaRG
LTEA_HepaRG_PEG10_up	Active	2.01	12.83903	Inducible reporter	HepaRG
LTEA_HepaRG_PPP2R4_up	Active	3.31	23.18532	Inducible reporter	HepaRG
LTEA_HepaRG_TGFA_up	Active	3.96	21.42175	Inducible reporter	HepaRG
LTEA_HepaRG_TGFB1_up	Active	1.48	18.53422	Inducible reporter	HepaRG
LTEA_HepaRG_TP53_up	Active	5.61	13.70365	Inducible reporter	HepaRG
TOX21_ARE_BLA_agonist_ratio	Active	4.79	39.41989	Inducible reporter	HepG2
ATG_Ahr_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_AP_1_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_AP_2_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_AR_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_BRE_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_C_EBP_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_CAR_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_CRE_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_DR4_LXR_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_DR5_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_E_Box_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_E2F_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_ERE_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_ERRa_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2

Assay name	Activity call	Scaled activity	AC50 (µM)	Assay design	Cell line
ATG_ERRg_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_Ets_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_FoxA2_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_FoxO_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_GATA_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_GLI_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_GR_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_GRE_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_HIF1a_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_HNF6_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_IR1_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_ISRE_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_LXRa_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_LXRb_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_Myb_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_Myc_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_NF_kB_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_NFI_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_NRF1_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_Oct_MLP_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_p53_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_PBREM_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_PPARd_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_PXRE_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_RARa_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_RARb_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_RORb_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_RORg_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_RXRa_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_Sox_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_Sp1_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_SREBP_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_STAT3_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_TCF_b_cat_CIS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_THRa1_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2
ATG_VDR_TRANS_up	Inactive	NA	NA	Inducible reporter	HepG2

Assay name	Activity call	Scaled activity	AC50 (μM)	Assay design	Cell line
LTEA_HepaRG_ABCB1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_ABCB11_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_ABCC2_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_ACLY_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_ACOX1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_ADK_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_ALPP_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_APOA5_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_BAD_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_BID_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CASP3_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CAT_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP1A2_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP24A1_1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP2B6_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP2C8_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP2C9_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP2E1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP3A4_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP3A5_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP3A7_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_CYP7A1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_EGF_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_FABP1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_FASN_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_FMO3_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_FOXO1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_GADD45A_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_GSTA2_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_GSTM3_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_HGF_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_HIF1A_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_HMGCS2_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_IGF1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_IL6R_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_LIPC_up	Inactive	NA	NA	Inducible reporter	HepaRG

Assay name	Activity call	Scaled activity	AC50 (μM)	Assay design	Cell line
LTEA_HepaRG_MIR122_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_MMP3_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_NFKB1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_NQO1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_PTEN_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_SDHB_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_SLC10A1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_SLC22A1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_SLC22A6_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_SLCO1B1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_STAT3_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_SULT2A1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_THRSP_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_TIMP1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_TNFRSF1A_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_UGT1A1_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_UGT1A6_up	Inactive	NA	NA	Inducible reporter	HepaRG
LTEA_HepaRG_XBP1_up	Inactive	NA	NA	Inducible reporter	HepaRG
TOX21_AhR_LUC_Agonist	Inactive	NA	NA	Inducible reporter	HepG2
TOX21_CAR_Agonist	Inactive	NA	NA	Inducible reporter	HepG2
TOX21_CAR_Antagonist	Inactive	NA	NA	Inducible reporter	HepG2
TOX21_PXR_Agonist	Inactive	NA	NA	Inducible reporter	HepG2

NA = not applicable.

[a]Data were sourced from EPA's CompTox Chemicals Dashboard ((U.S. EPA, 2022a), accessed November 3, 2022).

[b]Background control assays and nonspecific responses from inducible reporter gene assays analyzed in the negative fitting direction relative to the control ("_dn") are not presented herein.

Table E-2. Bioactivity summary for PFDA from in vitro HTS assays evaluating nuclear receptor-related activities from ToxCast/Tox21 across multiple endpoints and cell types[a,b,c]

Assay name	Activity call	Scaled Activity	AC50 (µM)	Biological target	Assay design	Organism	Tissue	Cell line
ATG_CAR_TRANS_up	Inactive	NA	NA	CAR (NR1I3)	Inducible reporter	human	liver	HepG2
ATG_PBREM_CIS_up	Inactive	NA	NA	CAR (NR1I3)	Inducible reporter	human	liver	HepG2
TOX21_CAR_Agonist	Inactive	NA	NA	CAR (NR1I3)	Inducible reporter	human	liver	HepG2
TOX21_CAR_Antagonist	Inactive	NA	NA	CAR (NR1I3)	Inducible reporter	human	liver	HepG2
TOX21_ERR_Antagonist	Active	1.31	6.62	ERR (ESRRA)	Inducible reporter	human	kidney	HEK293T
ATG_ERRa_TRANS_up	Inactive	NA	NA	ERR (ESRRA)	Inducible reporter	human	liver	HepG2
ATG_ERRg_TRANS_up	Inactive	NA	NA	ERR (ESRRA)	Inducible reporter	human	liver	HepG2
TOX21_ERR_Agonist	Inactive	NA	NA	ERR (ESRRA)	Inducible reporter	human	kidney	HEK293T
TOX21_PGC_ERR_Agonist	Inactive	NA	NA	ERR (ESRRA)	Inducible reporter	human	kidney	HEK293T
TOX21_PGC_ERR_Antagonist	Inactive	NA	NA	ERR (ESRRG)	Inducible reporter	human	kidney	HEK293T
ATG_FXR_TRANS_up	Active	2.28	19.00	FXR (NR1H4)	Inducible reporter	human	liver	HepG2
NVS_NR_hFXR_Agonist	Active	5.52	0.52	FXR (NR1H4)	Binding reporter	human	NA	NA
ATG_IR1_CIS_up	Inactive	NA	NA	FXR (NR1H4)	Inducible reporter	human	liver	HepG2
OT_FXR_FXRSRC1_0480	Inactive	NA	NA	FXR (NR1H4)	Binding reporter	human	kidney	HEK293T
OT_FXR_FXRSRC1_1440	Inactive	NA	NA	FXR (NR1H4)	Binding reporter	human	kidney	HEK293T
TOX21_FXR_BLA_agonist_ratio	Inactive	NA	NA	FXR (NR1H4)	Inducible reporter	human	kidney	HEK293T
TOX21_FXR_BLA_antagonist_ratio	Inactive	NA	NA	FXR (NR1H4)	Inducible reporter	human	kidney	HEK293T
ATG_GR_TRANS_up	Inactive	NA	NA	GR (NR3C1)	Inducible reporter	human	liver	HepG2
ATG_GRE_CIS_up	Inactive	NA	NA	GR (NR3C1)	Inducible reporter	human	liver	HepG2
NVS_NR_hGR	Inactive	NA	NA	GR (NR3C1)	Binding reporter	human	NA	NA
TOX21_GR_BLA_Agonist_ratio	Inactive	NA	NA	GR (NR3C1)	Inducible reporter	human	cervix	HeLa
TOX21_GR_BLA_Antagonist_ratio	Inactive	NA	NA	GR (NR3C1)	Inducible reporter	human	cervix	HeLa
ATG_HNF4a_TRANS_up	Active	1.59	80.32	HNF4A	Inducible reporter	human	liver	HepG2
ATG_LXRb_TRANS_up	Inactive	NA	NA	LXR (NR1H2)	Inducible reporter	human	liver	HepG2

Assay name	Activity call	Scaled Activity	AC50 (µM)	Biological target	Assay design	Organism	Tissue	Cell line
ATG_DR4_LXR_CIS_up	Inactive	NA	NA	LXR (NR1H2|NR1H3)	Inducible reporter	human	liver	HepG2
ATG_LXRa_TRANS_up	Inactive	NA	NA	LXR (NR1H3)	Inducible reporter	human	liver	HepG2
ATG_NURR1_TRANS_up	Active	1.87	25.57	NURR1 (NR4A2)	Inducible reporter	human	liver	HepG2
ATG_PPARa_TRANS_up	Active	1.30	18.13	PPAR (PPARA)	Inducible reporter	human	liver	HepG2
NVS_NR_hPPARa	Inactive	NA	NA	PPAR (PPARA)	Binding reporter	human	NA	NA
ATG_PPARd_TRANS_up	Inactive	NA	NA	PPAR (PPARD)	Inducible reporter	human	liver	HepG2
TOX21_PPARd_BLA_agonist_ratio	Inactive	NA	NA	PPAR (PPARD)	Inducible reporter	human	kidney	HEK293T
TOX21_PPARd_BLA_antagonist_ratio	Inactive	NA	NA	PPAR (PPARD)	Inducible reporter	human	kidney	HEK293T
ATG_PPARg_TRANS_up	Active	1.31	11.98	PPAR (PPARG)	Inducible reporter	human	liver	HepG2
NVS_NR_hPPARg	Active	5.15	13.73	PPAR (PPARG)	Binding reporter	human	NA	NA
OT_PPARg_PPARgSRC1_0480	Inactive	NA	NA	PPAR (PPARG)	Binding reporter	human	kidney	HEK293T
OT_PPARg_PPARgSRC1_1440	Inactive	NA	NA	PPAR (PPARG)	Binding reporter	human	kidney	HEK293T
TOX21_PPARg_BLA_Agonist_ratio	Inactive	NA	NA	PPAR (PPARG)	Inducible reporter	human	kidney	HEK293T
TOX21_PPARg_BLA_antagonist_ratio	Inactive	NA	NA	PPAR (PPARG)	Inducible reporter	human	kidney	HEK293
ATG_PPRE_CIS_up	Active	2.29	25.89	PPAR (PPARA|PPARD|PPARG)	Inducible reporter	human	liver	HepG2
TOX21_PR_BLA_Agonist_ratio	Inactive	NA	NA	PR (PGR)	Inducible reporter	human	kidney	HEK293T
TOX21_PR_BLA_Antagonist_ratio	Inactive	NA	NA	PR (PGR)	Inducible reporter	human	kidney	HEK293T
ATG_PXR_TRANS_up	Active	1.42	30.15	PXR (NR1I2)	Inducible reporter	human	liver	HepG2
NVS_NR_hPXR	Active	2.34	32.07	PXR (NR1I2)	Binding reporter	human	NA	NA
ATG_PXRE_CIS_up	Inactive	NA	NA	PXR (NR1I2)	Inducible reporter	human	liver	HepG2
TOX21_PXR_Agonist	Inactive	NA	NA	PXR (NR1I2)	Inducible reporter	human	liver	HepG2
ATG_RARa_TRANS_up	Inactive	NA	NA	RAR (RARA)	Inducible reporter	human	liver	HepG2
TOX21_RAR_LUC_Agonist	Inactive	NA	NA	RAR (RARA)	Inducible reporter	mouse	embryo	C3H10T1/2
TOX21_RAR_LUC_Antagonist	Inactive	NA	NA	RAR (RARA)	Inducible reporter	mouse	embryo	C3H10T1/2
ATG_RARb_TRANS_up	Inactive	NA	NA	RAR (RARB)	Inducible reporter	human	liver	HepG2
ATG_RARg_TRANS_up	Active	1.50	21.20	RAR (RARG)	Inducible reporter	human	liver	HepG2
ATG_DR5_CIS_up	Inactive	NA	NA	RAR (RARA|RARB|RARG)	Inducible reporter	human	liver	HepG2

Assay name	Activity call	Scaled Activity	AC50 (µM)	Biological target	Assay design	Organism	Tissue	Cell line
ATG_RORb_TRANS_up	Inactive	NA	NA	ROR (RORB)	Inducible reporter	human	liver	HepG2
ATG_RORg_TRANS_up	Inactive	NA	NA	ROR (RORC)	Inducible reporter	human	liver	HepG2
TOX21_RORg_LUC_CHO_Antagonist	Inactive	NA	NA	ROR (RORC)	Inducible reporter	Chinese hamster	ovary	CHO-K1
ATG_RORE_CIS_up	Active	1.41	21.07	ROR (RORA|RORB|RORC)	Inducible reporter	human	liver	HepG2
ATG_RXRa_TRANS_up	Inactive	NA	NA	RXR (RXRA)	Inducible reporter	human	liver	HepG2
OT_NURR1_NURR1RXRa_0480	Inactive	NA	NA	RXR (RXRA)	Binding reporter	human	kidney	HEK293T
OT_NURR1_NURR1RXRa_1440	Inactive	NA	NA	RXR (RXRA)	Binding reporter	human	kidney	HEK293T
ATG_RXRb_TRANS_up	Active	4.26	16.95	RXR (RXRB)	Inducible reporter	human	liver	HepG2
ATG_THRa1_TRANS_up	Inactive	NA	NA	TR (THRA)	Inducible reporter	human	liver	HepG2
TOX21_TR_LUC_GH3_Agonist	Inactive	NA	NA	TR (THRA|THRB)	Inducible reporter	rat	pituitary gland	GH3
TOX21_TR_LUC_GH3_Antagonist	Inactive	NA	NA	TR (THRA|THRB)	Inducible reporter	rat	pituitary gland	GH3
ATG_VDRE_CIS_up	Active	1.25	19.38	VDR	Inducible reporter	human	liver	HepG2
ATG_VDR_TRANS_up	Inactive	NA	NA	VDR	Inducible reporter	human	liver	HepG2
TOX21_VDR_BLA_agonist_ratio	Inactive	NA	NA	VDR	Inducible reporter	human	kidney	HEK293T
TOX21_VDR_BLA_antagonist_ratio	Inactive	NA	NA	VDR	Inducible reporter	human	kidney	HEK293T

NA = not applicable.

[a]Data were sourced from EPA's CompTox Chemicals Dashboard ((U.S. EPA, 2022a), accessed November 3, 2022).

[b]Nonspecific responses from inducible reporter gene assays analyzed in the negative fitting direction relative to the control ("_dn") are not presented herein.

[c]In vitro bioactivity data for the AR and ER are summarized in detail in Appendix E.2 and, therefore, are not presented herein.

E.2. IN VITRO BIOACTIVITY DATA RELEVANT TO THE POTENTIAL MECHANISMS OF REPRODUCTIVE TOXICITY

HTS screening ToxCast assays profiling in vitro activities for the AR, ER, and steroid hormone biosynthesis were sourced from EPA's CompTox Chemicals Dashboard ((U.S. EPA, 2019), accessed November 3, 2022) to investigate potential mechanisms of disruption of steroid hormone receptor activation and steroidogenesis that may be important for the reproductive toxicity of PFDA.

The suite of ToxCast assays and model predictions for the ER and AR encompass several endpoints in the signaling pathway of these receptors (e.g., receptor binding, receptor dimerization, cofactor recruitment, DNA binding, gene expression, and cell proliferation) across multiple in vitro models. PFDA was active in 2 of 17 AR assays (13%), demonstrating binding to the AR in rat prostrate tissue and AR-induced cell proliferation in a human prostate carcinoma cell line (22Rv1), but no activity in assays for cofactor recruitment and AR agonist/antagonist transactivation conducted primarily in human cell lines (see Table E-3). In ER assays, PFDA was active in 2 of 21 assays (11%), demonstrating activity for the ERα (ESR1) in 1 of 2 assays measuring RNA transcription in human hepatoma HepG2 cells and in an antagonist transactivation assay measuring protein expression in human embryonic kidney HEK293T cells (see Table E-3). PFDA was inactive in receptor binding assays for the ERα in human, bovine, and mouse tissues and in ER α/β assays for receptor dimerization, transcription factor-DNA binding, agonist transactivation, and ER-induced cell proliferation in different human cell lines. The AC50 values for the active ER and AR assays ranged from 8.40 to 62.3 μM, which are above the lower bound of the estimated ToxCast cytotoxicity limit (7.108 μM) ((U.S. EPA, 2022a), accessed November 3, 2022). ToxCast model predictions incorporating in vitro assay results and nonspecific responses such as cytotoxicity suggest that PFDA is inactive for both ER/AR agonist and antagonist pathways (AUC = 0) (see Table E-4).

The ToxCast database also included in vitro assays related to the regulation of steroidogenesis. PFDA showed a lack of activity in a single assay measuring inhibition of transcriptional activity for the aromatase gene (CYP19A1) in human breast cancer MCF-7 cells and several assays measuring biosynthesis of steroid hormones including glucocorticoids, androgens, estrogens, and progestogens in adrenal gland H295R cells (see Table E-5).

Table E-3. Bioactivity summary for PFDA from in vitro HTS assays evaluating activities for the AR, ER[a,b]

Assay name	Activity call	Scaled activity	AC50 (µM)	Biological target	Assay design	Organism	Tissue	Cell line
ACEA_AR_antagonist_80hr	Active	9.34	62.3	AR	Growth reporter	human	prostate	22Rv1
NVS_NR_rAR	Active	2.47	8.40	AR	Binding reporter	rat	prostate	NA
ACEA_AR_agonist_80hr	Inactive	NA	NA	AR	Growth reporter	human	prostate	22Rv1
ATG_AR_TRANS_up	Inactive	NA	NA	AR	Inducible reporter	human	liver	HepG2
OT_AR_ARELUC_AG_1440	Inactive	NA	NA	AR	Inducible reporter	Chinese hamster	ovary	CHO-K1
OT_AR_ARSRC1_0480	Inactive	NA	NA	AR	Binding reporter	human	kidney	HEK293T
OT_AR_ARSRC1_0960	Inactive	NA	NA	AR	Binding reporter	human	kidney	HEK293T
TOX21_AR_BLA_Agonist_ratio	Inactive	NA	NA	AR	Inducible reporter	human	kidney	HEK293T
TOX21_AR_BLA_Antagonist_ratio	Inactive	NA	NA	AR	Inducible reporter	human	kidney	HEK293T
TOX21_AR_LUC_MDAKB2_Agonist	Inactive	NA	NA	AR	Inducible reporter	human	breast	MDA-kb2
TOX21_AR_LUC_MDAKB2_Agonist_3uM_Nilutamide	Inactive	NA	NA	AR	Inducible reporter	human	breast	MDA-kb2
TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881	Inactive	NA	NA	AR	Inducible reporter	human	breast	MDA-kb2
TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881	Inactive	NA	NA	AR	Inducible reporter	human	breast	MDA-kb2
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Agonist	Inactive	NA	NA	AR	Binding reporter	human	bone	U2OS
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist	Inactive	NA	NA	AR	Binding reporter	human	bone	U2OS
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Cytoplasm_Ratio_Agonist	Inactive	NA	NA	AR	Binding reporter	human	bone	U2OS
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Cytoplasm_Ratio_Antagonist	Inactive	NA	NA	AR	Binding reporter	human	bone	U2OS
ATG_ERa_TRANS_up	Active	1.50	16.44	ER (ESR1)	Inducible reporter	human	liver	HepG2
TOX21_ERa_BLA_Antagonist_ratio	Active	3.32	22.7	ER (ESR1)	Inducible reporter	human	kidney	HEK293T
ACEA_ER_80hr	Inactive	NA	NA	ER (ESR1)	Growth reporter	human	breast	T47D
ATG_ERE_CIS_up	Inactive	NA	NA	ER (ESR1)	Inducible reporter	human	liver	HepG2
NVS_NR_bER	Inactive	NA	NA	ER (ESR1)	Binding reporter	bovine	uterus	NA
NVS_NR_hER	Inactive	NA	NA	ER (ESR1)	Binding reporter	human	NA	NA

Assay name	Activity call	Scaled activity	AC50 (µM)	Biological target	Assay design	Organism	Tissue	Cell line
NVS_NR_mERa	Inactive	NA	NA	ER (Esr1)	Binding reporter	mouse	NA	NA
OT_ER_ERaERa_0480	Inactive	NA	NA	ER (ESR1)	Binding reporter	human	kidney	HEK293T
OT_ER_ERaERa_1440	Inactive	NA	NA	ER (ESR1)	Binding reporter	human	kidney	HEK293T
OT_ERa_EREGFP_0120	Inactive	NA	NA	ER (ESR1)	Inducible reporter	human	cervix	HeLa
OT_ERa_EREGFP_0480	Inactive	NA	NA	ER (ESR1)	Inducible reporter	human	cervix	HeLa
TOX21_ERa_BLA_Agonist_ratio	Inactive	NA	NA	ER (ESR1)	Inducible reporter	human	kidney	HEK293T
TOX21_ERa_LUC_VM7_Agonist	Inactive	NA	NA	ER (ESR1)	Inducible reporter	human	ovary	VM7
TOX21_ERa_LUC_VM7_Antagonist_0.1nM_E2	Inactive	NA	NA	ER (ESR1)	Inducible reporter	human	ovary	VM7
TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2	Inactive	NA	NA	ER (ESR1)	Inducible reporter	human	ovary	VM7
OT_ER_ERbERb_0480	Inactive	NA	NA	ER (ESR2)	Binding reporter	human	kidney	HEK293T
OT_ER_ERbERb_1440	Inactive	NA	NA	ER (ESR2)	Binding reporter	human	kidney	HEK293T
TOX21_ERb_BLA_Agonist_ratio	Inactive	NA	NA	ER (ESR2)	Inducible reporter	human	kidney	HEK293T
TOX21_ERb_BLA_Antagonist_ratio	Inactive	NA	NA	ER (ESR2)	Inducible reporter	human	kidney	HEK293T
OT_ER_ERaERb_0480	Inactive	NA	NA	ER (ESR1|ESR2)	Binding reporter	human	kidney	HEK293T
OT_ER_ERaERb_1440	Inactive	NA	NA	ER (ESR1|ESR2)	Binding reporter	human	kidney	HEK293T

NA = not applicable.

[a]Data were sourced from EPA's CompTox Chemicals Dashboard ((U.S. EPA, 2022a), accessed November 3, 2022).

[b]Nonspecific responses from inducible reporter gene assays analyzed in the negative fitting direction relative to the control ("_dn") are not presented herein.

Table E-4. ToxCast model predictions for the ER and AR pathways for PFDA[a, b]

	Agonist AUC values (95% CI)	Antagonist AUC values (95% CI)
ER pathway	0 (0–0.0051)	0 (0–0.019)
AR pathway	0 (0–0.063)	0 (0–0.00016)

AUC = area under the curve score ranging from 0 to 1. An AUC value of 0 indicates that the chemical is inactive.
CI = confidence interval.
[a]Data for ER and AR pathways were sourced from Judson et al. (2015) and Kleinstreuer et al. (2017), respectively.
[b]95% CI for the ER activity model was sourced from a subsequent publication to the Judson et al. (2015) study (Watt and Judson, 2018).

Table E-5. Bioactivity summary for PFDA from in vitro HTS assays related to steroidogenesis[a]

Assay name	Activity call	Scaled activity	AC50 (μM)	Biological target	Assay design	Organism	Tissue	Cell line
CEETOX_H295R_11DCORT_noMTC_dn	Inactive	NA	NA	11-Deoxycortisol	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_11DCORT_noMTC_up	Inactive	NA	NA	11-Deoxycortisol	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_ANDR_noMTC_dn	Inactive	NA	NA	Androstenedione	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_ANDR_noMTC_up	Inactive	NA	NA	Androstenedione	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_CORTIC_noMTC_dn	Inactive	NA	NA	Corticosterone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_CORTIC_noMTC_up	Inactive	NA	NA	Corticosterone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_CORTISOL_noMTC_dn	Inactive	NA	NA	Cortisol	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_CORTISOL_noMTC_up	Inactive	NA	NA	Cortisol	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_DOC_noMTC_dn	Inactive	NA	NA	11-Deoxycorticosterone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_DOC_noMTC_up	Inactive	NA	NA	11-Deoxycorticosterone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_ESTRADIOL_noMTC_dn	Inactive	NA	NA	Estradiol	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_ESTRADIOL_noMTC_up	Inactive	NA	NA	Estradiol	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_ESTRONE_noMTC_dn	Inactive	NA	NA	Estrone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_ESTRONE_noMTC_up	Inactive	NA	NA	Estrone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_OHPREG_noMTC_dn	Inactive	NA	NA	17alpha-hydroxypregnenolone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_OHPREG_noMTC_up	Inactive	NA	NA	17alpha-hydroxypregnenolone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_OHPROG_noMTC_dn	Inactive	NA	NA	17alpha-hydroxyprogesterone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_OHPROG_noMTC_up	Inactive	NA	NA	17alpha-hydroxyprogesterone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_PROG_noMTC_dn	Inactive	NA	NA	Progesterone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_PROG_noMTC_up	Inactive	NA	NA	Progesterone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_TESTO_noMTC_dn	Inactive	NA	NA	Testosterone	inducible reporter	human	adrenal gland	H295R
CEETOX_H295R_TESTO_noMTC_up	Inactive	NA	NA	Testosterone	inducible reporter	human	adrenal gland	H295R
TOX21_Aromatase_Inhibition	Inactive	NA	NA	CYP19A1	inducible reporter	human	breast	MCF7

NA = not applicable.

[a]Data were sourced from EPA's CompTox Chemicals Dashboard (U.S. EPA, 2022a), accessed November 3, 2022).

APPENDIX F. ADDITIONAL CONFOUNDING CONSIDERATIONS

F.1. SPECIFIC PFAS CONFOUNDING CONSIDERATIONS FOR FETAL GROWTH RESTRICTION

As noted in the PFAS protocol, the potential for bias in effect estimates due to confounding is a source of uncertainty in epidemiological studies and was a focus during study evaluation and as part of the overall weight-of-evidence determination. Hemodynamic changes occur during pregnancy, such as increased blood plasma volume as a result of decreased mean arterial pressure, increased cardiac output, and systemic vasodilation (Sagiv et al., 2018; Sanghavi and Rutherford, 2014; Chapman et al., 1998). These changes could lead to lower PFAS levels in plasma, due to dilution and increased renal filtration. In line with this, several studies have noted decreasing serum or plasma concentrations for many slowly cleared PFAS during pregnancy, although this finding is not consistent for perfluorodecanoic acid (PFDA) (Oh et al., 2022a; Chen et al., 2021; Mamsen et al., 2019; Pan et al., 2017; Glynn et al., 2012). One study, however, noted an increase in serum concentration with pregnancy, suggesting either an increase in exposure to some PFAS during pregnancy or gestational changes in pharmacokinetics (e.g., decreased renal clearance) that are unique to this cohort (Taibl et al., 2023). These hemodynamic changes have been proposed as a potential confounder for associations between PFDA and neonatal and early childhood growth measures. This is suggested by the association between glomerular filtration rate (GFR), a marker of renal function and, indirectly, of plasma volume expansion, and fetal growth independent of gestational age and other maternal covariates (Morken et al., 2014; Gibson, 1973). Because PFDA concentration in serum is expected to decrease during pregnancy due to plasma volume expansion, increased renal excretion, and transplacental transfer, time windows earlier in pregnancy prior to this decrease may reflect the largest insult to a developing fetus. Potential confounding is one possible explanation for the effects of pregnancy hemodynamics, but in their meta-analysis of PFOA, Steenland et al. (2018) also proposed that GFR may lead to reverse causality if increased fetal growth leads to increased maternal blood expansion and glomerular filtration rate. This potential source of bias related to pregnancy hemodynamics is anticipated to be of greater concern when maternal serum PFAS samples are collected later in pregnancy. Therefore, as part of the study quality evaluations, more confidence was placed in studies that adjusted for different pregnancy hemodynamic markers or if they considered this potential source of confounding by sampling PFAS levels earlier in pregnancy. In an attempt to address this uncertainty, a few studies also adjusted for sample timing in their regression models. As noted in the syntheses, pattern analyses of study

results were also considered according to biomarker sampling timing to determine if pregnancy hemodynamics may be a source of between-study heterogeneity.

Only 1 of the 22 PFDA birth weight-related studies included in the discussion on developmental effects in Section 5.2.1 of the Toxicological Review collected and analyzed maternal hemodynamic data such as GFR and/or albumin (i.e., a marker of plasma volume expansion). Gyllenhammar et al. (2018) did not find any evidence of confounding following statistical adjustment of different GFR measures for any of the PFAS examined. Except for one study that showed some differences in PFOA results following adjustment for albumin, the Gyllenhammar et al. (2018) results are consistent with a lack of confounding demonstrated by either adjustment for albumin (Sagiv et al., 2018) or different GFR measures (Manzano-Salgado et al., 2017; Whitworth et al., 2012) for different PFAS examined in other studies. Nonetheless, existing meta-analyses for both PFOA (Steenland et al., 2018) and PFOS (Dzierlenga et al., 2020) only detected birth weight deficits for later trimester sampling (e.g., beyond the first trimester). A more recent meta-analysis of PFNA (Wright et al., 2023) reported nonmonotonic differences for early, late, and postpartum biomarkers, but these results were all statistically significant irrespective of sample timing windows examined. One limitation of the earlier two meta-analyses is they had no ability to differentiate late pregnancy from postpartum measures. Only 5 of the 22 PFDA studies of mean birth weight in the overall population examined any first trimester measures, which precluded a more detailed examination here. But there was some qualitative evidence of sample timing differences for the birth weight findings in the overall population. For example, 9 of the 11 studies reporting birth weight deficits had biomarkers based on later sampling during or after pregnancy. The patterns by sample timing were not consistent across endpoints, but the evidence of larger birth weight associations with later sample timing for PFDA measures may be indicative of potential impacts of pregnancy hemodynamics. However, the ability to more fully evaluate this was limited given the available data and disparate exposure measures, distributions, and contrasts examined across studies. Future research is needed to further elucidate these complexities, especially in studies with early samples and/or with repeated measures during different stages of pregnancy.

F.2. PFAS COEXPOSURE STATISTICAL APPROACHES AND CONFOUNDING DIRECTIONALITY

In general, an additional source of uncertainty in epidemiological studies is the potential for confounding by other PFAS (and other co-occurring contaminants). Although scientific consensus on how best to address PFAS coexposures remains elusive, this was considered in the study quality evaluations and as part of the overall weight-of-evidence determination. To be a confounder, the co-occurring PFAS would need to be associated with both the PFAS of interest and the outcome, but not an intermediate in the causal pathway; such PFAS would be considered positive confounders if their effect estimate with the endpoint of interest is in the same direction as the primary PFAS of

interest. If positive confounders are not accounted for, the anticipation is that any resultant bias would be away from the null.

A source of uncertainty in the epidemiological database was the potential for confounding by other PFAS (and other co-occurring contaminants) that co-occur and are actual confounders (i.e., associated with both the PFAS of interest and the outcome, but not an intermediate in the causal pathway between the two). In this example, such PFAS are considered positive confounders if their effect estimate with the endpoint of interest is in the same direction as the primary PFAS of interest. If positive confounders are not accounted for in the epidemiological study design or analysis phase, the anticipation is that any resultant bias would be away from the null. Certain statistical approaches can help address the challenges of evaluating the effects of numerous (often correlated) PFAS that may be present in the environment and estimated via different biomarkers and other measures. (i.e., those that adjust for at least one co-occurring exposure) can provide an estimate of the independent association for specific pollutants with the endpoint of interest. However, these models may not perform well when co-occurring exposures are highly correlated. Such correlation can lead to collinearity concerns and instability of modeling results. When exposures are highly correlated and additionally subject to different potential confounding factors (which may occur, e.g., when PFAS arise from different sources), coexposure amplification bias may be a concern (Weisskopf et al., 2018). Under this scenario, estimated associations from multi-PFAS adjusted models would be subject to greater bias compared with results from single-PFAS models.

Other mixture approaches are employed in epidemiological studies to characterize overall mixture effects and in some cases to "screen" large groups of exposures to identify exposure patterns and/or contributions, which may help determine which exposure(s) are most important to retain in further analyses. These statistical methods using dimension-reduction (e.g., principal component analysis, penalized modeling based on elastic net regression) and mixture methods (e.g., Bayesian kernel machine regression) are increasingly being used for identifying patterns among large groups of chemical exposures and help prioritize specific components/chemicals that contribute the highest proportion to the mixture. However, as noted by Meng et al. (2018), these approaches might be better suited as "prediction models to screen for a wide range of chemicals from different sources, and the interpretation of results might become less straightforward due to the necessary standardization of exposure values." These regression model outputs also do not provide confidence intervals, which precludes evaluations of precision. Given these interpretation difficulties and the potential for coexposure amplification bias, which statistical approach best represents independent effects of specific pollutants within complex PFAS mixtures is unclear. An evaluation of single-pollutant (i.e., PFDA alone) models and other approaches is detailed below.

The objective herein is to assess whether there is any direct evidence for confounding in the studies by comparing results from multipollutant (mutually adjusted for other PFAS) models and results from single-pollutant (i.e., PFDA alone with other confounders adjusted for) models. Additional objectives were to compare relationships between co-occurring PFAS and to evaluate

the extent to which these PFAS may be associated with a primary endpoint of interest (e.g., birth weight-related measures).

F.3. PFDA AND PFAS COEXPOSURE STUDY RESULTS

In general, the stronger an association between coexposures, and the larger the effect sizes seen for the coexposure of interest, the more concern there would be for potential confounding. Table F-1 shows correlations between PFAS coexposures and PFDA reported from five studies with mutually adjusted PFAS data, including four *medium* confidence (Meng et al., 2018; Woods et al., 2017; Lenters et al., 2016; Robledo et al., 2015), one *high* Luo et al. (2021), and one *low* confidence study (Starling et al., 2017). As shown in the PFAS Systematic Review Protocol (see Appendix A) and in Table F-1, PFNA and PFDA often co-occur (as expected given some similar anticipated sources) across studies with a consistent correlation of 0.6 or higher. These results also show that other PFAS may not consistently co-occur with PFDA, as the magnitude of these relationships can vary significantly across studies.

Table F-1. PFAS correlation coefficients in mutually adjusted studies

Reference	Study setting	Confidence	Correlations with PFDA			
			PFOS	PFOA	PFNA	PFHxS
Luo et al. (2021)	Guangzhou, China	High	**0.68**	**0.13**	**0.85**	**−0.03**
Woods et al. (2017)	Cincinnati, Ohio, USA	Medium	0.3	0.1	0.6	0.1
Lenters et al. (2016)	Greenland; Kharkiv, Ukraine; Warsaw, Poland	Medium	0.78	0.50	0.60	0.35
Meng et al. (2018)	Denmark	Medium	0.48	0.28	0.73	0.17
Robledo et al. (2015)	Michigan and Texas, USA	Medium	N/A	N/A	N/A	N/A
Starling et al. (2017)	Colorado, USA	Low	0.49	0.56	0.65	0.27

The results for the six studies based on continuous PFDA data (expressed as change in mean birth weight per unit change in exposure) are compared and summarized below in Table F-2. Three of the studies included multiple PFAS as predictors in ordinary least squares regression models (Meng et al., 2018; Woods et al., 2017; Robledo et al., 2015). Luo et al. (2021) examined multipollutant associations on the basis of nonparametric Bayesian kernel machine regression modeling. Two studies (Starling et al., 2017; Lenters et al., 2016) examined multiple PFAS using elastic net regression models. Elastic net regression is a modeling approach to select independent predictors (from an initial group of potentially correlated predictors) for inclusion in the model using penalized shrinkage methods (Lenters et al., 2016). As shown in Table F-2, two of the six studies (Luo et al., 2021; Lenters et al., 2016) reported inverse associations for birth weight and PFDA from single-pollutant models. However, PFDA was not associated with birth weight changes in multipollutant models for either study. For example, Lenters et al. (2016) reported null results

for PFDA in both their single-pollutant model and elastic net regression model, with only PFOA retained in the latter model. Starling et al. (2017) did not report birth weight deficits associated with PFDA on the basis of either single-pollutant or multipollutant models nor was PFDA selected for inclusion using elastic net regression. Meng et al. (2018) reported largely null results for PFDA in single-pollutant models but detected increases in mean birth weight with adjustment for PFOS, PFOA, PFNA, perfluoroheptane sulfonic acid (PFHpS), and PFHxS. Luo et al. (2021) reported large birth weight deficits (β = −97 g; 95% CI: −178, −16 per ln-unit PFDA increase) in single-pollutant PFDA model, but results were null in the multipollutant model. Lastly, Robledo et al. (2015) did not report results from single-pollutant models (or correlations) but in multipollutant models, they detected birth weight deficits associated with PFDA in female neonates only.

Given the moderate and strong correlations between PFDA and other PFAS, the magnitude of any associations that exist between these co-occurring PFAS and birth weight-related measures (and other developmental effects) may inform the potential for confounding of PFDA associations. For example, Lenters et al. (2016) reported birth weight deficits associated with increased levels of PFNA (β = −44.7 g; 95% CI: −92.0, 2.7 per 2SD ln-unit increase), PFOS (β = −68.8 g; 95% CI: −152.9, 15.2) and PFOA (β = −78.5 g; 95% CI: −137.0, −20.0) in single-pollutant models although only PFOA (β = −63.8 g; 95% CI: −122.8, −4.7) was retained in the elastic net regression model. Although birth weight deficits were not seen for PFDA in any of the regression models used by Starling et al. (2017), there were large mean birth weight deficits associated with increased exposure evaluated in single-pollutant models for both PFNA (β = −58 g; 95% CI: −104, −11 per ln-unit PFDA increase) and PFOA (β = −51 g; 95% CI: −97, −6). These deficits were larger in multipollutant models for both PFNA (β = −92 g; 95% CI: −167, −18) and PFOA (β = −70 g; 95% CI: −148, −9) but were attenuated when included in a penalized elastic net regression model (β = −33 g and −14 g, respectively). In the Woods et al. (2017) study, none of the five PFAS examined contributed greatly to the overall changes in mean birth weight when other environmental contaminants were considered in their elastic net model. Although Luo et al. (2021) reported birth weight deficits in single-pollutant models for several PFAS including PFDA and PFNA, only PFOS showed a large reduction based on Bayesian kernel machine regression results (β = −105.0 g; 95% CI: −209.4, −0.6 for Q4). Meng et al. (2018) reported similar deficits in birth weight associated with increased exposure to PFNA (β = −54.2 g; 95% CI: −105.8, −2.7 per $\log_2$-unit PFDA increase) and PFOS (β = −55.5 g; 95% CI: −145.6, 34.5) in their model containing mutually adjusted PFAS; however, effects were seen in the opposite direction (increase in mean birth weight) for PFDA (β = 48.0 g; 95% CI: −0.6, 96.5) and PFOA (β = 49.5 g; 95% CI: −8.7, 107.9) in the same model. Finally, Robledo et al. (2015) reported that only PFOA was associated with large deficits in mean birth weight (β = −61.6 g; 95% CI: −159.2, 35.9 per SD ln-unit increase) among girls in multipollutant models, while among boys deficits were only seen for perfluorooctane sulfonamide (PFOSA) (β = −104.2 g; 95% CI: −194.2, −14.3) and PFDA (β = −53.4 g; 95% CI: −161.0, 54.2).

In the six studies using mutually adjusted PFAS approaches to address coexposures, there was no consistent evidence for birth weight deficits associated with increased exposure to PFDA. Among the five studies that examined both single and multipollutant models, none of the studies that showed birth weight deficits in single-pollutant models reported greater or more precise associations following statistical adjustment for other PFAS. Of the three studies showing some adverse effects (Luo et al., 2021; Lenters et al., 2016; Robledo et al., 2015), only one (Robledo et al., 2015) showed deficits in multipollutant models and this was limited to females only. Among the three studies that provided correlations among co-occurring PFAS and showed some evidence of adverse effects for any PFAS, the largest birth weight deficits were seen for PFNA (Meng et al., 2018; Starling et al., 2017), PFOA (Robledo et al., 2015), and PFOS (Luo et al., 2021). The correlation coefficients for PFDA and these three coexposures across these studies were all at least 0.50.

As noted in Section 3.2.3 of the Toxicological Review, 11 of 22 studies showed evidence of some associations with PFDA and mean birth weight in the overall population. Among these 11 studies, which included the 3 highlighted above, 7 (Yao et al., 2021; Kashino et al., 2020; Wikström et al., 2020; Li et al., 2017b; Swedish Environmental Protection Agency, 2017; Valvi et al., 2017; Lenters et al., 2016) showed deficits comparable in magnitude for PFNA and PFDA. Two studies showed larger deficits for PFDA compared with PFNA (Kwon et al., 2016; Robledo et al., 2015), and two studies showed larger deficits for PFNA compared with PFDA (Luo et al., 2021; Workman et al., 2019). Given these comparable results seen in most of these studies for both PFNA and PFDA and the moderately high correlations consistently reported between PFDA and PFNA, there is considerable uncertainty due to potential confounding by co-occurring PFAS in the existing literature. It remains unclear, however, if the consistency of birth weight deficits demonstrated from (categorical and continuous) results in the full set of 22 mean birth weight PFDA studies could be fully attributed to confounding by PFAS coexposures.

Table F-2. Impact of coexposure adjustment on estimated change in mean birth weight per unit change (ng/mL) in PFDA levels[a]

Reference	Study confidence	Single-PFAS model results (in grams) with 95% CIs[a]	Multi-PFAS results (in grams) with 95% CIs[a]	Elastic net regression results	Exposure comparison[b]	Effect of adjustment on PFDA birth weight results	PFAS adjustments
Starling et al. (2017)	High	11.5 (−37.3, 60.4)	97.5 (31.5, 163.6)	15.7	ln-unit (ng/mL) increase	Slightly Strengthened	PFOS, PFOA, PFNA, PFHxS
Lenters et al. (2016)	Medium	−43.9 (−104.8, 17.0)	N/A	N/S	2 SD ln-unit (ng/mL) increase	Attenuated	PFOS, PFOA, PFNA, PFUnDA, PFDoDA, PFHxS

Luo et al. (2021)	High	−96.8 (−178.0, −15.5)	6.6 (−84.2, 97.3)[b]	N/A	ln-unit (ng/mL) increase	Attenuated	PFOA, PFOS, PFBA, PFBS, PFHxS, PFNA, PFUnDA, PFDoDA, PFTrDA, 6:2 Cl-PFESA, 8:2 Cl-PFESA
Meng et al. (2018)	Medium	−9.0 (−43.2, 35.2)	48.0 (−0.6, 96.5)	N/A	log_2-unit (ng/mL) increase	Changed from Null to Positive	PFOS, PFOA, PFNA, PFHxS, PFHpS
Robledo et al. (2015)	Medium	N/A	−53.4 (−161.0, 54.2) Girls −1.8 (−90.6, 87.1) Boys[c]	N/A	1 SD ln-unit (ng/mL) increase	N/A	PFOA, PFOS, PFNA, PFOSA, Et-PFOSA-AcOH, Me-PFOSA-AcOH
Woods et al. (2017)	Medium	−12.6 (−56.8, 40.4)[d]	N/A	N/S	log_{10}-unit (ng/mL) increase	Attenuated	PFOS, PFOA, PFNA, PFUnDA, PFDoDA, PFHxS

N/A: not available; N/S: PFAS not selected in elastic net regression model.

[a]Models were based on ordinary least squares regression.

[b]Beta and 95% CIs estimated from Figure 3 of (Luo et al., 2021).

[c]The birth weight results tabulated here are all for the overall population (i.e., male, and female neonates combined), except for Robledo, which only reported sex-specific findings.

[d]The posterior 95% credible intervals reported for Woods et al. (2017) are based on a Bayesian hierarchical linear model.

APPENDIX G. DETAILED PHARMACOKINETIC ANALYSES

This appendix provides two detailed pharmacokinetic analyses. The first is a Bayesian analysis of perfluorodecanoic acid (PFDA) pharmacokinetics in laboratory animals to estimate key pharmacokinetic (PK) parameters. The second is the description and evaluation of a one-compartment PK modeling approach for estimating internal doses, evaluated against rat PFDA PK data using the mean parameter values estimated for male rats in the Bayesian estimation.

G.1. PARTIAL POOLING OF PFDA PHARMACOKINETIC DATA FOR HIERARCHICAL BAYESIAN ANALYSIS

We estimated the sex-specific pharmacokinetic parameters (half-life, volume of distribution, and clearance) of PFDA in rats by fitting one- and two-compartment models to the available concentration-versus-time data. A Bayesian hierarchical methodology was developed to fit these models because of the need to pool time-course concentration data across numerous studies with varying exposure scenarios within each study. This allowed for each concentration-versus-time dataset to be fit to each pharmacokinetic model for which fitted parameters for each dataset are sampled from a population-level distribution, which models the similarities between each dataset. In addition, the Bayesian analysis allowed for the generation of central estimates and credible intervals for the PK parameter of interest, e.g., half-life, volume of distribution and clearance, using posterior distributions from the estimated variables. Finally, the Bayesian methodology allowed for hypothesis testing of the one- and two-compartment formulations to decide which model more appropriately fit the data.

G.1.1. Pharmacokinetic Model

To determine pharmacokinetic parameters for PFDA, we estimated constants for both one- and two-compartment model assumptions. For a one-compartment model assumption, the following exponential decay functions were fit to the available data:

$$C_{1-cmpt}^{IV}(t) = \frac{D}{V} e^{-k_e t} \quad \text{(G-1)}$$

$$C_{1-cmpt}^{oral}(t) = \frac{D}{V}\left(\frac{k_a}{k_a - k_e}\right)\left(e^{-k_e t} - e^{-k_a t}\right) \quad \text{(G-2)}$$

where D represents the administered dose and V, k_e, and k_a represent the central compartment volume, elimination constant, and absorption constant (for oral only) to be fit. From these fitted constants, pharmacokinetic parameters are derived:

$$V_d = \frac{V}{BW} \quad \text{(G-3)}$$

$$t_{½} = \frac{\ln 2}{k_e} \quad \text{(G-4)}$$

$$CLC = V_d * k_e \quad \text{(G-5)}$$

where V_d, $t_{1/2}$, and CLC represent the volume of distribution, terminal half-life, and clearance, respectively, and BW represents the animal body weight.

For the two-compartment model assumption, the following exponential decay functions were fit to available data

$$A^{IV} = \frac{\alpha - k_{dc}}{\alpha - \beta};\ A^{oral} = k_a\left(\frac{k_{dc} - \alpha}{(k_a - \alpha)(\beta - \alpha)}\right) \quad \text{(G-6)}$$

$$B^{IV} = \frac{\beta - k_{dc}}{\beta - \alpha};\ B^{oral} = k_a\left(\frac{k_{dc} - \beta}{(k_a - \beta)(\alpha - \beta)}\right) \quad \text{(G-7)}$$

$$C^{IV}_{2-cmpt}(t) = \frac{D}{V}\left(A^{IV}e^{-\alpha t} + B^{IV}e^{-\beta t}\right) \quad \text{(G-8)}$$

$$C^{oral}_{2-cmpt}(t) = \frac{D}{V}\left(A^{oral}e^{-\alpha t} + B^{oral}e^{-\beta t} - \left(A^{oral} + B^{oral}\right)e^{-k_a t}\right) \quad \text{(G-9)}$$

where D represents the administered dose and V, α, β, k_{dc}, and k_a represent central compartment volume, alpha-phase elimination constant, beta-phase elimination constant, deep-to-central compartment rate constant, and absorption constant (for oral only) to be fit. From these fitted constants, the remaining two-compartment constants (k_{cd}: central-to-deep compartment rate constant and k_e: elimination constant) and the deep compartment volume (V_{deep}) are derived by solving:

$$\alpha + \beta = k_{cd} + k_{dc} + k_e \quad \text{(G-10)}$$

$$\alpha * \beta = k_{dc} * k_e \quad \text{(G-11)}$$

$$V_d = V\frac{k_{cd}}{k_{dc}} \quad \text{(G-12)}$$

which allows for the desired pharmacokinetic parameters to be derived using the following equations:

$$V_{d-ss} = \frac{V + V_{deep}}{BW} = \frac{V}{BW}\left(\frac{k_{cd} + k_{dc}}{k_{dc}}\right) \quad \text{(G-13)}$$

$$t_{½} = \frac{\ln 2}{\beta} \quad \text{(G-14)}$$

$$CLC = \frac{V}{BW} * k_e \quad \text{(G-15)}$$

where V_{d-ss}, $t_{1/2}$, and CLC represent the steady-state volume of distribution, terminal half-life, and clearance, respectively, and BW represents the animal body weight.

G.1.2. Bayesian Inference

The fitted constants for each model structure (described above) were estimated using available time-course concentration data reported in rats with parameters for each model estimated using a hierarchical Bayesian calibration approach. This hierarchical Bayesian approach

pooled the time-course concentration data for male and female rats from multiple studies Ohmori et al. (2003), Kim et al. (2019), Dzierlenga et al. (2019). For the two-compartment model, to ensure parameter identifiability, α and β were constrained to be ordered such that $\alpha > \beta$. This constraint ensures the exponential terms are identifiable and do not "flip" while exploring the parameter space during Markov-chain Monte-Carlo (MCMC) sampling. Finally, priors for each pharmacokinetic parameter were chosen to be "weakly informative" on the basis of prior knowledge of PFAS pharmacokinetics (ATSDR, 2021) with 95% equal-tailed intervals spanning multiple orders of magnitude.

Priors for pharmacokinetic parameters are presented in Table G-1 with corresponding model-specific parameter prior distributions presented below. Finally, a sensitivity analysis on the model priors is shown in Section G.1.3.

Table G-1. Weakly informed prior distributions for pharmacokinetic parameters used in the Bayesian analysis

	median	mad	eti_3%	eti_97%
Half-life (d)	15	12	0.88	250
Clearance (mL/kg-d)	50	49	0.32	6,000
V_{d-ss} (mL/kg)	900	811	9.3	32,822

For the hierarchical approach, the concentration-versus-time data comprised a population level and dataset level for which model parameters were estimated. Here, each dataset represented each study/sex/dose concentration-versus-time dataset extracted from the literature and were fit using the model.

$$C_{ij} = \begin{cases} C_{1-cmpt}^{route} & \text{for 1-compartment model,} \\ C_{2-cmpt}^{route} & \text{for 2-compartment model} \end{cases} \quad \text{(G-16)}$$

$$C_{ik} \sim LN(\bar{x}_{ij}, \tilde{\sigma}_k) \quad \text{(G-17)}$$

where $\bar{x}_{ij}$ is the sample mean of the observed concentrations at time t_{ij} for dataset j, and $\tilde{\sigma}_k$ is the study-level log-transformed standard deviation for the relative errors based on study k. Study-level priors for $\tilde{\sigma}_k$ were determined using the average log-transformed standard deviations.

$$\bar{\sigma}_{i,j}^2 = \ln\left(1 + \frac{s_{i,j}^2}{\bar{x}_{i,j}^2}\right) \quad \text{(G-18)}$$

$$\gamma_k = \frac{\sum_i \bar{\sigma}_{i,j \in k}}{n_k} \quad \text{(G-19)}$$

where $s_{i,j}$ is the sample standard deviation on the observed concentrations at time $t_{i,j}$ for study k. If s_{ij} was available, $\bar{\sigma}_{i,j}$ is the log-transformed standard deviation using the sample mean and standard deviation. For studies from which sample standard deviations could not be extracted, an

average of all log-transformed standard deviations was used. This allowed for study-level prior distributions on the error model log-transformed standard deviation:

$$\tilde{\sigma}_k \sim \begin{cases} \text{Exp}(1/\gamma_k) & \text{if } \gamma_k \text{ available,} \\ \text{Exp}(1/\gamma) & \text{otherwise.} \end{cases} \quad \text{(G-20)}$$

Using this model, dataset-level fitted constants were assigned priors on the basis of a noncentered parameterization of a population-level distribution. This reparameterization of a typical hierarchical Bayesian model allows for increased sampling efficiency and can be more efficient for sampling when there is limited data (Betancourt and Girolami, 2013). Finally, nonelimination rate constants (k_a and k_{dc}) were assigned a unit normal, weakly informative prior to aid parameter identifiability (Gelman et al., 2015).

$$\ln \mu_{k_a} \sim N(0,1) \quad \text{(G-21)}$$
$$\ln \mu_V \sim N(0,1) \quad \text{(G-22)}$$
$$\ln \mu_{k_e} \sim N(-3,1.5) \; one\ compartment\ model \quad \text{(G-23)}$$
$$\ln \mu_{k_{dc}} \sim N(0,1) \; two\ compartment\ model \quad \text{(G-24)}$$
$$\ln \mu_{\alpha,\beta} \sim N(-3,1.5), \mu_\beta < \mu_\alpha \; two\ compartment\ model \quad \text{(G-25)}$$
$$\sigma_{k_a,V,k_e,\alpha,\beta,k_{dc}} \sim \text{Exp}(3) \quad \text{(G-26)}$$
$$\ln(k_a, V, k_e, \alpha, \beta, k_{dc})_j \sim N\left(\mu_{k_a,V,k_e,\alpha,\beta,k_{dc}}, \sigma_{k_a,V,k_e,\alpha,\beta,k_{dc}}\right) \quad \text{(G-27)}$$

One- and two-compartment model goodness of fits were compared using the widely applicable information criteria (WAIC). Pharmacokinetic parameters from the most appropriate model, as judged by the WAIC comparison, were reported. To estimate the population-level pharmacokinetic parameters, we examined posterior probability densities of the parameters from the WAIC-determined model and calculated distributional estimates of the half-life, volume of distribution, and clearance using the equations described above. The parameter space was sampled using PyMC (Salvatier et al., 2016) using four independent Markov chains run for 10,000 iterations per chain. Posterior parameter distributions were determined using the final 5,000 iterations of each chain ensuring an effective sample size (ESS) greater than 10,000 (Kruschke, 2021). Convergence was assessed using a potential scale reduction factor with a maximum threshold of $\hat{R} = 1.05$ (Kruschke, 2021).

G.1.3. Prior Sensitivity Analysis

To investigate the impact of prior selection on posterior pharmacokinetic parameter estimation, we conducted a sensitivity analysis on the priors used in the Bayesian analysis. Priors were classified into three categories: weakly informed, broad, and uninformed. Weakly informed priors are defined using the half-life, clearance, and volume of distribution, described above on the basis of reported ranges of PFDA pharmacokinetics with a prior predictive check demonstrating available data for fitting fall within the prior 90% credible interval (see Figure G-1).

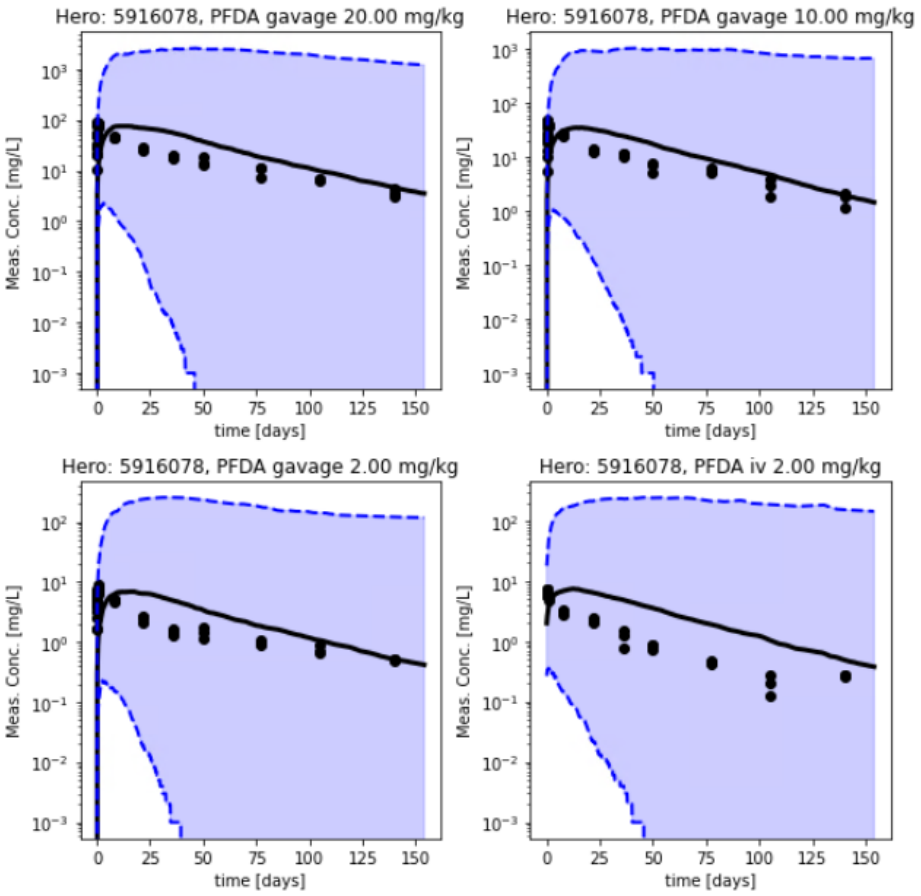


Figure G-1. Prior predictive check to ensure equal-tailed interval from prior distributions encompass the available time-course concentration data for fitting.

In addition to these weakly informed priors, we also characterized a set of broad priors, defined as uniform distributions spanning the 3% and 97% equal tailed interval (ETI, analogous to corresponding percentiles) from the weakly informed priors, and completely uninformed priors, representing uniform priors spanning multiple orders of magnitude (i.e., flat priors). Figure G-2 (prior sensitivity) compares these three classes of priors and their impact on the posterior pharmacokinetic parameter distributions,

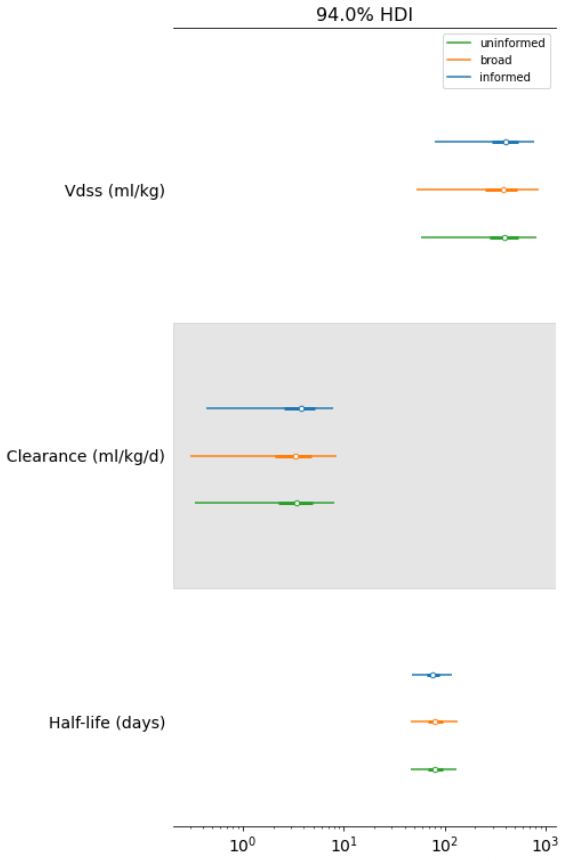


Figure G-2. Prior sensitivity on half-life, steady-state volume of distribution, and clearance to ensure weakly informed priors do not bias posterior distributions of the pharmacokinetic parameters.

Based on these findings, we used the weakly informed pharmacokinetic priors for fitting available time-course concentration data.

G.1.4. Study-Specific Clearance Values and Model Fits

Three datasets were used for the sex-specific parameter estimation, which had a mixture of gavage and i.v. exposure routes and follow-up times extending up to 150 days (Dzierlenga et al., 2019; Kim et al., 2019; Ohmori et al., 2003). The sex-specific clearance value distribution obtained from fitting the three datasets together had a mean and 90% credible interval of 4.06 (2.05–6.05) mL/kg-day in female rats and 4.14 (0.68–7.02) mL/kg-day in male rats. For these data, a two-compartment PK model was deemed superior. Visual inspection shows some of the data have a distinguishable distribution and excretion phase, which is appropriate for a two-compartment model (see Figure G-3). A two-compartment model can fit data that appear linear as is evidenced in fits to other datasets (see Figure G-4). Credible intervals for the fits to individual datasets are qualitatively small showing good model fits to the data from individual studies. The relatively large credible interval for the pooled data is due to the large variation between studies. For example, in

male rats the mean clearance values for individual studies ranged from 1.51 to 7.45 mL/kg-day, with a similar range in female rats.

Trends comparing the terminal clearance following i.v. and gavage doses appeared within studies but did not hold for the whole dataset. For example, in Kim et al. (2019) i.v. doses resulted in smaller, but similar clearance to gavage doses (see Figure G-4). However, these clearance values were consistently smaller than clearance values calculated from the two other datasets. In the analysis of the Dzierlenga et al. (2019) dataset, i.v. doses resulted in clearly greater clearance than the three dose levels administered by gavage, which all had similar clearance within each sex (see Figures G-5 and G-6). There was a difference in clearance between sexes in this study, but only for gavage doses. In this study, the gavage doses resulted in mean clearance values between 3.57 and 3.77 mL/kg-day in female rats and 5.12 and 5.74 mL/kg-day in male rats. However, the clearance calculated from the single i.v. dose was similar between female and male rats. Likewise, the two other studies showed similar mean clearance values for male and female rats (see Figure G-3 and Figure G-4). That most of the difference in PFDA PK between male and female rats is related to a difference in absorption, which can be moderated by active transport, is possible. Additional experiments designed to carefully evaluate these factors would be needed to resolve this question.

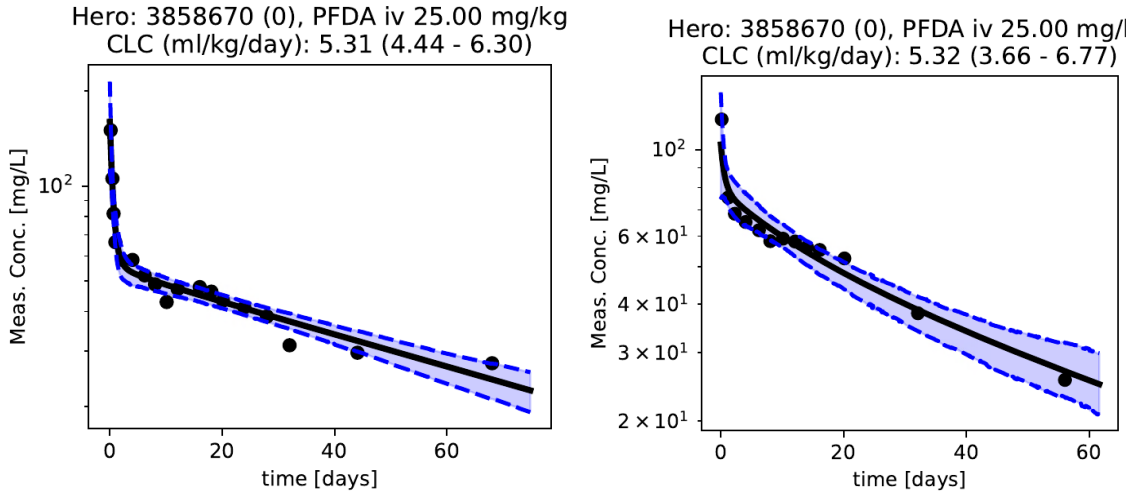


Figure G-3. Predicted (black line with blue 90% credible interval) and observed (black circles) serum time-courses for female (left) and male (right) rats after a 25 mg/kg i.v. bolus of PFDA. Observed data from (**Ohmori et al., 2003**).

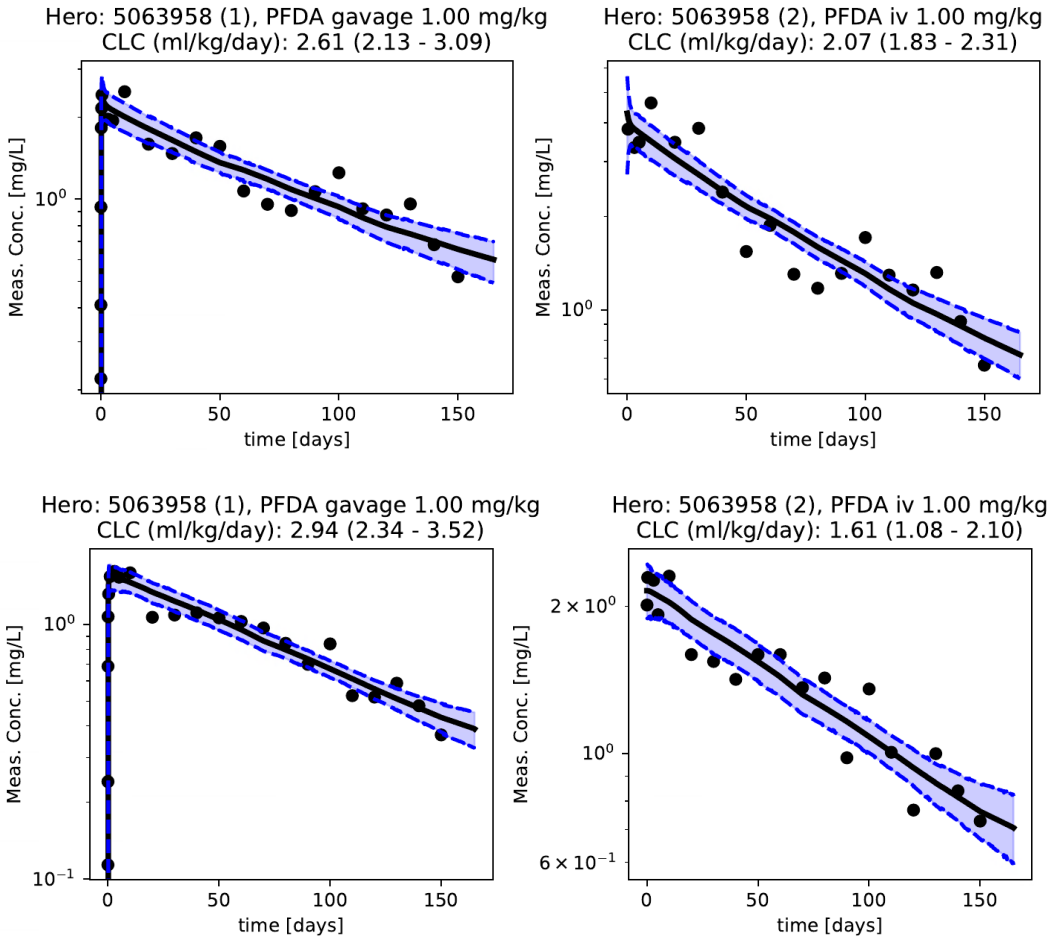


Figure G-4. Predicted (black line with blue 90% credible interval) and observed (black circles) serum time-courses for female (top 2 panels) and male (bottom 2 panels) rats after a 1 mg/kg gavage or i.v. bolus of PFDA. Gavage exposures are on the left, and i.v. exposures are on the right. Observed data from (**Kim et al., 2019**).

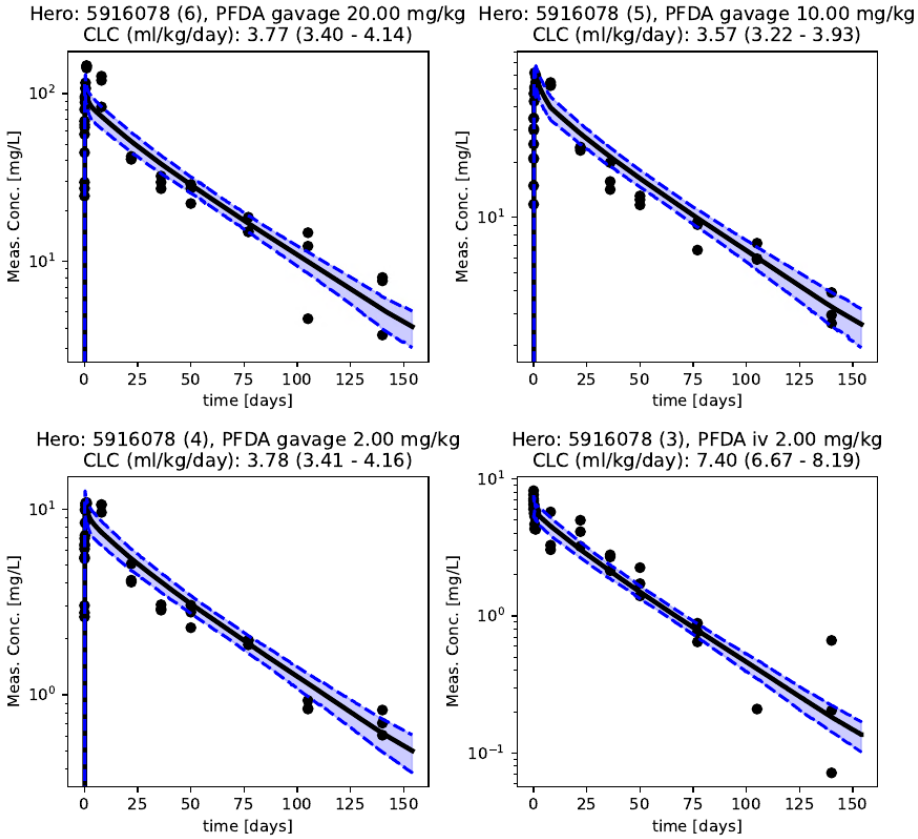


Figure G-5. Predicted (black line with blue 90% credible interval) and observed (black circles) serum time-courses for female rats after a 2 mg/kg i.v. or 2, 10, or 20 mg/kg gavage bolus of PFDA. Observed data from (**Dzierlenga et al., 2019**).

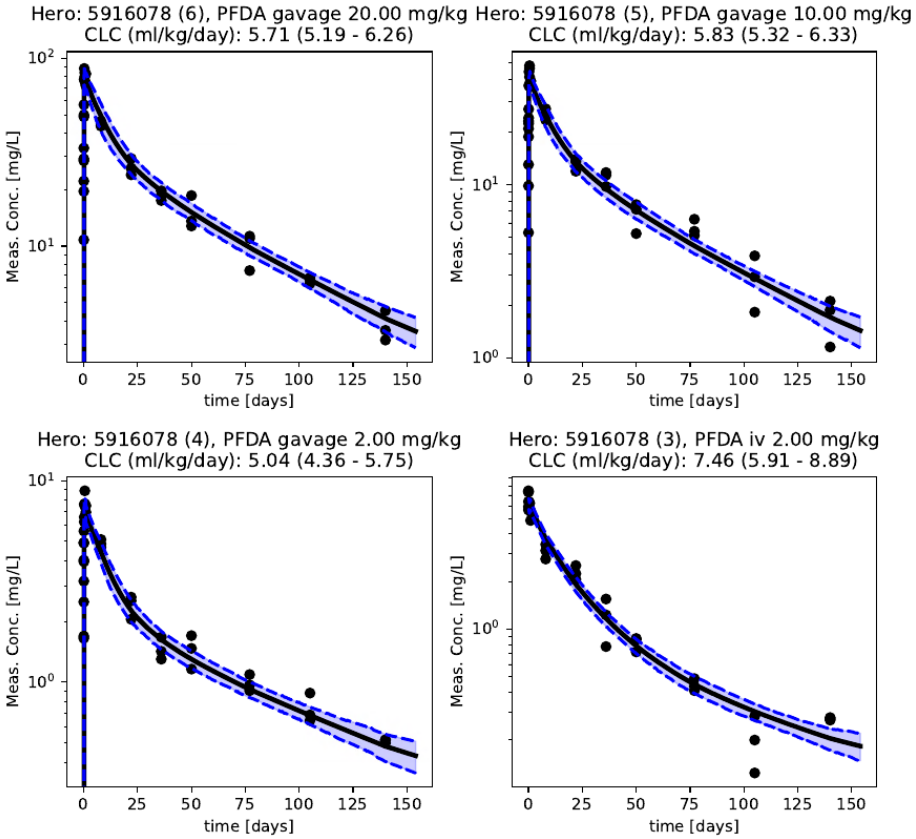


Figure G-6. Predicted (black line with blue 90% credible interval) and observed (black circles) serum time-courses for male rats after a 2 mg/kg i.v. or 2, 10, or 20 mg/kg gavage bolus of PFDA. Observed data from (**Dzierlenga et al., 2019**).

G.2. DESCRIPTION AND EVALUATION OF A TWO-COMPARTMENT PK APPROACH FOR ESTIMATION OF INTERNAL DOSES IN RATS

For PFDA, the clearance values obtained in the preceding Bayesian analysis are low enough that internal doses will not reach steady-state for shorter-term studies, in particular for the rat NTP 28-day bioassay. In this case a PK model could be used to account for the growth of the animal, the intrinsic elimination, and the accumulation of PFDA over the period of dosing. The two-compartment PK model is given by:

$$dA_c/dt = F_{abs} \times dose \times BW - CL_{tot} \times A_c/V_c - k_{12} \times A_c + k_{21} \times A_t \text{ and} \quad \text{(G-29)}$$

$$dA_t/dt = k_{12} \times A_c - k_{21} \times A_t, \quad \text{(G-30)}$$

where A_C and A_t are the total amounts of PFDA in the animal's central (c) and tissue (t) compartments (mg), F_{abs} is the fraction absorbed for an oral dose (bioavailability), BW is the body weight (kg), CL_{tot} is the total clearance (L/kg-d), V_c is the volume of distribution for the central compartment (L/kg), and k_{12} and k_{21} are the rate constants for transfer from the central to tissue

and tissue to central compartments, respectively (d^{-1}). The concentration in plasma (central compartment) is:

$$C_{plasma} = A_c/(V_c \times BW). \quad \text{(G-31)}$$

The differential equation for the amount of chemical in the central compartment can then be rewritten:

$$dA_c/dt = F_{abs} \times dose \times BW - CL_{tot} \times BW \times C_{plasma} - k_{12} \times A_c + k_{21} \times A_t, \quad \text{(G-3)}$$

which leads to the interpretation that the volume of plasma cleared of the chemical per unit time per kg BW is CL_{tot}.

Computational model code to implement this PK model for the specific analyses described below is available from (Schlosser, 2024).

PK parameters for male and female rats (F_{abs}, CL_{tot}, k_{12}, k_{21}, and V_c) were taken from the preceding Bayesian analysis. Specifically, samples containing 1000 parameter sets from the posterior distribution of the Bayesian analysis for the population-level parameters for male and female rats were used for subsequent analysis. The algebraic mean of each parameter from the sex-specific sample was calculated independently from each sample to generate model predictions for an average male or female rat, respectively. Given the slow clearance of PFDA, the growth of rats during toxicity studies lasting multiple weeks can be a significant factor as increases in BW dilute the body burden from earlier exposures. The highest doses tested in the NTP bioassay significantly reduced animal BW, which compounds this effect. Therefore, time-dependence in BW based on the empirical data for BW at the doses evaluated was incorporated into the model evaluation, to account for this time dependence and dose dependence. For illustration, the change in male rat BW observed in the NTP bioassay (28-day exposure (NTP, 2018)) is shown in Figure G-7. Doses of 0.625 mg/kg-day and below did not significantly affect BW gain during the bioassay, but higher dose levels caused a significant decline after 7 days of exposure.

G.2.1. Evaluation of the Two-Compartment PK Model Predictions for Rats

The internal dose of PFDA predicted by the PK model as a function of exposure day, normalized to the dose for comparison, is shown in Figure G-8. For example, the model simulated concentrations obtained using a dose of 0.625 mg/kg-day were divided by 0.625 before plotting. If the BW curve was the same for all doses, all the resulting normalized curves would be superimposed. The predicted concentration increases steadily throughout the study for all dose levels, showing no sign of saturation. However, the increase in animals receiving the highest doses becomes relatively faster after day 7, deflecting above the lower-dose curves. This occurs because the decreasing BW at these doses concentrates the PFDA already administered into a smaller total animal mass. For model simulations, the dose is assumed to be adjusted continuously on the basis of the time-interpolated weights as shown in Figure G-7. (The study report states that animals were

weighed daily, but only weekly values are provided there.) For example, if an animal loses weight between day 7 and day 21, the daily dose is assumed to be adjusted accordingly. Since the animals were necropsied on day 29, 1 day after the final dose, the model simulations include a final day with zero exposure. Mean (±SD) serum PFDA concentrations from the NTP study, collected at time of necropsy, are shown for comparison.

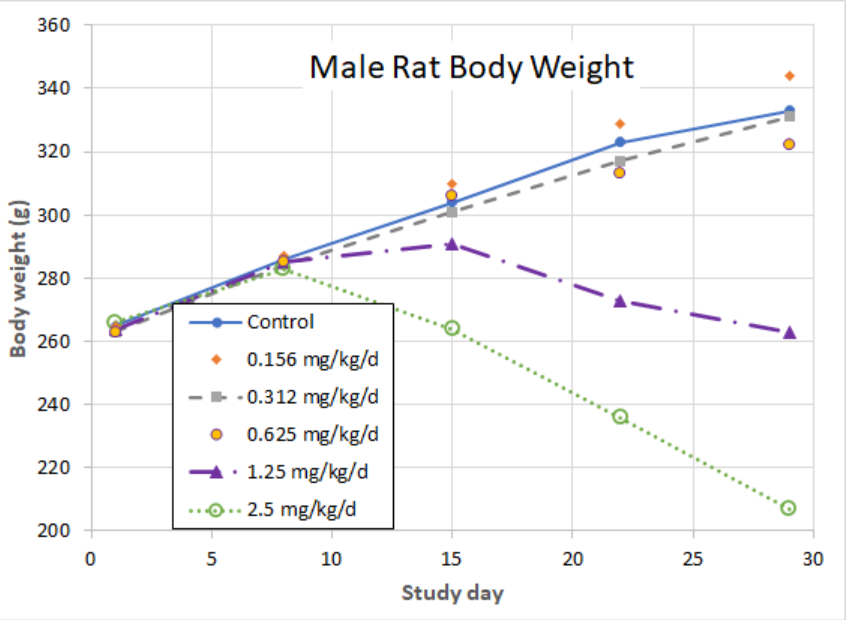


Figure G-7. Male rat body weight changes during 28-day PFDA bioassay (NTP, 2018). Datasets are identified by the dose (mg/kg-d).

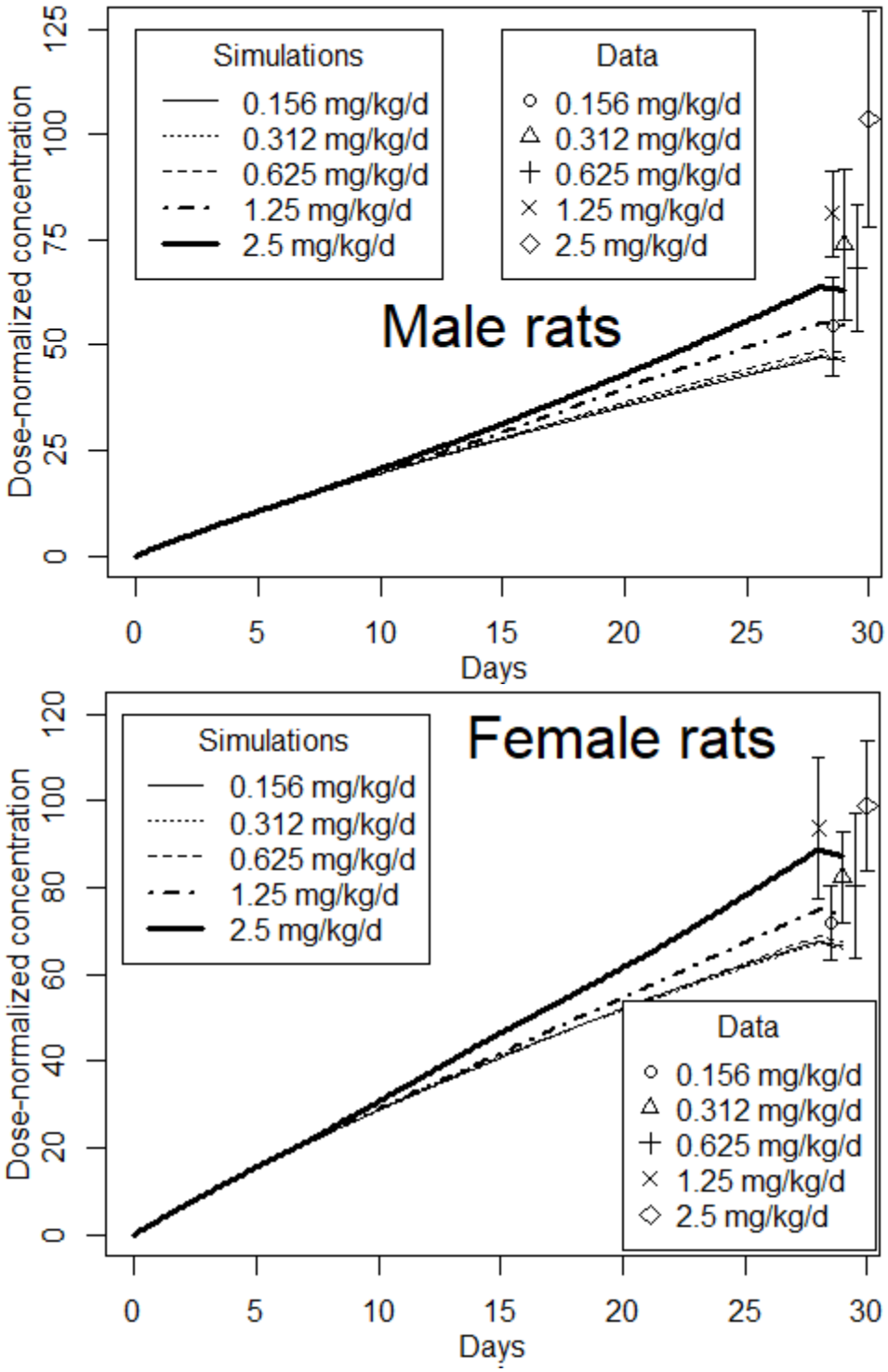


Figure G-8. Predicted accumulation and observed end-of-study of PFDA in male and female rats in the NTP bioassay (NTP, 2018) as a function of study day. Predicted and measured concentrations (mg/L) were normalized to respective doses (mg/kg-d). Data are mean ± SD, jiggered from day 29 (when samples were collected) to make error bars distinct.

In Figure G-8 the model consistently underpredicts the male rat data by 15%–40% and, while the prediction for the lowest dose is within 1 SD of the mean, the predictions for higher doses are 30%–40% below the mean with more than 1SD difference. The results for female rats are considerably better, no more than 22% below the mean measured levels and three of the five predictions are within 1 SD. While EPA generally considers this much discrepancy acceptable for a comparison of PK model predictions to data, that there is systematic bias rather than some predictions being above and some below the data raises concern. One might also note the data point for 0.625 mg/kg-day is less than that for 0.312 mg/kg-day, whereas the model simulations show only increasing normalized concentration with dose. For male rats there is a greater overall effect of dose in the data (distance between lowest and highest point) than that predicted by the model, although for female rats the model performs well in this regard. To further evaluate the extent of nonlinearity, the end-of-study plasma concentrations from NTP (2018), PK-model-estimated concentrations, and the estimated steady-state concentration (dose/clearance) are plotted against the dose in Figure G-9. The exposure-dose relationship is essentially linear for the three lowest doses (to 0.625 mg/kg-day), with some variation, and then increases a bit more rapidly than linearly with dose above that. As indicated by the BW data in Figure G-7 and resulting simulations in Figure G-8, this upward inflection could be due to dose-related BW losses, which are assumed to concentrate the previously administered PFDA into a smaller total volume. While this assumption is incorporated into PK model, the model systematically underpredicts the mean concentration at the end of the study, even at the lowest dose. This under-prediction of the PK data suggests that a secondary factor, not incorporated into the model, is involved. The PK model also incorporates the assumption that the distribution of PFDA to body tissues (ratio of amount in tissues vs. serum) is constant, but the data suggest less distribution at the end of the study than estimated from single-dose PK studies. If the growing rats, especially the males, have a disproportionate increase in volumes of tissues in which PFDA distributes poorly, such as fat, the overall volume of distribution will decrease over time, resulting in higher than predicted serum levels. This type of shift would require a valid PBPK model in which growth of specific tissue types is not assumed to be directly proportional to total body weight.

Also, from Figure G-8, there is no evidence of saturation of renal resorption, which would result in downward curvature in the exposure-dose relationship (at higher doses). Instead, the discrepancy between the NTP data and the model simulations can be explained if clearance is somewhat lower than estimated from the PK studies or as suggested above, the volume of distribution decreases with growth of the rats over the 28 days.

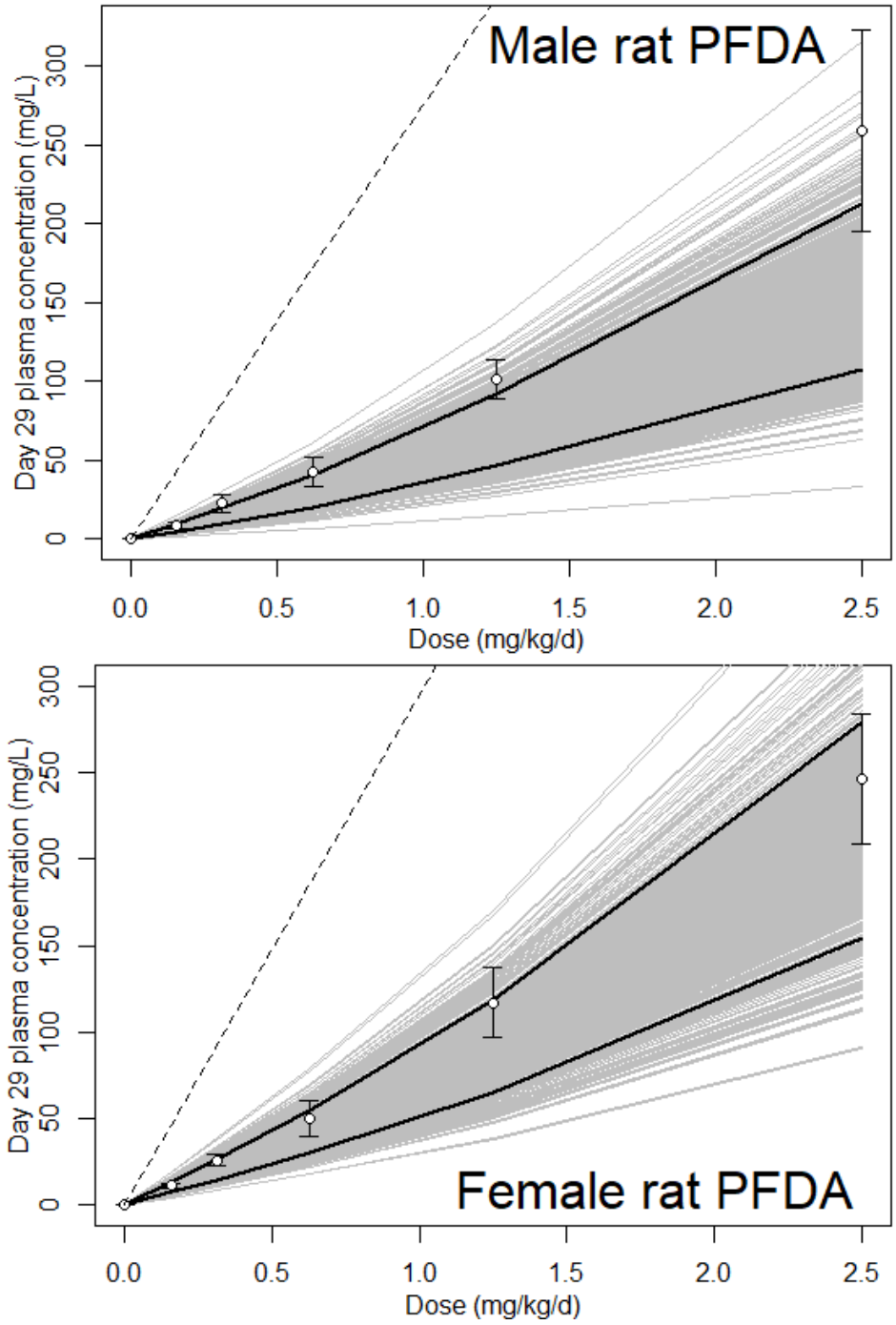


Figure G-9. Measured end-of-study of PFDA in male and female rats in the NTP bioassay (NTP, 2018) as a function of dose versus model predictions. Points are mean ± SD serum concentrations measured at the end of the bioassay (NTP, 2018). Gray lines are results from the PK model using 1,000 posterior parameter samples from the Bayesian analysis (see Appendix G.1). Solid black lines are 5th and 95th percentiles of the samples. Dashed line is the steady-state serum concentration, i.e., dose/clearance.

G.2.2. Alternate Approach for PFDA PK Evaluation of Rats – Data Interpolation

As shown in Figures G-8 and G-9 and discussed above, the two-compartment PK model does a generally good job of predicting the serum concentrations observed in the NTP bioassay but has a systematic bias, consistently underpredicting the observed mean concentrations. However, the qualitative shape of the model prediction suggests an alternative approach for estimating the average concentrations from the measured serum concentrations. In particular, due to the low clearance in both male and female rats, the PK model predicts an almost linear increase in serum concentration with time during the bioassay and that the observed concentrations could be accurately predicted with only a small change in the parameters. One can expect, then, a more accurate model would also predict an essentially linear increase in concentration with time, but one that increases with a slope sufficient to match the observed means. If in fact the serum concentration increases linearly over the study duration, the average concentration during the study will simply be 50% of the final observed concentration. Further, given the near linearity in the exposure-dose relationship (see Figure G-9), one could reasonably estimate the final concentration for other doses (e.g., PODs identified from EPA's dose-response analyses) by linear interpolation between the two nearest reported concentrations. The resulting values are informed by the PK model results while using the observed concentrations, which likely reflect mechanisms not included in the PK model.

While Frawley et al. (2018) did not measure and report end-of-study serum concentrations from their toxicity study, they used the same strain of rats, study duration, and dosing and sacrifice schedule as (NTP, 2018). Therefore, the average serum levels will be imputed from the NTP end-of-study serum concentrations in the same way, using the serum concentrations reported in (NTP, 2018).

A computational script implement that performs the internal dose interpolation described here is contained in the model package available from (Schlosser, 2024).

G.2.3. PK Evaluation for Gestational Exposure to PFDA in Mice

A toxicity POD was also determined for decreased fetal body weight in mice, reported by Harris and Birnbaum (1989). However, PFDA serum concentrations were not measured as part of this study so interpolation such as was done with the rat NTP data is not possible. Further, no gestational PK data are available for PFDA in mice or rats that might otherwise be used to evaluate model extrapolation to that lifestage, leading to high uncertainty for use of the PK model. Also noted is that the POD for this endpoint, 1 mg/kg-d, is high enough that the result is not a sensitive or critical observation. Therefore, the POD is extrapolated to humans using the data-derived extrapolation factor (DDEF) approach, i.e., $POD_{HED} = POD_A \times (F_{abs,A}/F_{abs,H}) \times CL_H/CL_A$. This follows from the PK prediction that given exposure to POD_A, the serum concentration in the animal at steady-state is $C_{S,A} = POD_A \times F_{abs,A}/CL_A$ and the human equivalent dose occurs when $C_{S,H} = POD_{HED} \times F_{abs,H}/CL_H = C_{S,A}$, i.e., when humans have the same serum concentration as the animal.

Hence, $POD_{HED} = C_{S,A} \times CL_H/F_{abs,H} = (POD_A \times F_{abs,A}/CL_A) \times CL_H$. In this case, CL_A is set to the CL for female mice (2.2 mL/kg-d, (Fujii et al., 2015)), because dosimetry in the dam is what determines exposure to the mouse fetus. As discussed in Section 3.1.1, F_{abs} is estimated to be 100% for mice from the available data and is assumed to be 100% in humans given the lack of data to the contrary. In conjunction with UF_H = 0.147 mL/kg-d (see Table 3-3), the resulting POD_{HED} = (1 mg/kg-d)*(0.147 mL/kg-d)/(2.2 mL/kg-d) = 0.067 mg/kg-d.

APPENDIX H. SUMMARY OF PUBLIC AND EXTERNAL PEER-REVIEW COMMENTS AND EPA'S DISPOSITION

The Toxicological Review has undergone a formal external peer review in accordance with U.S. Environmental Protection Agency (EPA) guidance on peer review (U.S. EPA, 2015). A public, external peer-review meeting was held July 10, 11, and 13, 2023, which included an opportunity for public comment. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment approach, key conclusions, and areas of scientific controversy or uncertainty. Prior to the external peer review, the Toxicological Review of Perfluorodecanoic Acid and Related Salts was released for public comment (in April 2023). Public comments on the assessment were submitted to the U.S. Environmental Protection Agency (EPA) by June 9, 2023.

Comments made by the external peer reviewers and public commenters, as well as EPA's responses to these comments, are arranged by charge question below. In addition, each charge question includes an overarching summary of the peer-review panel's comments, drawing from the summary language provided in Section 2 of the final peer-review report. In many cases, the comments of the individual reviewers have been synthesized and paraphrased for brevity but when feasible this appendix uses direct language from the external peer-review report and public comments (please consult the final peer-review report for the full text of the panel's comments and consult the EPA docket for the full text of the public comments received: EPA-HQ-ORD-2019-0287). external peer reviewers were asked to prioritize their comments to indicate their relative importance as follows. The prioritization instructions are duplicated below from the IRIS PFDA charge questions to the peer reviewers, which can be found in the public EPA docket (EPA-HQ-ORD-2019-0287):

- **Tier 1: Necessary Revisions –** Use this category for any revisions you believe are necessary to adequately support and substantiate the analyses or scientific basis for the assessment conclusions, or to improve the clarity of the presentation in the PFDA Toxicological Review.

- **Tier 2: Suggested Revisions –** Use this category for any revisions you encourage EPA to implement to strengthen the analyses or scientific basis for the assessment conclusions, or to improve the clarity of the presentation in the PFDA Toxicological Review.

- **Tier 3: Future Considerations –** Use this category for any advice you have for scientific exploration that might inform future work. While these recommendations are generally

outside the immediate scope or needs of the PFDA Toxicological Review, they could inform future reviews or research efforts.

Appendix H lists all Tier 1 Recommendations and Tier 2 Suggestions from the external peer reviewers, organized by charge question. Tier 1 Recommendations and Tier 2 Suggestions were considered in light of the extent to which those suggestions would impact the conclusions or quantitative analyses of the assessment, consistency across committee members in raising the suggestion, and the level of effort to implement. For this assessment, Tier 2 suggestions deemed impactful to the toxicity value conclusions were implemented. Tier 3 Future Considerations are generally outside the immediate scope or need of the Toxicological Review but may inform future analyses; for the specific Tier 3 considerations received, these comments ultimately did not inform the analyses in this assessment and are therefore not discussed in this appendix (these comments are included in the external peer-review report linked above). Text from the report that could be interpreted as advice or suggestions to EPA, but which was not tiered, was considered by EPA as Tier 3.

Public comments made on topics raised by the external peer reviewers are noted along with the external peer-review comments. When comments from the public and peer-review panel on a topic conflicted, the recommendations of the panel are prioritized. Additional public comments not raised by the peer reviewers are included in a separate section at the end of each charge question section.

External peer-review and public comments regarding requests for additions of clarifying text or editorial or grammatical corrections have been made throughout the assessment as appropriate; these comments and responses have not been tracked in this appendix.

H.1. CHARGE QUESTION 1 – LITERATURE SEARCH METHODS AND DOCUMENTATION

1. The Toxicological Review for PFDA describes and applies a systematic review protocol for identifying and screening pertinent studies. The protocol is described in brief detail in Section 1.2.1 and in full detail in Appendix A. Please:

    a) Comment on whether the literature search strategy and screening criteria for PFDA are appropriate and clearly described.

    b) Identify additional peer-reviewed studies of PFDA that EPA should consider incorporating prior to finalizing the assessment.

EPA synthesized the literature published through April 2022 in the external review draft and has been monitoring newly identified studies (i.e., studies identified by EPA or the public that meet the PECO criteria or otherwise inform key assessment conclusions, but which were not addressed in the external review draft, for example due to publication after April 2022). EPA will characterize these studies in a document that will be provided to the peer-review panel and the

public and, following the review, included as an appendix to the assessment prior to finalization (see Appendix I). The characterization will focus on EPA's judgment of whether the studies would have a material impact on the conclusions (i.e., identified hazards or toxicity values) in the external review draft. Following receipt of this additional document after the review is underway, please:

c) Review EPA's characterization and provide tiered recommendations regarding which studies, if any, would have a material impact on the draft's conclusions and should be incorporated into the assessment before finalizing, as well as your interpretation of the impact of those studies to be incorporated.

H.1.1. External Peer-Review Comments on Literature Search Methods and Documentation

Peer-Review Comment Summary

- *Charge question 1a*: While several reviewers provided Tier 1 and Tier 2 comments with suggested specific revisions (see below), all nine reviewers agreed that the literature search strategy and screening criteria are clearly described, and the process is well documented.
- *Charge question 1b*: The majority of the panel (five reviewers) did not identify any additional studies of PFDA for EPA to consider prior to finalizing the assessment, while several reviewers provided specific Tier 1 or Tier 2 comments (see below, noting there were also Tier 3 comments on this topic) on additional studies.
- *Charge question 1c*: The majority of the panel (eight reviewers) did not note any material impact of the new studies on the draft's conclusions, although several Tier 1 and Tier 2 comments were provided (see below, noting there were also Tier 3 comments on this topic), primarily related to clarifying how newer literature will be tracked or incorporated.

Tier 1 Necessary Revisions

Comment: A reviewer provided the following comments on the PECO criteria for literature screening: 1) "In the Evidence column for Populations, Human: Recommend adding text similar to for Animal, '*(including preconception, in utero, lactation, peripubertal, and adult stages)*' or otherwise assure inclusion of sensitive developmental windows including those relevant to mammary gland development (e.g., duration of lactation as an outcome), fertility, infant exposure, etc."; 2) "In the Evidence column for Outcomes, *'(Note: Other than genotoxicity studies, studies including only molecular endpoints [e.g., gene or protein changes; receptor binding or activation] or other nonphenotypic endpoints addressing the potential biological or chemical progression of events contributing towards toxic effects will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol])*.' Recommend clarifying here or elsewhere how vaccine immune titer is a functional measure of adaptive immune response and therefore a phenotypic outcome."

EPA Response: EPA believes the wording of the existing PECO statement for "Population" captures the literature noted by the reviewer and did not revise this portion of the PECO statement.

Similar to the description of the animal population, the PECO statement in Table 1-4 makes note of sensitive human populations stating, "any population and lifestage (occupational or general population, including children and other sensitive populations)." Regarding the use of antibody responses for immunotoxicity, a sentence was added to the "Outcome" portion of the PECO table to clarify that functional immune measures (e.g., antibody response) are considered phenotypic outcomes. Further, the section on immune effects (see Section 3.2.2) provides a justification for using reduced antibody levels as a functional measure of immunosuppression that could indicate a general increase in susceptibility to infectious diseases (i.e., not limited to those studied).

Comment: Four reviewers asked for clarification on the reasons for excluding 595 studies as "Not Relevant to PECO" at the title and abstract level as done for studies that were excluded at full text screening (Figure 2-1).

EPA Response: Studies are excluded if they do not meet all PECO criteria and text has been added to the assessment for clarification. During screening, most studies are excluded because they do not meet any or only meet a few of the PECO criteria. Thus, a single screened out study typically has multiple reasons for exclusion which is unwieldy to document, especially at the title and abstract level when screening may be needed for thousands of studies. Some of the studies that did not meet all PECO criteria were considered to have potentially relevant supplemental information and were tagged as such. The annotation used in the assessment is consistent with the convention in the IRIS Handbook (U.S. EPA, 2022b). A sentence was added to Section 2.1 to clarify that excluded studies "did not meet the PECO and did not contain potentially relevant supplemental information," …

Comment: A reviewer suggested further explanation on the health effects that were included and excluded for extraction into HAWC for epidemiological studies (Section 1.2.4. page 33, line 34).

EPA Response: The IRIS Handbook provides for flexible approaches for data extraction, to include extraction into HAWC or tabular presentation. Details on how data extraction is approached for studies that meet PECO criteria can be found in Section 1.2.4 of the Toxicological Review and Section 8 of the PFAS protocol cited in Appendix A of this document. Specifically, the decision whether to extract epidemiological studies in HAWC was based primarily on whether data visualizations were determined necessary to understand the evidence synthesis judgments. Text has been added to clarify this rationale in Section 1.2.4. When visualizations were not used, results were instead extracted into tables in the assessment when relevant.

Comment: One reviewer identified three additional epidemiological studies for consideration, including a study on developmental effects (Padula et al., 2023) and two studies on lactation duration (Timmermann et al., 2017; Romano et al., 2016).

EPA Response: EPA identified the (Padula et al., 2023) study in its March 2023 literature search update and determined that its incorporation into the draft is not necessary given the results of the study do not influence the current draft judgment for either gestational duration or fetal

growth restriction (see Appendix I). In addition, the methods describing consideration of newly identified studies are laid out in Section 1.2.1 of the main document, which has been edited for clarity.

Studies on breastfeeding duration (Rosen et al., 2018; Timmermann et al., 2017) and others identified on this outcome) have been added to Section 3.2.5. These studies were incorporated because they were viewed as relevant to a key assessment uncertainty. Namely, few studies (for any PFAS) are available on this specific outcome and potential effects of PFAS on this outcome have been hypothetically linked to impacts on mammary gland development, an outcome affected by certain PFAS in experimental studies (e.g., reviewed in: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7968861/). Specifically, for the Toxicological Review, a subsection was added to synthesize the results of the epidemiological studies focusing on PFDA effects on breastfeeding duration. Additionally, a table (see Table 3-27) was added to present the results of these studies. Finally, this outcome was added to the evidence profile table for PFDA exposure and female reproductive effects.

Comment: A reviewer provided the following comment on the additional studies submitted during public comment, "EPA should consider (Tier 1 necessary revision) whether or not the results of the studies recommended by public commenters should be incorporated into the assessment utilizing relevant practical criteria (e.g., based on their potential impact (if any) on data gaps, assessment conclusions, and/or toxicity values). However, it appears that EPA has already done so (see *EPA characterization of studies identified after public release of the draft IRIS Toxicological Review of Perfluorodecanoic Acid (PFDA, CASRN 335-76-2) and Related Salts*)."

EPA Response: EPA confirms the reviewer is correct that EPA has already reviewed new studies identified during public comment and provided a judgment on the material impact on key assessment decisions (i.e., hazard identification and dose-response assessment) to decide whether to include such studies in the revised assessment prior to finalization. This information was provided to peer reviewers and added to the public docket and has also been included in the supplemental information to the Toxicological Review of PFDA (see Appendix I).

Comment: Regarding EPA's characterization of the studies identified after release of the External Peer-review Draft, a reviewer noted "Tier 1 recommendation is to clarify overall how the recent updated literature review will be incorporated into the current draft, Tier 2 recommendation deals with clarification of how those papers identified as YES [i.e., materially impactful], will be incorporated into the specific relevant sections of the report"

EPA Response: Text was edited in Section 1.2.1 of the Toxicological Review to clarify the strategy for incorporating new studies, "The literature through March 2023 was screened while the document was undergoing public comment. The results of this literature update and any additional unscreened studies identified during public comment and external peer review were screened against the PECO criteria and presented in Tables I-1, I-2, and I-3 of Appendix I in the assessment. The tables provide the identified studies that met PECO criteria or certain supplemental evidence

categories (i.e., in vivo mechanistic or MOA studies, including non-PECO routes of exposure and populations; in vitro and in silico models; and absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetic [PK] studies) and EPA's judgment and supporting rationale on whether the studies would have a material impact on the assessment conclusions (i.e., identified hazards or toxicity values) presented in the public comment draft. New studies judged influential in informing assessment conclusions and data gaps were incorporated into the relevant section of the assessment prior to finalization."

Comment: Three reviewers suggested that the exposure section be updated and expanded. One reviewer provided several references for inclusion.

EPA Response: Several updates were made to this section using information from the following recent information sources: https://echo.epa.gov/trends/pfas-tools, (Holder et al., 2023), and NHANES - National Health and Nutrition Examination Survey Homepage (cdc.gov). However, EPA notes that a comprehensive evaluation of exposure is outside the scope of IRIS assessments. The background information described in Section 1.1 is an overview and is not intended to provide a comprehensive description of the available information on PFDA and related salts, and information on human exposure is tagged as supplemental information during the screening process.

Tier 2 Suggested Revisions

Comment: A reviewer suggested "Listing any major research activities that are [on]going would be useful (Tier 2) because of the number of people contributing to the literature."

EPA Response: The Agency's broader strategic roadmap (https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024) documents ongoing activities at EPA aimed at safeguarding communities from PFAS contamination, including evaluation of potential human health effects associated with exposure to individual PFAS and PFAS mixtures. Text was added to the Executive Summary of the draft Toxicological Review to better emphasize this resource and highlight a few relevant activities.

H.1.2. Public Comments on Literature Search Methods and Documentation

Comment: A commenter noted discrepancies in the number of studies identified in the Toxicological Review and in the PFAS-Tox Database and provided a list of additional human studies for EPA's consideration noting, "… In order to better, understand where the differences in results arise, it would be helpful if EPA would provide a list of all excluded studies and the reason for exclusion." The reviewer added, "… While some of the studies that were included in the PFAS-Tox Database may be out of the scope of the EPA's analysis, it would be helpful to understand EPA's decision process on these studies. For example, Pan et al., found that PFDA was associated with increased DNA fragmentation index and high DNA stainability (a marker of the percentage of sperm with immature chromatin) in semen. (9) Semen evaluations were considered in Section 3.2.4 of the Draft Toxicological Review, and it is unclear why this study was not included. EPA should review

the submitted attachment and evaluate if additional human studies should be included in the Toxicological Review."

EPA Response: EPA has reviewed the studies included in the PFAS-Tox Database, including the available human studies, against the PFDA assessment PECO criteria; the results of this screening are included on the HERO project page (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2614). As mentioned above, EPA reviewed the studies identified during public comment and provided a judgment on the material impact on key assessment decisions (i.e., hazard identification and dose-response assessment) to decide whether to include such studies in the revised assessment prior to finalization. This information was provided to peer reviewers and has also been included in the supplemental information to the Toxicological Review (see Appendix I). Regarding the study highlighted by the commenter, EPA documented the decision not to include Pan et al., (2019, 6315783) since the evidence for semen parameters was inconsistent and this study would not influence the assessment's conclusions for male reproductive effects or otherwise impact key assessment conclusions or uncertainties (see rationale in Table I-1 in Appendix I).

Comment: The same commenter noted seven missing studies on breastfeeding duration and the overall lack of evaluation of this human health endpoint in the draft Toxicological Review, "Given the importance of breastfeeding and its association with many other health impacts, this is a major oversight. EPA should review the submitted attachment and consider summarizing the available evidence that PFDA may be associated with shortened duration of breastfeeding."

EPA Response: As described above, EPA evaluated studies identified after release of the draft Toxicological Review for public comment and as a result of this analysis, several studies on breastfeeding duration have been added to the Toxicological Review (see Section 3.2.5). Specifically, a subsection was added to synthesize the results of the eight epidemiological studies focusing on PFDA effects on breastfeeding duration. Additionally, a table (see Table 3-27) was added to present the results of these studies. Finally, this outcome was added to the evidence profile table for PFDA exposure and female reproductive effects.

Comment: Regarding supplemental evidence, the same commenter suggested, "In general, studies presumably marked as supplemental materials are not consistently referred to and discussed in the document. EPA should provide further guidance for when it will make use of available supplemental materials. The list of nonmammalian models or animals that were exposed through non-oral routes, for example, is not readily accessible, or cited in the Draft Toxicological Review. EPA should better mention and summarize these mechanistic studies, in order to more fully describe the potential effects of PFDA." The commenter also identified 72 studies in animals exposed to PFDA via intraperitoneal (i.p.) injection that were submitted to EPA for consideration.

EPA Response: EPA has reviewed the 72, i.p. injection studies and found that only one study had not been previously identified (Reo and Adinehzadeh, 2000). EPA decided not to incorporate this study into the assessment because it does not impact assessment conclusions or inform key

data gaps (see Table I-3 in Appendix I for more details). Screening decisions for the remaining studies can be found in the HERO project page for PFDA (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2614), which provides a list of all studies tagged as supplemental.

Section 1.2 of the Toxicological Review and the *Systematic Review Protocol for the PFAS IRIS Assessments* (see Appendix A) outline consideration of supplemental materials. Not every study tagged as supplemental is described or cited in the Toxicological Review draft. Cited supplemental material studies are typically those that address specific scientific issues and uncertainties. This is the case for some of the i.p. studies submitted by the commenter that were not included because they would not help bring clarity to the core areas of uncertainty in the assessment (see Section 2.4 of the PFAS protocol). Additional information on the IRIS Program approach to categorizing and considering supplemental studies can be found in the IRIS Handbook (https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=356370).

Comment: The same commenter added, "The use of studies tagged as supplemental is also important in the evaluation of carcinogenicity. Notably, we identified several studies that likely were tagged as supplemental studies by EPA, which may be informative, especially should EPA consider using the Key Characteristics of Cancer framework, which has been used by agencies such as California's Office of Environmental Health Hazards. In particular, the study by Benninghoff et al. 2012, which evaluated tumor promotion in trout, was important in OEHHA's analysis of the carcinogenicity of PFOS. PFDA was also evaluated in the study by Benninghoff et al." The commenter listed seven studies specific to PFDA that may be of interest for the evaluation of carcinogenicity.

EPA Response: As is the case for most PFAS evaluated by EPA to date, EPA concluded that there is ***inadequate information to assess the carcinogenic potential*** of PFDA by any route of exposure based primarily on consideration of the human and animal toxicological literature. As stated in Section 3.3.1, the scope of the mechanistic analysis for carcinogenicity focused on genotoxicity based on the sparse and *low* confidence human and animal studies available and the insufficient information for evaluation of alternative carcinogenic mechanisms. EPA notes that one of the seven studies (Liu et al., 2019) identified by the commenter has already been synthesized as part of the genotoxicity evaluation for PFDA. The remaining studies on other potential mechanisms are tagged as potentially relevant supplemental information but are not cited in the Toxicological Review because they would not impact assessment hazard or dose-response conclusions. A list of supplemental studies for PFDA can be found in the HERO project page for this Toxicological Review (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2614).

H.2. CHARGE QUESTION 2 – NONCANCER HAZARD IDENTIFICATION

2. For each health effect considered in the Toxicological Review and outlined below, please comment on whether the available data have been clearly and appropriately

synthesized to describe the strengths and limitations, including whether the presentation and analysis of study results are clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. Please comment on whether the study confidence conclusions for the PFDA studies are scientifically justified, giving appropriate consideration to important methodological features of the assessed outcomes.[7] Please specify any study confidence conclusions that are not justified and explain any alternative study evaluation decisions. For each, please also comment on whether the weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified. Note that the data from studies considered informative to the Toxicological Review are synthesized in the relevant health effect-specific sections and available in the Health Assessment Workspace Collaborative (HAWC).

a) For liver effects, the Toxicological Review concludes that the available ***evidence indicates*** PFDA exposure is likely to cause liver effects in humans given sufficient exposure conditions, on the basis of a series of short-term studies in rats and mice demonstrating consistent and coherent effects with a clear biological gradient. The liver findings for PFDA were similar to those for other structurally related long-chain PFAS and determined to be adverse and relevant to humans.

    i. Additional considerations influenced the liver effects hazard identification decisions. Appendix A (*Systematic Review Protocol for the PFAS IRIS Assessments*) outlines the human relevance of hepatic effects in animals that involve PPARα receptors as a key science issue. To the extent supported by the PFDA literature (and to a lesser extent, literature for other PFAS), the Toxicological Review evaluates the evidence relevant to the potential involvement of PPARα and non-PPARα pathways with respect to the reported liver effects. The Toxicological Review ultimately concludes evidence from in vivo and in vitro studies support a potential role for multiple pathways operant in the induction of hepatic effects from PFDA exposure, although how those pathways interact within a mode of action (MOA) cannot be specifically determined.

b) For immune effects, the Toxicological Review concludes that the available ***evidence indicates*** PFDA exposure is likely to cause immunosuppression in humans given sufficient exposure conditions, primarily on the basis of consistent evidence of reduced antibody responses from two epidemiological studies in children and one study in adults. Although some evidence for coherent immunomodulatory responses consistent with immunosuppression was identified in short-term animal studies, the animal evidence overall is uncertain. The Toxicological Review concludes the immune effects are considered relevant to humans as the judgment is based on studies in humans.

[7]The Toxicological Review provides an overview of individual study evaluations within each evidence synthesis section, and the results of those outcome-specific evaluations are made available in the Health Assessment Workplace Collaborative (HAWC). Note that a HAWC Frequent Questions page, linked here, is available to help the reviewer navigate this online resource.

i. For nearly all epidemiology studies of PFDA, there is potential that exposure to other highly correlated PFAS could contribute to the observed effects. The evidence synthesis for potential PFDA-induced immune effects included evaluation of the potential for confounding across PFAS as well as other sources of confounding and, on the basis of the available data, determined that residual confounding could explain part of the observed effect, but concern was minimal, and it was unlikely to fully explain the associations seen in the literature.

c) For developmental effects, the Toxicological Review concludes that the available ***evidence indicates*** PFDA exposure is likely to cause developmental effects in humans given sufficient exposure conditions, based primarily on consistent findings of dose-dependent decreases in fetal weight in mice gestationally exposed to PFDA supported by some coherent evidence of decreased birth weight from studies of exposed humans in which PFDA was measured during pregnancy, although uncertainties in the available epidemiological evidence reduced the impact of these latter findings. The Toxicological Review concludes the developmental effects in mice are considered relevant to humans given similar findings of fetal growth restriction in mice and humans.

i. As described in question 3.c and the footnote to 3.c, the evidence synthesis for potential PFDA-induced developmental effects considered potential confounding factors and concluded that confounding across PFAS or from other potential sources of bias (e.g., pregnancy hemodynamics in studies that measured PFDA during or after pregnancy) introduce significant uncertainty. These sources of uncertainty ultimately reduce the strength of the available human evidence to *slight* for an evidence base that might otherwise be interpreted as *moderate*.

d) For male reproductive effects, the Toxicological Review concludes that the available ***evidence indicates*** PFDA exposure is likely to cause male reproductive effects in humans given sufficient exposure conditions, based on coherent evidence in adult male rats exposed to PFDA for 28 days. Although no direct information on the human relevance of the animal evidence is available, the findings in animals are presumed to be relevant on the basis of the conserved role of androgen-dependent pathways in male productive functions across species.

e) For female reproductive effects, the Toxicological Review concludes that the available ***evidence indicates*** PFDA exposure is likely to cause female reproductive effects in humans given sufficient exposure conditions, based primarily on coherent evidence from a 28-day study in adult female rats. Although human studies are available examining associations between PFDA and female reproductive toxicity (e.g., fecundity), the results were mostly null, possibly due to their low sensitivity

for observing effects. The Toxicological Review concludes the female reproductive effects are considered relevant to humans given that mechanisms of female reproduction are similar between rats and humans. Available ***evidence indicates*** PFDA exposure is likely to cause male reproductive effects in humans given sufficient exposure conditions, on the basis of coherent evidence in adult male rats exposed to PFDA for 28 days. Although no direct information on the human relevance of the animal evidence is available, the findings in animals are presumed to be relevant on the basis of the conserved role of androgen-dependent pathways in male productive functions across species.

f) For cardiometabolic effects, the Toxicological Review concludes that the available ***evidence suggests*** but is not sufficient to infer that PFDA exposure may have the potential to cause cardiometabolic effects in humans given sufficient exposure conditions, on the basis of associations between PFDA and serum lipids, adiposity, cardiovascular disease, and atherosclerosis in a few epidemiological studies. However, the evidence is largely inconsistent across studies, which adds considerable uncertainty. Evidence in experimental animals was *indeterminate*.

g) For neurodevelopmental effects, the Toxicological Review concludes that the available ***evidence suggests*** but is not sufficient to infer that PFDA exposure may have the potential to cause neurobehavioral effects in humans given sufficient exposure conditions, on the basis of associations between PFDA and outcomes related to attention and behavior in epidemiological studies. However, the evidence is largely inconsistent across studies, which adds considerable uncertainty. No evidence was found in experimental animals to inform this outcome (*indeterminate)*.

h) For endocrine, urinary, and other noncancer effects (i.e., hematological, respiratory, digestive, dermal, musculoskeletal, and nervous systems), the Toxicological Review concludes there is ***inadequate evidence*** to determine whether PFDA exposure has the potential to cause these effects in humans on the basis of the sparsity of available evidence.

H.2.1. External Peer-Review Comments on Hepatic Effects

Peer-Review Comment Summary

The majority (seven) of reviewers commented that the available data are clearly and appropriately synthesized, and study confidence conclusions are scientifically justified and appropriate, including with regard to characterizing the PPPRα. One reviewer disagreed with the characterization of evidence of liver effects in human studies as *slight* and noted that evidence is between *slight* and *moderate* and provided related tiered comments (see below). While another reviewer generally noted agreement with EPA's text on liver effects, they provided tier 1 and 2 comments on EPA's analyses of human relevance and adversity (see below).

Tier 1 Necessary Revisions

Comment: A reviewer commented, "… EPA should: (1) attempt to scientifically justify whether the mouse or rat is likely most biologically representative of humans such that the same or similar effects (hepatic, etc.) are expected in humans at similar doses when converted to human equivalent doses (HEDs); and (2) in the event (1) cannot be established with sufficient scientific confidence, acknowledge within the assessment that the choice of the most appropriate laboratory animal model for prediction of PFDA-induced adverse effects in humans has not been scientifically established (i.e., is not 'settled science') but rather species selection is based on policy."

EPA Response: In response to the reviewer's comment and to highlight adherence to Agency guidelines, the following sentence was added to Section 3.2.1, "This assumption is consistent with EPA's review of RfD/RfC methodology from 2002 (EPA, 2002)." For additional context, Section 3.2.1 and Appendix D provide a detailed synthesis and evaluation of the available mechanistic evidence from in vivo and in vitro model systems for PFDA-induced liver effects to address specific issues surrounding the adversity and human relevance of the hepatic responses observed in rodents. Specifically with regards to the issue of using rats as a human health model for PFDA-induced hepatic effects, the Toxicological Review concluded, "Given that the precise role of PPARα in the non-cancer liver effects of PFDA remains largely unknown and the evidence supporting involvement of both PPARα-dependent and independent pathways, the effects observed in animals are considered potentially relevant to humans."

Comment: The same reviewer noted that the statement from Section 2.4.2 of Appendix A, "Activation of the peroxisome proliferator-activated receptor alpha (PPARα) by PFAS has been reported, with in vitro evidence that the potency of human and mouse PPARα activation is positively correlated with increasing PFCA chain length up to C9 (no human receptor activation was noted for PFDA…)," contradicts with another statement in the main Toxicological Review (page 3–50, line 5) which indicates, "PFDA can activate the human PPARα in vitro…." The reviewer recommended that "EPA should resolve this apparent discrepancy."

EPA Response: The text on evidence integration in Section 3.2.1 has been edited for clarity, "PFDA can activate the human PPARα in vitro but it exhibits less/no sensitivity toward the human isoform in comparison with other mammalian species in some studies." As noted in the section on summary of mechanistic studies for PFDA under Section 3.2.1, evidence of human PPARα activation in vitro by PFDA has been demonstrated across multiple studies using primary and immortalized human liver cell lines and in a receptor binding study. However, reduced or no sensitivity toward the human PPARα compared with the mouse, Baikal seal and polar bear isoforms has also been documented in some studies (including the one referenced by the reviewer).

Tier 2 Suggested Revisions

Comment: A reviewer disagreed with evidence synthesis judgement for hepatic effects from human studies stating, "The overall determination that 'slight evidence' of liver effects in human

[studies] (exposure duration not mentioned) is inconsistent given the positive effects observed 4 of 5 studies for PFDA and ALT. The finding of an association is largely consistent with the extensive literature on other longer chain legacy PFAS and liver effects. The subsequent sentence saying that the results lack coherence and mentions other (unnamed) biomarkers in humans is therefore unclear. Overall, this section would be clearer if it clearly separated the determinations from the human and the animal data, then discussed integration and consistency (or lack thereof) between the two data types." The reviewer thought the descriptor probably lay between *slight* and *moderate* for the human evidence given the extensive evidence for related PFAS. The reviewer also added, "I agree that the mechanistic evidence supports the observed effects, which increases confidence in the conclusions and thus the writing on weight-of-evidence determination (WOE) needs to be clearer on what a scientifically justified finding is for this endpoint."

EPA Response: EPA agrees that the human evidence for this health effect is borderline between *slight* and *moderate*. Based on the reviewer's feedback, the studies identified in the literature search update were added to see if they clarified the judgment. Discussion was also included on the potential for confounding across PFAS, which is typically included for effects with *moderate* or higher evidence. This analysis indicated that there is serious concern for potential confounding by PFNA exposure. Overall, the judgment remains *slight* for human studies, but the text has been revised to clarify the rationale (see Section 3.2.1 of the Toxicological Review).

Comment: The same reviewer suggested clearly stating the human relevance of the 28-day animal study given the time needed to reach steady state.

EPA Response: The PK analysis indicates that rats in the NTP 28-day study probably did not reach steady state. However, determinations regarding human relevance depend on whether or not the mechanism of toxicity is relevant to humans, not the dosimetric extrapolation across species. The text in Section 3.2.1 has been edited to clarify statements regarding the human relevance of the animal data. Additionally, Section 3.1.7 discusses the approach for dosimetric extrapolation from the 28-day rat study to humans, which has been revised to use observed PFDA concentrations in rats from the NTP study and to no longer assume steady state. But the revision of the dosimetric adjustment only has a quantitative impact on the value of the HED and does not alter the relevance of the endpoint to human health.

Comment: While in agreement with EPA's overall hazard characterization for PFDA-induced liver effects, a reviewer suggested that "… EPA should explicitly show how the Hall et al. (2012) criteria for adversity are met. For example, while fold increases in biomarkers of liver dysfunction (e.g., bilirubin, bile acids/salts) are explicitly discussed on p. 3–48 (lines 36–38), the fold increases needed to fulfill a second criterion (e.g., in ALT or ALP) are not explicitly discussed on lines 34–35 (e.g., a two- to threefold increase in ALT or biomarkers of hepatobiliary damage such as AST, ALP and γ-glutamyltranspeptidase [γGT]; Tables 3-7 and 3-8 are relevant)."

EPA Response: Section 3.2.1 provides specific considerations for evaluating potentially adaptive versus adverse liver effects induced by PFDA exposure, including the (Hall et al., 2012)

criteria. Hall et al. (2012) indicated that concordant histopathological evidence of degenerative or necrotic changes (e.g., hepatocyte necrosis, fibrosis, inflammation, steatosis, biliary degeneration, necrosis of resident cells within the liver) can be used to support the argument that liver weight/hepatocyte enlargement are adverse (Hall et al., 2012). The evidence for PFDA across two *high* confidence 28-day gavage studies in rats shows a clear pattern of increased hepatocyte damage/injury with dose, ranging from cytoplasmic changes to hypertrophy to necrosis (Frawley et al., 2018; NTP, 2018). The necrotic lesions were accompanied in some cases by evidence of an initial inflammatory response (NTP, 2018) and, although these changes were characterized as minimal, the findings indicate some degree of structural degeneration considered adverse. Consistent with these observations, steatosis, necrosis, edema, and degeneration were reported in low confidence short-term oral studies in rats and mice at higher PFDA doses (Wang et al., 2020; Kawashima et al., 1995).

Hall et al. (2012) also indicated that clinical markers of liver injury can provide evidence in support of adversity, considering dose-dependent and biologically significant changes in at least two of the following parameters: two- to threefold increase in ALT; a biologically significant change in biomarkers of hepatobiliary damage (e.g., AST, ALP and γ-glutamyltranspeptidase [γGT]); a biologically significant change in biomarkers of liver dysfunction (e.g., albumin, bilirubin, bile acids/salts and coagulation factors). PFDA increased ALT levels in female rats in a *high* confidence short-term study (NTP, 2018) and although the increases in circulating ALT levels were relatively small (20%–44% or 1.2- to 1.4-fold), concordant changes in other clinical biomarkers occurred in rats that are consistent with the (Hall et al., 2012) criteria for adversity (i.e., dose-dependent increases in levels of AST, ALP, bile salts/acids and bilirubin). Correspondingly, large increases in ALT (338% or 4.4-fold) and AST (649% or 7.5-fold) were reported in mice that exhibited liver lesions after exposure to a high dose of PFDA (13 mg/kg-day) (Wang et al., 2020).

Overall, the evidence for PFDA-induced liver effects in animals meets all the Hall (2012, 2718645) criteria for adversity. Text was added to Section 3.2.1 for clarity and discussion of specific fold changes for all liver clinical markers.

Comment: The same reviewer recommended, "… EPA should consider additional tables and/or figures that would help readers visualize important EPA conclusions, such as 'coherent changes in serum biomarkers, histopathology, and liver weights' cited in Table 3-11 [Table 3-10 in the revised Toxicological Review]."

EPA Response: A figure displaying changes across histopathology, serum biomarkers and organ weight endpoints has been added to Section 3.2.1 in the discussion on evidence integration and to Table 3-10.

H.2.2. Public Comments on Hepatic Effects

Comment: One commenter agreed with the overall conclusion that the liver effects of PFDA in rodents should be considered adverse and relevant to humans but disagreed with the statement that the biological significance of the small increases in alanine aminotransferase (ALT) in humans

is unclear, noting that "… The EPA Science Advisory Board (SAB) PFAS Review Panel provided a detailed rationale as to why relatively small increases in ALT, including those associated with PFAS, should be considered adverse… " Similarly, another commenter recommended the following "When evaluating the epidemiological data for hepatic effects, I encourage EPA to consider reviewing a new paper regarding the use of clinical consensus cutoffs compared with statistical cutoffs for abnormal values."

EPA Response: Text was added to the synthesis regarding the adversity of the changes in ALT (see Section 3.2.1 of the Toxicological Review).

Comment: Another commenter raised concerns about "… Uncertainty surrounding the utility of animal data for human health assessment based on interspecies differences in the interaction of PFAS with peroxisome proliferator-activated receptor (PPAR)-alpha (PPARα) and the applicability of environmental concentrations in this interaction."

EPA Response: A detailed evaluation of the available mechanistic evidence, including the potential role of PPARα in the liver toxicity of PFDA and its implications to human health was provided in Appendix D and in the synthesis of mechanistic studies and supplemental information under Section 3.2.1. The available data indicate a likely role for both PPARα-dependent and -independent mechanisms in the liver effects of PFDA in animals. Existing evidence from in vitro studies and animal models considered more relevant to humans with respect to PPARα sensitivity suggest that some responses may be conserved across species (including activation of relevant nuclear receptor pathways [PPARα/γ, PXR and FXR] and outcomes related to hepatocellular stress, mitochondrial damage, lipid accumulation and liver enlargement). As such, the Toxicological Review concluded "Given that the precise role of PPARα in the noncancer liver effects of PFDA remains largely unknown and the possible involvement of PPARα-dependent and independent pathways, the effects observed in animals are considered potentially relevant to humans." A majority of the external reviewers agreed with this conclusion as noted in the external peer-review report, "Seven [of nine] reviewers commented that available data are clearly and appropriately synthesized and study confidence conclusions are scientifically justified and appropriate, including with regard to characterizing the PPPRα."

Comment: A commenter noted, "it is unclear why the evidence of PFDA-induced liver effects was judged to be 'moderate' rather than 'robust.' As discussed in the paragraph that begins on line 12, the available animal data supports the conclusion that PFDA causes liver toxicity. Although there are no studies with exposure durations of longer than 28 days, the results of such studies would not be anticipated to contradict the conclusion that PFDA causes liver toxicity based on the shorter studies."

EPA Response: The ORD Staff Handbook for Developing IRIS Assessments (U.S. EPA, 2022b) defines the evidence synthesis judgment of *robust* animal evidence as follows, "The set of *high* or *medium* confidence, independent experiments (i.e., across laboratories, exposure routes, experimental designs [for example, a subchronic study and a multigenerational study], or species)

reporting effects of exposure on the health outcome(s)." While the database for PFDA-induced liver effects includes several short-term studies via the oral route, the primary evidence for coherent liver effects comes from two high/medium confidence studies from the same laboratory, species, and exposure design. The text in Section 3.2.1 has been edited to clarify the database limitations and uncertainties that resulted in an evidence synthesis judgment of *moderate* for animal studies, "The evidence base for liver effects in animals consists primarily of two *high*/*medium* confidence 28-day studies in S-D rats conducted by NTP that showed concordant effects. Other available short-term studies provided support for PFDA-induced liver effects across different laboratories and species but had issues with incomplete reporting that resulted in a *low* confidence rating, evaluated limited endpoints, and/or tested higher doses associated with general systemic toxicity, which add some uncertainty. Additional studies via relevant exposure routes and experimental designs (most prominently subchronic and chronic exposure studies) examining potential liver effects of PFDA exposure are needed to increase confidence in the evidence base." It is also worth noting that a majority of the external peer reviewers (seven of nine reviewers) agreed with EPA's synthesis and characterization of the available evidence for liver effects.

H.2.3. External Peer-Review Comments on Immune Effects

Peer-Review Comment Summary

In reference to charge question 2b, the majority of the peer reviewers agreed with EPA. Two reviewers agreed with EPA's overall conclusions and had no Tier 1 or 2 recommendations. Five reviewers agreed with EPA's overall conclusions but also made some suggestions for improvement (see below) and one reviewer also provided tiered recommendations but did not state explicit agreement or disagreement with EPA on this charge question. One reviewer disagreed with EPA's conclusion on the strength of evidence for human immunosuppression from the epidemiological studies, citing several Tier 1 and Tier 2 concerns (see below).

During the peer-review meeting, five reviewers noted that discussion of mixtures and confounding needed to be further developed in the document (Tier 1 or 2), with one reviewer noting that confounding effects from other exposures could impact estimates used to derive RfDs (see charge questions 3 and 4), and another reviewer (the reviewer that disagreed with EPA's evidence integration judgment) arguing that issues of mixtures and confounding in the epidemiology studies, combined with the low effect estimates, precluded the use of these studies for quantitative risk assessment and derivation of toxicity factors. This reviewer also did not agree that the measures of immunosuppression (titers) were clinically significant, particularly for rare diseases such as diphtheria and tetanus, although other reviewers agreed with the use of immune titers as an appropriate endpoint (see below and charge questions 3 and 4).

Tier 1 Necessary Revisions

Comment: Two reviewers asked for more clarity/explanation on the issue of confounding from PFAS co-exposures and the justification for using vaccine responses as a measure of immunotoxicity. One reviewer commented specifically "… a clearer characterization of the effect of multiple correlated PFAS on the immunosuppression attributed to PFDA is needed … The pros (and cons) of multiple confounder adjustment/potential for overfitting are all issues that should be explicitly addressed in the write up as part of the summary judgment. A more explicit discussion of the strengths, for example, of looking at titers of multiple vaccine endpoints in more than one cohort are needed as well as a discussion of how relatively small changes in titer levels at the measurement timepoints are justifiable as key endpoints."

EPA Response: Revised text in Section 3.2.2 provides additional clarity on potential confounding by other PFAS:

"It is plausible that the observed associations with PFDA exposure could be partially explained by confounding across the PFAS or cumulative effects, although several analyses and observations indicate that this is unlikely. Exposure levels to other PFAS in the Faroe Islands populations were considerably higher (PFOS 17 ng/mL, PFOA 4 ng/mL, PFNA 1 ng/mL, PFDA 0.3 ng/mL at age 5 years in Grandjean et al. (2012), and there was a high correlation between PFDA and PFNA (r = 0.78) and moderate correlations with PFOS and PFOA (r = 0.39 and 0.35, respectively). The authors assessed the possibility of confounding in a follow-up paper (Budtz-Jørgensen and Grandjean, 2018a) that reanalyzed data from both Grandjean et al. (2012) and (Grandjean et al., 2017) for benchmark analysis. In this reanalysis, effect estimates for PFDA were adjusted for PFOS and PFOA. Details of the analytic results were provided to EPA by the authors (Budtz-Jørgensen and Grandjean, 2018b). There was variable attenuation of the observed effect estimates across the different analyses, and PFNA (the PFAS with the strongest correlation with PFDA) was not adjusted for in these models. However, details of the regression modeling results (Budtz-Jørgensen and Grandjean, 2018b) show that PFNA was a nonsignificant predictor of either tetanus or diphtheria antibody concentrations with associations just 15% the strength of the PFDA association and thus PFNA could not have been a meaningful confounder. Further, adjustment of the PFDA association by PFOS and PFOA did not eliminate the association, so confounding by co-occurring PFAS is unlikely to fully explain the associations. The details of the effects of PFDA with, and without, control of PFOS and PFOA are shown in Appendix C.1.1 with discussion of the impact and implications of multiple confounder control. Overall, while it is not possible to rule out confounding across PFAS, the available ***evidence suggests*** that it is unlikely to explain the observed effects. Other sources of potential confounding, including possible coexposures such as PCBs, were controlled appropriately. However, Grandjean et al. (2012) showed the correlation of PCBs with PFDA in their Table 2 at age 5 years as r = 0.14; the low correlation with PFDA means that PCBs could not have been a meaningful confounder of the PFDA effect estimate."

These studies describing children's effects of PFDA on antibody concentrations were based on two birth cohorts using two types of antibodies and multiple exposure windows. Changes in titer levels in young children can be a key endpoint. Reduced antibody production is an indication of immunosuppression and may result in increased susceptibility to infectious diseases generally (i.e., not limited to those specifically studied). Regarding the last part of the comment on the need for "a discussion of how relatively small changes in titer levels at the measurement timepoints are justifiable as key endpoints," it is not clear to EPA if this refers to the magnitude of the observed change in tetanus antibodies of −22.3% (−35.8% to −5.8%) per twofold increase in concentrations of PFDA in 5-year olds serum ((Grandjean et al., 2012); see Table 3) which is not a small change in titer levels, or if this refers to the size of the BMR (½ SD) used in the derivation of the BMDL. Note that Appendix C.1 shows that the SD of the $\log_2$(tetanus antibody concentration in IU/mL) is 2.09 $\log_2$(IU/mL) and thus ½ SD is 1.05 $\log_2$(IU/mL). Exponentiating this back to the natural scale, ½-SD change in $\log_2$(IU/mL) is equivalent to a 2.07 IU/mL change (i.e., $2^{1.05}$). The interquartile range of PFDA in 5-year-olds serum was (0.65 IU/mL, 4.60 IU/mL), so a change of 2.07 IU/mL is approximately equal to a 25% change in the distribution which is also not a small change in titer levels.

Comment: In regard to the human evidence, a reviewer noted "What is also missing from this write up is a clear statement on why the findings from the Faroe Islands cohorts, which have several unique characteristics and dietary habits, are generalizable to the United States."

EPA Response: Similar immune suppression results have been reported in populations outside the Faroe Islands, which supports that they are relevant to U.S. populations (see Section 3.2.2; additional studies are available for other PFAS). Further, unless there is a clear explanation for why a population is not relevant to the United States (e.g., a genetic polymorphism unique to that population), relevance is assumed in order to ensure protection for vulnerable and susceptible groups. In the case of dietary habits, while diet in the Faroe Islands may be different than most of the general population in the United States, there is potential for similar high risk dietary habits within the United States

Comment: A reviewer provided the following recommendations on the presentation and synthesis of the human immune studies: 1) clarify the number and quality of studies on immunostimulation and autoimmunity to explain why these outcomes were excluded; 2) include the dichotomous titer outcome from Timmerman et al. (2021) in Table 3-12 [Table 3-11 in the revised Toxicological Review]; 3) display the results from final dataset used for the derivation of the reference dose for immune effects in Table 3-12 and clarify any differences in units; 4) state that vaccine immune titer is a functional measure of adaptive immune response and therefore an important indicator of clinically relevant immunotoxicity.

EPA Response: (1) Regarding the studies of immunostimulation and autoimmunity, please see Appendix I for the list of studies identified after public release of the draft (Qu et al., 2022; Gaylord et al., 2020; Ammitzbøll et al., 2019). Rationale for not incorporating these studies is

provided there. In brief, these outcomes were not excluded from consideration in the assessment but were not discussed in the Toxicological Review due to a lack of studies in the original search; studies identified in subsequent searches were not influential on assessment conclusions due to lack of association in the small number of available studies. (2) and (3) The dichotomous titer results were not included in the table because the results in the table are expressed as percent change in antibodies. The dichotomous results are described in the text. (4) The suggested text on vaccine titer as a functional measure of adaptive immune response has been included in the section.

Comment: A reviewer recommended "EPA should reevaluate the adversity of these presumed antibody level effects, including the association with PFDA itself, and do so within the context of potential confounding [including co-exposures to other PFAS], other limitations [e.g., weak/non-statistically significant associations], and available human/animal data on disease incidence (Tier 1 necessary revision), as this has important implications for the hazard judgment and the strength of human evidence descriptor for immunosuppression (listed as 'moderate' in Table 3-19 [Table 3-18 in the revised Toxicological Review])."

EPA Response: Revised text from Section 3.2.2 provides additional clarity on potential confounding by other PFAS (see response above for more details). There has been ongoing debate regarding the biological significance of small changes in serum antibody levels (DeWitt et al., 2016; IPCS, 2012; Agarwal and Cunningham-Rundles, 2007; Luster et al., 2005), but EPA's interpretation was supported during the external peer review of the PFOS and PFOA Toxicological Reviews (U.S. EPA, 2022c) as well as by the majority of reviewers of this Toxicological Review (seven out of nine). Vaccine immune titers are functional measures of adaptive immune response and important indicators of clinically relevant immunotoxicity; reduced antibody production is an indication of immunosuppression and may result in increased susceptibility to infectious diseases generally (i.e., not limited to those specifically studied). EPA defines an "adverse effect" as a "biochemical change, functional impairment, or pathologic lesion that *affects* the performance of the whole organism or reduces an organism's ability to respond to an additional environmental challenge" (italics added) (U.S. EPA, 2002). Reduced antibody titers are thus considered an appropriate effect for the derivation of a candidate RfD.

Appendix C.1 provides analyses and explanations regarding the potential for confounding and confidence judgments on the resulting PODs based on the uncertainty in the POD that might be attributable to potential confounding.

Comment: The same reviewer disagreed with the evidence integration judgment for immune effects stating, "Table 3-19 [Table 3-18 in the revised Toxicological Review] indicates that human data provide 'moderate' evidence and that 'the inconsistent and low confidence evidence on infectious disease did not influence this judgment.' However, this points to the fact that EPA has not duly considered the implications of the null findings on human and laboratory animal infectious disease and other relevant considerations (e.g., some discussed above) for the scientific WOE, which is not a scientifically supportable approach as it does not consider all relevant data, directly

relevant human data in particular. EPA should consider such null findings (and other relevant considerations) in their WOE (Tier 1 necessary revision). Combined with the 'slight' human data for sensitization and allergic response, the 'slight' laboratory animal data for immunosuppression, and the 'indeterminate' animal data for sensitization and allergic response (Table 3-19) [Table 3-18 in the revised Toxicological Review], it does not appear that PFDA exposure is 'likely to cause' adverse immune effects in humans is sufficiently supported. EPA should reevaluate this determination (Tier 1 necessary revision) as 'may cause' might very well be the better supported hazard judgement."

EPA Response: The judgment that PFDA exposure is likely to cause adverse immune effects in humans was supported by the majority of reviewers of this Toxicological Review (seven out of nine). The conclusion was primarily driven by consistent evidence of reduced antibody responses across two birth cohort studies with supportive evidence of possible immunosuppressive effects in animals and limited mechanistic studies. The organization of the analyses of immune effects into different categories of immune dysfunction follows the WHO/IPCS guidance on the evaluation of immunotoxicity, an approach repeatedly supported by external reviewers. Text has been added to the evidence synthesis and integration sections describing why the infectious disease evidence does not reduce certainty in the vaccine response data. Specifically, the infection disease studies are expected to be biased toward the null due to the difficulty in measuring infectious disease and reduced study sensitivity. Similarly, the human evidence for hypersensitive-related outcomes had poor sensitivity and a small number of studies and lacked support from animal evidence. Therefore, the finding was interpreted as less certain; however, they do not influence or reduce confidence in the vaccine response data since immunosuppression and sensitization and allergic responses are considered different forms of immunotoxicity with distinct mechanisms and considerations for risk assessment according to the immunotoxicity guidelines from the WHO/IPCS (IPCS, 2012) and as described in the methodological section for immune effects (see Section 3.2.2).

Tier 2 Suggested Revisions

Comment: A reviewer suggested "Table 3-12 [Table 3-11 in the revised Toxicological Review]: Recommend also including a figure showing effect estimates and 95% confidence intervals, ranked by confidence level."

EPA Response: EPA believes a table is more appropriate for this set of studies as different transformations were used in the studies, leaving the quantitative estimates not directly comparable (e.g., may represent a doubling of exposure or a ln-unit increase). Given that all but one of the studies in the table are *medium* confidence, sorting by confidence level is not necessary.

Comment: A reviewer suggested, "EPA should consider additional tables and/or figures that would help readers visualize important EPA conclusions, such as 'coherent evidence of potential immunosuppression in rats and mice at doses ≥0.089 mg/kg-d across two high/medium confidence studies' cited in Table 3-19 [Table 3-18 in the revised Toxicological Review]."

EPA Response: An interactive HAWC visual summarizing coherent immune responses in PFDA-exposed animals is now cited in the evidence integration section for immune effects in Section 3.2.2 and in Table 3-18.

Comment: A reviewer suggested, "Some indication if the suspected effects on the immune system were in line with data from other PFOA [PFAS] would be helpful."

EPA Response: The evidence integration section under immune effects (see Section 3.2.2) discusses immune hazard conclusions for PFOS and PFOA, stating "Overall, the evidence [for PFDA] supports an association with immunosuppressive-type effects. These results are consistent with hazard identification conclusions from the NTP (2016) monograph on immunotoxicity associated with exposure to PFOS and PFOA, which concluded that PFOS and PFOA are *presumed to be an immune hazard to humans* based largely on evidence of suppression of antibody responses in both human and animal studies (NTP, 2016). Additionally, the conclusions from the Science Advisory Board (SAB) report, *Review of EPA's Analyses to Support EPA's National Primary Drinking Water Rulemaking for PFAS,* are now discussed in the integration section for immune effects. The SAB panel agreed with EPA that the human evidence for PFOS and PFOA showed consistent associations between exposure and reduced antibody responses in children indicative of potential immunosuppression (U.S. EPA, 2022c). The SAB panel stated that "Decreased antibody responses to vaccines is relevant to clinical health outcomes and likely to be predictive of risk of disease. The conclusion that suppression of vaccine responses is an adverse finding is widely accepted in the field of immunotoxicology…Moreover, the immunosuppression indicated by the observed antibody decreases are not limited to those specific antigens (e.g., tetanus and diphtheria only), but rather are indicative of modulation of the general immune response." Additionally, the SAB panel concluded that "decreased antibody responses to vaccinations are adverse effects, and that this effect is an appropriate critical effect for deriving RfDs for PFOA and PFOS."

Comment: Two reviewers made specific suggestions regarding the issue of PFAS mixtures. One reviewer mentioned, "Although it is the goal of this assessment to develop an RfD for PFDA as if it is the only chemical causing an effect, mixture risk analysis procedures are being developed to explain the combined effect of more than one chemical exposure. The Agency may choose to strengthen their argument about PFDA using some of this logic." The second reviewer added, "A more rigorous methodology [e.g., direct experimentation with a similar mixture, either in vivo or in vitro] is required to examine the potencies of PFAS for specific endpoints. Statistics should be supported by biological plausibility."

EPA Response: This assessment is specific to PFDA and its related salts and the consideration of a potential additive effect of exposure to multiple PFAS chemicals would be out of scope for the current IRIS assessment. The consideration of potential additive effects of exposure to multiple PFAS could be part of subsequent risk assessment and risk management activities addressing human exposure to multiple PFAS. The draft human health toxicity values for PFDA are part of a broader PFAS strategic roadmap (https://www.epa.gov/pfas/pfas-strategic-roadmap-

epas-commitments-action-2021-2024) at EPA for protecting human health and the environment from PFAS contamination. Text was added to the Executive Summary of the Toxicological Review draft to provide examples from the PFAS strategic roadmap aimed at evaluating health effects from individual PFAS and PFAS mixtures or groups. Additionally, Table 4-1 in Section 4.1 of the Toxicology Review compares health effects for PFDA and other PFAS across EPA human health assessments, highlighting notable data gaps for PFDA and PFAS in general.

H.2.4. Public Comments on Immune Effects

Comment: One commenter agreed with EPA's conclusion that reduced antibody responses are indicative of immunosuppression and may indicate increased susceptibility to infectious disease in general. This commenter also agreed with the conclusions regarding an association between PFDA and decreased antibody responses in children based on the available epidemiological data. Conversely, two commenters raised concerns regarding the interpretation of the epidemiological data for PFDA and immunotoxicity, noting that: 1) the evidence for immunomodulation of PFAS in humans and animals is "at best 'suggestive' of an association but…too weak to draw a solid conclusion" (reviewed by Antoniou et al., [2022]); 2) lack of studies supporting the clinical relevance of reduced antibody responses and the clinically relevant impacts of PFDA exposure on reduced antibody responses; and 3) absence of accounting for likely confounding factors such as exposure to methylmercury and polychlorinated biphenyls (PCBs), which have been found to be elevated in the diet of the studied, Faroe Islands population.

EPA Response: There has been ongoing debate about the biological significance of small changes in antibody levels following vaccination, but EPA's interpretation that the changes are adverse was supported during the external peer review of the PFOS and PFOA Toxicological Reviews (EPA 2022) and by the majority of peer reviewers for this Toxicological Review (seven of nine reviewers). Immunomodulatory effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards.

With regard to confounding by coexposures such as PCBs, this was considered as part of the study evaluation process. The studies in the Faroe Islands considered the potential for confounding by PCBs. They reported that there were only weak correlations between PFAS and PCBs exposure (0.14 for PFDA at age 5 years), and that associations did not materially change with adjustment for PCBs. Thus, confounding by PCBs exposure was not considered likely to significantly bias the effect estimates.

H.2.5. External Peer-Review Comments on Developmental Effects

Peer-Review Comment Summary

In reference to charge question 2c, the majority of peer reviewers agreed with EPA's evidence integration judgment. Three reviewers agreed with what EPA had written in this section

and had no suggested Tier 1 or 2 revisions. Five reviewers agreed with EPA's conclusions but provided Tier 1 and 2 comments to strengthen those conclusions (see below). One reviewer agreed with EPA's conclusion of *slight* evidence for developmental effects in human studies and on that basis recommended that EPA use the Harris and Birnbaum (1989) mouse study to develop RfDs rather than a single epidemiology study (see also charge questions 3 and 4).

Tier 1 Necessary Revisions

Comment: A reviewer stated that on "Page 156, line 24: The sentence starting with 'Although' discussing potential bias needs to be rewritten for clarity as it is unclear what it means."

EPA Response: The text was edited as follows: "The degree of consistency across the observational epidemiological studies varied depending on the developmental endpoints examined, with more mixed findings for non-BWT measures. In addition, the evidence of inverse associations between PFDA exposure and birth weight and birth length was less compelling when based on early or prepregnancy measures of PFDA. This might be indicative of potential bias due to the impact of pregnancy hemodynamics on PFDA levels. Thus, despite the reasonably consistent evidence of an association between PFDA and different BWT-related measures, and more mixed findings for some other endpoints, there is considerable uncertainty given that sample timing differences may explain at least some of the reported fetal growth restriction deficits."

Comment: A reviewer stated that EPA should include a new study from Padula et al. 2023 that reported a significant inverse association for PFDA with birthweight.

EPA Response: EPA has reviewed the Padula et al. (2023) study and determined that its inclusion is not necessary given the overlap of this pooled analysis with data from populations examined in other ECHO cohort studies (Chang et al., 2022; Eick et al., 2020; Sagiv et al., 2018; Starling et al., 2017). Thus, the results were not anticipated to influence the judgments for either gestational duration or fetal growth restriction. For transparency, this characterization and rationale have been added to the Appendix I table describing the studies identified after release of the draft for public comment (see Table I-1 of Appendix I). We have also highlighted the Padula et al. study and noted the other overlapping ECHO cohort studies considered in a footnote in Table 3-19.

Comment: A reviewer stated that "Bach et al. (2016) did not log transform maternal PFDA serum levels in the continuous analysis, therefore the confidence rating in that analysis should be reduced or excluded."

EPA Response: EPA does not automatically downgrade a study especially given more recent thinking that cautions against automatically transforming exposure data due to skewness (Choi et al., 2022).

Comment: A reviewer stated that EPA "Should note that gestational age adjusted birth weight is the preferred measure for the birth weight outcome, and indicate which studies utilized that approach."

EPA Response: EPA could find no consensus reference on whether gestational age adjusted birth weight is the preferred measure of fetal growth restriction; this is due in part to documented concern over whether adjusting for gestational age may be a causal intermediate (Ananth and Schisterman, 2017). Given that term births represent a more etiologically homogeneous population, studies that restricted to term births only were considered more directly interpretable to estimate potential impacts on birth weight.

Comment: A reviewer stated that "Pubertal development is one of the four endpoints of developmental toxicity and should be discussed in this section."

EPA Response: The developmental toxicity synthesis is focused on a narrower etiological window for early childhood effects (e.g., within the first 2 years of life for epidemiological studies). Given that the reproductive system is further differentiated later in childhood, EPA evaluated and grouped evidence on pubertal endpoints with other reproductive endpoints. Thus, the available epidemiological data for pubertal development are discussed in the sections on male (see Section 3.2.4) and female (see Section 3.2.5) reproductive effects. On a related note, text from the Toxicological Review's synthesis of developmental effects indicates that "pubertal development is discussed in the reproductive sections (see Sections 3.2.4 and 3.2.5)."

Comment: A reviewer stated that "Given the multiple possible windows of exposure for this outcome [birth weight], I recommend stating when possible whether the individual study evaluated and conclusions drawn were concerning maternal and/or neonatal PFDA exposure, especially in the Table 3-24 [Table 3-23 in the revised Toxicological Review] summary of the weight of evidence."

EPA Response: EPA agrees that differentiating and highlighting differences across etiological windows, especially given the noted challenges with different sampling times for the exposure biomarkers, is essential to clarify the overall weight of evidence. Table 3-23 highlights this uncertainty in the "Factors that decrease strength" column, including descriptions of the sets of studies with the differing exposure biomarkers (which are delineated in greater detail by study in the evidence synthesis section): "Substantial uncertainty due to the potential impact of hemodynamic changes among studies showing birth weight deficits, especially based on late biomarker sampling defined at trimester 2 or later, e.g., 9 of 11 studies in the overall population and 6 of 9 studies in girls and 5 of 9 in boys." The revised Toxicological Review has also extended this evaluation of early versus late sampling timing to all developmental endpoints and these results are found in the summary statements for each individual endpoint.

Comment: A reviewer stated that "The argument that uncertainty around the relationship between sample timing differences and reported fetal growth restriction deficits needs to be strengthened. Is it more likely than not to be a confounding variable and what is the evidence for this opinion? If this isn't justified, then downgrading an important observation seems unwarranted."

EPA Response: This general issue is addressed in more detail in the Appendix F and specifics regarding potential impact on the current PFDA database are addressed in several sections on developmental epidemiological effects synthesis, dose response, and evidence integration and are highlighted for other publications (Dzierlenga et al., 2020; Steenland et al., 2018). For example, as we detail: "There was a definitive pattern by sampling timing as only 2 of the 11 studies (including 2 of 9 *medium*/*high* studies) reporting BWT deficits in the overall population had early sampling biomarker measures during pregnancy. The majority of sex-specific studies reporting BWT deficits were also based on later biomarker sampling (defined here as from the second trimester exclusive onward)." "Despite reasonably consistent evidence of an association between PFDA and different BWT-related measures, and more mixed findings for other endpoints, there is considerable uncertainty given that sample timing differences may explain at least some of the reported fetal growth restriction deficits."

Tier 2 Suggested Revisions

Comment: A reviewer stated that "The evidence integration section lacks coherence as its sequencing of topics and determinations could be clearer: it is easier to understand the findings by consulting Table 3-24 [Table 3-23 in the revised Toxicological Review]." The same reviewer also stated that on "Page 156, ~line 30–35: The text starting at line 30 seems like a more appropriate place to start the evidence integration section as it identifies the main endpoints and overall confidence."

EPA Response: It is standard IRIS practice to have the overall evidence integration judgment as the topic sentence in the opening paragraph of the evidence integration sections. Thus, while EPA has reorganized this section to offer more clarity, the starting place was not changed.

H.2.6. Public Comments on Developmental Effects

Comment: A commenter suggested that "considerations related to lower serum PFDA levels later in pregnancy due to hemodynamic factors discussed elsewhere in the document" be mentioned on p. 3–99, lines 19–20.

EPA Response: The comment is referencing discussion of considerations for evaluating the exposure domain of individual epidemiology studies of developmental effects. Text has been added in the revised Toxicological Review to clarify: "Although not considered as a factor influencing the exposure domain rating, the potential effects of hemodynamic factors later in pregnancy affecting serum PFDA levels and the result from individual studies is separately discussed in the evidence synthesis section and Appendix F."

Comment: A commenter suggested that the discrepancies in the mouse fetal body weight data (Harris and Birnbaum 1989) be discussed in the text section about the dose-response data from this study (p. 3–151, lines 1–9).

EPA Response: The following text was added to the developmental section as suggested: "It should be noted that the magnitude of fetal body weight changes was actually higher in the shorter

duration study (GD 10–13 vs. GD 6–15) at comparable doses. For example, decreases in fetal body weight were 4% and 10% at 0.25 and 1 mg/kg-day in the shorter (GD 10–13) experiment versus 1% and 4% at 0.3 and 1 mg/kg-day in the longer (GD 6–15) experiment. Although this dose-response trend is not expected, the reductions in fetal body weight observed in both experiments are still considered to be adverse."

Comment: One commenter stated that "Several *high* or *medium* confidence epidemiology studies of birth weight reported no association with PFDA exposure including studies limited to PFDA levels in the first trimester." The same reviewer stated that the evidence base for developmental effects "does not support a link between PFDA and lower birth weight in humans."
EPA Response: The majority of the external review panel (seven out of nine reviewers) agreed with the scientific justification for the selection of the studies/effects used in the derivation of the developmental RfD. EPA detailed the consistency between studies by confidence levels in Table 3-24 [Table 3-23 in the revised Toxicological Review] as well as evaluation of other potential sources of heterogeneity of results in the developmental epidemiological synthesis. And, as shown in that table and in other figures (e.g., see Figure 3-28), the majority of studies show inverse associations with mean BWT and PFDA exposures. Consistency in direction and magnitude of associations is also detailed in the various developmental effect syntheses and summary sections.

H.2.7. External Peer-Review Comments on Male Reproductive Effects

Peer-Review Comment Summary

In reference to charge question 2d, seven reviewers agreed with EPA's conclusions and would have liked to have longer-term data. Two of these reviewers included Tier 1 or Tier 2 comments to expand and clarify some discussions (see below). One reviewer did not comment on this topic.

Tier 1 Necessary Revisions

Comment: A reviewer commented, "Non-monotonicity of testosterone effects should be noted as a possible explanation for inconsistency among male reproductive endpoints in the human studies, which rarely considered such a dose response." The reviewer added that such considerations should be discussed as part of the literature review and evidence integration.

EPA Response: The following text on possible nonmonotonicity of testosterone effects was added to the evidence synthesis for human studies (see Section 3.2.4), "Given that the inverse associations were observed only in the studies with highest exposure concentrations in the participants, it is possible that the observed inconsistency is due to nonmonotonicity of the effect of PFDA exposure on testosterone, but the data are insufficient to determine whether this is likely, so the inconsistency decreases certainty."

Comment: In regard to the observed reductions in body weights in male rats, a reviewer noted, "While described as 'moderate' body weight changes, reductions of 21%–38% are well above

those typically considered adverse (e.g., >10% in adult animals) and would be associated with doses above the maximum tolerated dose (MTD) and overt toxicity. As such, the draft assessment should further elaborate upon, explain, and/or clarify the statement cited above (**Tier 1 necessary revision**)."

EPA Response: The body weight reductions (21%–38%) observed in the 28-day oral gavage studies were considered direct effects of PFDA on the male reproductive system on the basis of evidence from supplemental i.p. injection studies that examined potential effects of decreased body weight on PFDA-induced male reproductive toxicity. The i.p. injection studies used pair-fed controls that were weight-matched to PFDA treatment groups and showed that only large reductions in body weight (72%) are associated with confounding effects on male reproductive endpoints. The results are consistent with EPA's Guidelines for Reproductive Toxicity Risk Assessment that note only severe effects on body weight (e.g., >50% in some rodent studies) appear to preclude interpretations regarding reproductive effects. However, EPA agrees the term "moderate" may be confusing in this instance and has deleted it.

Comment: The same reviewer recommended that EPA scientifically justify the most human relevant laboratory species for evaluation of male reproductive effects or acknowledge this as an uncertainty.

EPA Response: The text in Section 3.2.4 has been edited: "In the absence of information to the contrary and given the conserved role of androgen-dependent pathways in male reproductive functions across species (including humans), the available evidence is considered to be relevant to humans. This assumption is based on *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996)." EPA's *Guidelines for Reproductive Toxicity Risk Assessment* state, "An agent that produces an adverse reproductive effect in experimental animal studies is assumed to pose a potential reproductive threat to humans." Supporting this, EPA's Review of the RfD and RfC processes (2002) document states, "Absent a clearly most-relevant species, the most sensitive mammalian species is used, that is, the species that shows toxicity at the lowest exposure level."

Comment: A reviewer identified a new study that evaluated sperm DNA fragmentation in humans (Pan et al., 2019) and recommended "There should be cross referencing with those finding under this toxicity endpoint. Tier 1 recommendation is to ensure that this cross-referencing is done and sited as mechanistic evidence supportive of the current conclusion in this IRIS review."

EPA Response: As stated in Section 1.2.1, EPA evaluated studies identified during the latest literature search update (through March 2023) and public comment and provided this to the peer reviewers. As part of this evaluation, EPA's disposition on whether the studies would be incorporated on the basis of their material impact on the assessment conclusions was provided. Pan et al., (2019) was included in this compilation provided to reviewers. Although this was not a new study and it had already been considered by EPA, the study was revisited given the reviewer recommendation above. The study authors reported an association with increased sperm DNA fragmentation; however, the results for semen parameters most informative for evaluating male

reproductive effects (i.e., motility, concertation, and morphology) were null, inconsistent, or imprecise (nonsignificant). Therefore, the mixed findings were not clearly supportive (nor clearly inconsistent) and EPA ultimately did not incorporate the study in the Toxicological Review since it does not influence the assessment conclusions for human or mechanistic evidence or the overall hazard judgment for male reproductive effects. Text was added to Table I-1 in Appendix I to clarify EPA's disposition on the DNA fragmentation findings from the Pan et al., (2019) study.

Tier 2 Suggested Revisions

Reviewers had no tier 2 revisions.

H.2.8. Public Comments on Male Reproductive Effects

No public comments were provided.

H.2.9. External Peer-Review Comments on Female Reproductive Effects

Peer-Review Comment Summary

In reference to charge question 2e, eight reviewers agreed with EPA's weight-of-evidence conclusion for PFDA-induced female reproductive and would have liked longer-term data and more mechanistic studies whereas a lone reviewer stated that the conclusion "may be too strong given the sparse data of the single rat study" (Tier 2). Of the eight reviewers who agreed, two provided Tier 1 and 2 comments for further justification and clarity. For example, one reviewer stated that "The lactation duration outcome should be included in this section, as the pregnancy and postpartum period is a sensitive developmental window for mammary gland development." Tier 1 Recommendations and Tier 2 Suggestions are provided below.

Tier 1 Necessary Revisions

Comment: A reviewer stated that "Figure 3-53: It is unclear which are male, and which are female, these should be labeled."

EPA Response: The labels on the forest plot have been added.

Comment: A reviewer stated that "The lactation duration outcome should be included in this section, as the pregnancy and postpartum period is a sensitive developmental window for mammary gland development (as is in utero/early life). It should also be noted that mammary gland development is a sensitive outcome for other PFAS (e.g., PFOA)." The reviewer also provided citations for two epidemiolocal studies (Timmerman et al. 2017 and Romano et al. 2016) that reported associations between PFAS exposure and lactation duration as examples.

EPA Response: The studies on breastfeeding duration have been added to the Toxicological Review (see Section 3.2.5). The following statement regarding mammary gland development can be found in Section 4: "Further, the absence of studies examining the potential for effects of PFDA exposure on the thyroid in developing organisms, or on mammary glands, represent data gaps in

light of associations observed for other PFAS, such as PFBS, PFOS, and PFOA (ATSDR, 2018; U.S. EPA, 2018)."

Comment: A reviewer stated that "The discussion and evidence stream (Table 3-29) should be revised to reflect the following points:

"The statement of 'unclear biological relevance of increases' in testosterone in the single (*high* confidence) animal study (NTP 2018) should be revised to note coherence with the finding of continuous diestrous as high levels of androgens in women causes infrequent, irregular, or non-existent menstruation.

The finding of high testosterone and a continuous phase of diestrous in the (NTP, 2018) study is also consistent with the finding of decreased progesterone in Leydig tumor cells (Zhao et al., 2017). High levels of testosterone in women are associated with levels of progesterone, irregular menstruation, and decreased fertility."

EPA Response: EPA responds to these comments as follows:

1. Text was added to Section 3.2.5 to highlight the possible coherence between increased testosterone levels and prolonged diestrus observed in PFDA-treated rats from the NTP 2018 study. The following text was added to the section on hormone levels: "studies have shown that high levels of androgens (e.g., testosterone) can cause irregular menstruation cycles in women. For example, Van Anders and Watson (2006) reported an association between high levels of testosterone and increased menstrual cycle length in healthy premenopausal women. Such findings could suggest possible coherence between increased testosterone levels and prolonged diestrus observed in PFDA-treated female rats from the (NTP, 2018) study given that the mechanisms responsible for regulating female reproductivity (e.g., estrous cyclicity in rats and menstrual cycling in humans) are similar between rats and humans (Goldman et al., 2007; Bretveld et al., 2006)." Subsequently, corresponding text was added to the evidence profile table for female reproductive effects.
2. As stated in Table 3-29, the evidence of PFDA-decreased progesterone production in mouse Leydig tumor cells (Zhao et al., 2017) cannot be corroborated with data from the lone reproductive study in rats (NTP, 2018), given that progesterone levels were not measured in the 2018 NTP study. Furthermore, the available scientific literature for links between progesterone and testosterone do not seem to provide support for the PFDA-induced effects on the female reproductive system. For example, decreased progesterone levels may actually lead to low testosterone given that progesterone is a precursor to the production of testosterone. Additionally, testosterone was shown to increase progesterone production under in vitro conditions (Rangel et al., 2007). Therefore, this recommendation was not incorporated into the Toxicological Review.

Comment: A reviewer asked for more clarification regarding the following statement: "Although decreased body weight in female rats was observed at the same doses (body weight

decreases were 12%–36% at ≥1.25 mg/kg-day; refer to Section 3.2.10 on General toxicity effects for more details) as effects on estrous cyclicity, it is unclear if these effects are related and the effect on female reproductive function is disproportionately more severe and concerning than the moderate changes in body weight." The body weight changes are described as "moderate" but reductions of 21%–38% are well above those typically considered adverse (>10% in adult animals).

EPA Response: It is noted that the EPA reproductive toxicity guidelines discuss body weight changes using the terms "mild," "moderate," and "severe," citing evidence that only "severe" effects on body weight (e.g., >50% in some rodent studies) appear to preclude interpretations regarding reproductive effects. It is also noted that a 10% change in body weight is not necessarily a cutoff for adversity, although it can be and has been used as an appropriate BMR (or minimally adverse change), depending on the study- and evidence-specific decision context. However, EPA agrees the term is confusing in this instance and thus deleted "moderate."

Comment: The same reviewer also asked for more clarification regarding the following statement: "Although body weight has been shown to fluctuate during the different estrous stages and weight loss has been shown to correlate with disrupted estrous cyclicity in rats (Tropp and Markus, 2001), it is not possible to determine if the decreases in body weight in female rats might be responsible for the effects on estrous cyclicity observed in the NTP (2018) study. Furthermore, even though no changes were observed on other stages of the estrous cycle (i.e., proestrus and metestrus), the effects of PFDA on estrus and diestrus are still considered biologically relevant given the potential influence that the lack of cyclicity may have on fertility, regardless of whether the observed decrease in body weight may have partially contributed to these changes." The reviewer further stated that "EPA should clarify how it was determined that decreases in body weight only 'may have partially contributed' to estrous cycle effects given the statement [emphasis added] that 'it is not possible to determine if the decreases in body weight in female rats might be responsible for the effects on estrous cyclicity observed.'"

EPA Response: As the reviewer points out above, the Toxicological Review indicates the PFDA-induced effects on estrous cyclicity are disproportionately more severe than changes in body weight. For example, the percentage of time spent in diestrus was statistically significantly increased by 27–63% at ≥1.25 mg/kg-day compared with 12%–36% decreases in body weight at the same doses. Given the PFDA-induced estrous effects are more severe than body weight changes, these data, at a minimum, are interpreted to indicate the effects on diestrus and estrus are not entirely due to decreased body weight. Furthermore, without time-course data showing effects on estrous cyclicity and body weight at multiple intervals, it is not possible to make definitive conclusions on the possible contribution of PFDA-induced body weight changes on estrous cyclicity effects.

Comment: The same reviewer commented that EPA should scientifically justify the rat as the most human relevant laboratory animal species for female reproductive effects or acknowledge this as an uncertainty.

EPA Response: The following text has been added to Section 3.2.5: "These findings are interpreted as relevant to humans in the absence of evidence to the contrary. This assumption is based on *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996)." As stated in EPA's review of the RfD and RfC processes (2002) document, "Absent a clearly most-relevant species, the most sensitive mammalian species is used, that is, the species that shows toxicity at the lowest exposure level." Furthermore, EPA's *Guidelines for Reproductive Toxicity Risk Assessment* states that "An agent that produces an adverse reproductive effect in experimental animal studies is assumed to pose a potential reproductive threat to humans." In the case of PFDA, no data were identified to suggest the rat data from the NTP 2018 study would not be relevant to humans.

Tier 2 Suggested Revisions

Comment: A reviewer stated that EPA "Should note that while the histopathology study found no effects on the mammary glands, timing of the exposure and outcome was not in a sensitive developmental window (in utero or pregnancy)."

EPA Response: The text in the evidence integration discussion of Section 3.2.5 has been edited as follows: "Given the short-term duration of the lone animal study, it cannot be reasonably ruled out that detectable histopathological effects could have become apparent with a longer study duration or during a sensitive developmental window (e.g., in utero or pregnancy)."

Comment: A reviewer stated that the hazard conclusion for female reproductive effects may be too strong given the sparse data of the single rat study and should be reconsidered.

EPA Response: The hazard conclusion for PFDA-induced female reproductive effects is based on considerations outlined in the IRIS Handbook where a single well-conducted study can be used to support an evidence synthesis conclusion of "*moderate*" and an evidence integration conclusion of "***evidence indicates (likely)***." In addition, this conclusion was supported by eight of the nine peer reviewers.

H.2.10. Public Comments on Female Reproductive Effects

No public comments were provided.

H.2.11. External Peer-Review Comments on Cardiometabolic Effects

Peer-Review Comment Summary

In reference to charge question 2f, all nine reviewers agreed with EPA's conclusions. Two reviewers provided Tier 2 suggestions for improvement (see below).

Tier 1 Necessary Revisions

Reviewers had no Tier 1 revisions.

Tier 2 Suggested Revisions

Comment: While in agreement with EPA's conclusions, a reviewer provided the following comment, "The justification of [cardiometabolic effects] would be more coherent if there was a logic tree that walked the reader through the various pathways by which PFDA affects each examined contributor to overall cardiometabolic health." Similarly, another reviewer suggested, "It would be useful to include some background on why this was evaluated based on MOA."

EPA Response: Given the limited nature of the evidence for cardiometabolic effects, it is not possible to accurately characterize the pathways as suggested without additional scientific study. The agreed-with conclusion of *slight* human evidence and ***suggestive*** evidence overall is intended to highlight the need for such additional research and thus, no additional MOA analysis or pathway examination was added.

Comment: A reviewer noted, "The clinical relevance of Tables 3-30, 3-33, and 3-34 could be made easier to interpret if the various measurement units for the various relevant outcomes (such the lipid subfractions, fasting blood glucose, and waist circumference, respectively) were provided for the studies that reported the ß effect estimate (Tier 2 Recommendation)." This reviewer added "It would be additionally helpful in Section 3.2.6 to provide more details of the lipid subfractions reported in the studies (e.g., the strength and directionality of association between PFDA exposure and not just total cholesterol levels, but also LDL, HDL, and triglycerides, since HDL elevation is protective while elevation of the other subfractions are adverse outcomes); similarly, the evidence integration section can be better clarified in this regard [Tier 2 Recommendation])."

EPA Response: Units for the outcome measures were added to Tables 3-30, 3-33, and 3-34. Results are presented for LDL and triglycerides in Table 3-30 and discussed in text. Data for HDL were also reviewed to determine whether they would influence assessment conclusions and were determined not influential to the assessment conclusions.

H.2.12. Public Comments on Cardiometabolic Effects

No public comments were provided.

H.2.13. External Peer-Review Comments on Neurodevelopmental Effects

Peer-Review Comment Summary

In reference to charge question 2g, all reviewers agreed with EPA's conclusions. Two reviewers provided Tier 2 suggestions (see below).

Tier 1 Necessary Revisions

Reviewers provided no Tier 1 revisions.

Tier 2 Suggested Revisions

Comment: A reviewer suggested "Table 3-38 can be strengthened if it has more information on participants ages/age categories and breaking out results by gender as feasible when clinically relevant (e.g., ADHD tends to be more often diagnosed in boys vs. girls)."

EPA Response: Additional detail has been added to the table on sex and age. None of the studies presented sex-stratified results, so results broken out by gender is not possible, but each of the studies either matched for sex or adjusted for it. Those that did not match did additionally examine potential interaction by sex but there was no clear indication of a difference in response by sex.

Comment: Another reviewer asked for clarification (with one or two sentences) on the availability of any supportive evidence such as coherence with mechanistic data (e.g., CompTox data) or animal data and a reference to possible consistency with other long-chain PFAS.

EPA Response: As described in Section 3.2.7, the database for PFDA-induced neurodevelopmental effects is limited to a few human studies that showed inconsistent results and no data in animals via any relevant route and duration. Given these uncertainties and the overall paucity of mechanistic data available to inform this health outcome, EPA did not perform a comprehensive evaluation of mechanistic data or supplemental information from other PFAS since it would not change the assessment conclusions or help address key data gaps.

H.2.14. Public Comments on Neurodevelopmental Effects

No public comments were provided.

H.2.15. External Peer-Review Comments on Endocrine, Urinary and Other Noncancer Effects

Peer-Review Comment Summary

In reference to charge question 2h, five reviewers agreed with EPA's conclusions for endocrine (i.e., thyroid), urinary, and other noncancer effects, whereas two reviewers agreed with the conclusions for only urinary and other noncancer effects. Three reviewers disagreed with EPA's conclusions for endocrine effects, with two reviewers providing Tier 1 comments questioning EPA's interpretations regarding potential thyroid toxicity (see below). One reviewer did not explicitly state whether they agreed with EPA's conclusion for endocrine effects but did provide Tier 1 and Tier 2 comments (see below).

Tier 1 Necessary Revisions

Comment: A reviewer stated that EPA should clarify that the conclusions of inadequate evidence for endocrine effects do not apply for reproductive hormones (male and female reproduction), which are also part of the endocrine system.

EPA Response: The following text was added to Section 3.2.8: "Potential PFDA effects on male and female reproductive organs (e.g., testes and ovaries) and reproductive hormones

(e.g., testosterone) that also encompass part of the endocrine system are discussed in the sections on male reproductive effects (Section 3.2.4) and female reproductive effects (Section 3.2.5."

Comment: A reviewer stated that EPA should reexamine the evaluation of epidemiological studies for thyroid effects for consistency in the interpretation of pathway responses from other IRIS Toxicological Reviews on related PFAS (e.g., PFHxA). Several factors that were used to identify studies as "deficient" are concerning, such as only studies with fasting thyroid measurements being deemed nondeficient and considering studies that did not take thyroid measures at the same time of day for all measurements deficient. Another concerning factor is the difference in interpretation of the human studies for PFDA versus other PFAS that found consistent effects for thyroid hormones that were not always associated with changes in TSH. This reviewer thought the designation for the human evidence for PFDA would be *slight* or higher on the basis of these considerations.

A second reviewer echoed the concerns about deficiencies identified by EPA on the basis of how thyroid measurements were taken, stating "In the methodological considerations of the endocrine studies, serum thyroid hormone concentrations are not usually regarded to have diurnal variation, and this may have thus been an unnecessary limitation" and "to not regard thyroid-related studies that failed to consider diurnal variation as deficient."

EPA Response: The text in the Toxicological Review has been revised to emphasize that lack of consideration of fasting or diurnal variation was not considered a major source of bias and that studies were not downgraded in overall study confidence if this was the primary limitation identified (i.e., such a limitation led to a rating of "*deficient*" only within the outcome domain, but this limitation alone did not decrease the potential influence of these studies on the evidence synthesis judgment as such studies were *medium* confidence overall, as shown in Figure 3-79; the outcome domain is the third column).

The statement about lack of coherence across thyroid measures has been removed.

Regarding the judgment of *slight* versus *indeterminate*, EPA feels the conclusion of *indeterminate* is justified because the majority of studies are null. There is a concern for reduced sensitivity due to limited exposure contrast in most of the studies, which reduces our ability to interpret null results (i.e., they are not interpreted as evidence of *no effect* given this limitation), however, there is not sufficient evidence to reach *slight* or higher at this time. A table of results has been added to the synthesis, which may make it easier for readers to get an understanding of the existing evidence.

Comment: The same reviewer also stated that EPA should reexamine the animal studies for thyroid effects as the designation of "lack of expected coherence across thyroid measures with any currently available understanding of adverse thyroid-related change" does not appear consistent with conclusions or observations for other PFAS. The reviewer suggested that an evidence stream judgment of "*moderate*" would be more appropriate based on the in vivo animal data.

EPA Response: It is important to note that the PFDA database for thyroid effects is more limited in the number of studies and less consistent when compared with other PFAS. For example, PFBS exposure was shown to decrease total T3, total T4, and free T4 in pregnant mice, nonpregnant adult male and female rats, and female offspring mice exposed through gestation (U.S. EPA, 2021a). Furthermore, other PFAS chemicals like PFHxA and PFHxS have been shown to cause similar effects to PFBS on the thyroid economy in rodents (decreased T3 and T4 with normal levels of TSH) (U.S. EPA, 2023b, 2021a). In the case of PFDA, effects on the thyroid economy in rodents were limited to a single study in adult rats (NTP, 2018). In the NTP 2018 study, PFDA caused a statistically significant decreasing trend in TSH in males only, a statistically significant increasing trend for T3 in male and female rats with significant increases reported at ≥1.25 mg/kg-day for females only, statistically significant decreases in free T4 in both sexes, and a lone statistically significant decrease in total T4 at the second lowest dose in males only. The lack of large or consistent decreases in total T4 and increases in T3 with PFDA exposure are key divergences from the findings for other PFAS with stronger evidence judgments; the reason for these inconsistencies requires further study. So not only are the effects of PFDA on thyroid homeostasis mixed and incoherent, but they are also not consistent with other PFAS. Further inconsistency is introduced by the mechanistic and supplementary data for PFDA. Taken together, EPA feels that the evidence synthesis of *indeterminate* for the endocrine effects in animals is justified for PFDA. Also as stated above, the majority of the peer-review panel (five of the nine reviewers) agreed with EPA's conclusions for endocrine effects; an additional reviewer did not explicitly state whether they agreed with EPA's conclusion for endocrine effects.

Comment: The same reviewer also stated that EPA should clarify in the animal study discussion and evidence integration table (Table 3-42 of the revised Toxicological Review) that histopathology findings would not necessarily be expected from the 28-day rat study given the short-term duration of the study. This is consistent with the observed null histopathological findings.

EPA Response: The following text was added to the evidence integration discussion of Section of 3.2.8: "Regarding the lack of PFDA-induced histopathological changes in endocrine tissues, it cannot be reasonably ruled out that detectable histopathological effects could have become apparent with a longer study duration."

Comment: The same reviewer also stated that EPA should reevaluate the evidence integration judgment of "inadequate evidence" given the previous comments and consider a designation of "suggestive" or higher.

EPA Response: The overall summary judgment is based on the separate evidence stream judgments, in this case *indeterminate* for evidence in both humans and animals and with limited, inconsistent mechanistic support (comment responses on these separate judgments are provided above). Given those judgments, ***inadequate evidence*** is the appropriate combined judgment (please see the PFAS protocol and IRIS Handbook for additional details).

Comment: A reviewer stated that "Any included studies of thyroid hormone status in pregnancy should not be combined with the same assessments as non-pregnant adults, given the different reference ranges for TSH and of changes in the binding of serum thyroid hormone levels in gestation."

EPA Response: The synthesis has been updated to separate general population adults and pregnant women.

Comment: The same reviewer also stated that "Given the multiple possible windows of PFDA exposure on thyroid effects, it would be clearer to state when possible whether the individual study evaluated, and conclusions drawn were concerning maternal and/or neonatal PFDA exposure."

EPA Response: A table that includes the timing of exposure measurement has been added to the synthesis of human evidence.

Comment: A reviewer stated that "The Agency should incorporate the concept of 'coherence' in terms of the response to chemicals that do not act as a goitrogen per se."

EPA Response: As suggested by the reviewer, EPA reviewed the available data for thyroid effects induced by nongoitrogenic chemicals (e.g., PCBs, PBDEs) to compare to PFDA effects. Goldey and Crofton (1998) reported that gestational exposure to Aroclor 1254 decreased levels of T4 in rats with no effect on T3. In adult rats, Aroclor 1254 decreased levels of T4 with no change in T3 or TSH (Hood and Klaassen, 2000). PBDE mixtures were reported to decrease levels of T4 and T3 in weanling rats with no effect on TSH (Zhou et al., 2001). In short, the effects of PCBs and PBDEs on thyroid economy do not appear to be comparable or add support to the results of PFDA, but they do exhibit results similar to those observed following exposure to PFAS other than PFDA (e.g., PFBS; PFBA; PFHxA; PFHxS). It should also be noted that EPA is not questioning the adversity of decreased T4 but rather concluding that that there is ***inadequate evidence*** to determine whether PFDA exposure might cause thyroid (or other endocrine) effects in humans. Also as stated above, the majority of the peer-review panel (five of the nine reviewers) agreed with EPA's conclusions for endocrine (i.e., thyroid) effects; an additional reviewer did not explicitly state whether they agreed with EPA's conclusion for endocrine effects.

Tier 2 Suggested Revisions

Comment: A reviewer stated that "Established determinations of thyroid effects for other PFASs (e.g., PFOA and PFOS) should be noted" and that the human evidence for the thyroid hormone section "would benefit from a table and/or figure grouped by confidence level and noting limitations."

EPA Response: This information can be found in Table 4-1 (see Section 4.1) that facilitates comparisons of endocrine toxicity hazard conclusions across EPA PFAS assessments. A table of results sorted by population and study confidence has been added to the human evidence synthesis.

Comment: A reviewer stated that "Animal studies and even human studies with compounds that target the thyroid system result in outcomes that are not classic (decrease in serum T4 and

increase in serum TSH). The results are often not considered or understood. It would be very useful for the EPA offices to have the same view about thyroid endpoints."

EPA Response: EPA's conclusions for PFDA-induced endocrine effects are not inconsistent with other EPA assessments. As noted above, the PFDA database for thyroid effects is not identical, is more limited with respect to the number of studies and is less consistent when compared with other PFAS and other chemical groups like PCBs and PBDEs. For example, were the PFDA-specific studies showing effects (that were at all consistent, large, or dose-dependent) only on serum total T4, with or without changes in TSH, the assessment would make a stronger evidence synthesis judgment.

H.2.16. Public Comments on Endocrine, Urinary, and Other Noncancer Effects

Comment: A commenter suggested "mentioning that the possible contribution of decreased food consumption to decreased body weight is unknown in some or all of these studies (e.g., NTP, 2018, and Frawley et al., 2018) because food consumption was not measured [page 3–283, lines 1–20]."

EPA Response: The suggested text was added to Section 3.2.10. Furthermore, the following text can be found in the section: "Key issues regarding study quality evaluation in the *medium* and *low* confidence studies were related to exposure sensitivity (no analytical verification methods or quantitative data on food consumption)."

H.3. CHARGE QUESTIONS 3 AND 4 – NONCANCER TOXICITY VALUE DATA SELECTION AND MODELING

3. For PFDA, no RfC was derived for inhalation exposures. An RfD is derived based on studies by Budtz-Jørgensen and Grandjean (2018) and Grandjean et al. (2012) showing decreased serum antibody concentrations for both tetanus and diphtheria in children (male and female) at age 7 years and PFDA measured at age 5 years and developmental effects (i.e., reduced birth weight in humans) from the Wikström (2020) study. Given the close proximity of the developmental and immune PODs and resulting osRfDs and because these effects are observed during the developmental period, they are selected as co-critical effects supporting the RfD. Is the selection of the studies for the immune (Budtz-Jørgensen and Grandjean, 2018) and developmental (Wikström, 2020) effects for use in deriving the RfD values for PFDA scientifically justified? Are the modeling approaches appropriate?

    a) If so, please provide an explanation.

    b) If not, please provide an alternative study(ies) or effect(s) that should be used to support the derivation of the lifetime RfD and detail the rationale for use of such an alternative.

    c) As part of the recommendations in "a" or "b" above, please comment on whether the effects selected are appropriate for use in deriving the lifetime RfD, including considerations regarding adversity (or appropriateness in representing an

adverse change) and the scientific support for their selection.[8] Please also see charge questions 2b and 2c.

d) EPA used benchmark dose modeling (BMD) (U.S. EPA, 2012) to identify points-of-departure (PODs) for PFDA. Are the BMD modeling approaches, selection and justification of benchmark response levels, and selection of the BMD models used to identify each POD for toxicity value derivation scientifically justified and clearly described?

e) For liver, male reproductive and female reproductive effects, quantitative information was limited to studies in animals exposed to PFDA for 28 days and little to no information was available to evaluate the effects of chronic exposure on these health hazards. Therefore, the derivation of lifetime organ-specific (os) RfD values was not attempted for liver, male reproductive and female reproductive effects. However, these endpoints were considered for the derivation of subchronic osRfDs. Does the provided scientific rationale support this decision? Please explain.

f) Given the lack of studies on inhalation exposure to PFDA, no reference concentration (RfC) is derived. Please comment on this decision.

4. In addition, for PFDA, an RfD for less-than-lifetime ("subchronic") exposures is derived. No subchronic RfC was derived. The same studies and outcomes were chosen for use in deriving the lifetime and subchronic RfDs. Are the selection of these studies and these effects for the derivation of the subchronic RfD for PFDA scientifically justified?

   a) If so, please provide an explanation.

   b) If not, please provide an alternative study(ies) or effect(s) that should be used to support the derivation of the subchronic RfD and detail the rationale for use of such an alternative.

   c) As part of the recommendations in "a" or "b" above, please comment on whether the effects selected are appropriate for use in deriving the subchronic RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for their selection.

[8]For the decreased antibody responses, Selgrade (Tox Sci 2007;100:328–332) suggests these specific immunotoxic effects may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards.
For developmental effects (i.e., fetal growth restriction), the human evidence was determined to be *slight*, primarily due to potential confounding by hemodynamic changes among studies showing birth weight deficits. For the study (i.e., Wikström, 2020), used to derive the developmental RfD, there is no presumed impact of pregnancy hemodynamics given the early sampling (96% from the first trimester). However, unlike the Wikström (2020) study, some uncertainty remains across many of the available human developmental studies given the predominance of associations that were detected were for studies with later pregnancy sampling.

d) Please comment on the other subchronic osRfDs (i.e., for liver, male reproductive, and female reproductive effects).

e) Given the lack of studies on inhalation exposure to PFDA, no subchronic RfC is derived. Please comment on this decision.

H.3.1. External Peer-Review Comments on Noncancer Toxicity Value Data Selection and Modeling for the Lifetime ("Chronic") Reference Dose (RfD)

Peer-Review Comment Summary

In reference to charge question 3, the majority (seven) of reviewers agreed that selection of Budtz-Jørgensen and Grandjean (2018) and Wikström (2020) for deriving the RfD values was scientifically justified and that the modeling approaches were appropriate for immune and developmental effects, respectively. One reviewer did not comment in response to this question. Another reviewer commented that the selection of the studies was not scientifically justified and appropriate for modeling RfDs for immune and developmental effects of PFDA, and recommended modeling of alternative studies. The reviewers provided several Tier 1 and Tier 2 comments (see below) on this topic, and several Tier 3 future considerations were identified (see peer-review report).

Specifically in reference to charge question 3a, one reviewer (with concurrence expressed by three other reviewers during the meeting) commented that while the studies were reliable sources given the underlying data, EPA should comment on clinical relevance, uncertainties, and justification of the choices made in the derivation of the RfDs, and another reviewer similarly recommended that EPA add additional discussion of confounding in the selected studies (see below). A fifth reviewer added that selection of co-critical effects is reasonable given the proximity of the points of departure for the effects and that they are both observed during the developmental period.

In reference to charge question 3b, while the majority of reviewers supported EPA's decisions, one reviewer did not agree and provided several Tier 1 recommendations (see below). In general, the reviewer commented that for immune effects, Budtz-Jørgensen and Grandjean (2018) reported inconsistent and/or insignificant associations between antibody concentrations and PFDA, did not adjust for confounding from associated other PFAS, defined the "clinically protective" level of serum antibodies as a higher concentration than typical for the assay, and also noted no evidence of increased incidence of tetanus or diphtheria, despite NHANES data indicating that the U.S. population geometric mean serum level of PFDA is higher than the POD. Further, the reviewer commented that EPA did not adequately consider other immune studies with null findings. For birth weight, the reviewer commented that EPA should use a metanalysis or data from Harris and Birnbaum (1989) rather than Wikström (2020), given the weight-of-evidence designation for the epidemiological literature as *slight*.

In reference to charge question 3c, three reviewers agreed that the selected effects are appropriate for use in deriving the lifetime RfD, with one of the reviewers suggesting EPA increase discussion of the pharmacokinetic differences associated with pregnancy that could affect the interpretation of the epidemiological studies (see below). Most reviewers did not comment on 3c.

Specifically in reference to charge question 3d, five reviewers agreed that the BMD modeling approaches, selection and justification of BMRs, and selection of BMD models used to identify each POD for toxicity value derivation are scientifically justified and clearly described and did not provide additional comments. Two reviewers commented that while some aspects were clearly described, the issue of mixtures and confounding from other associated PFAS was not adequately addressed (see below). One reviewer did not comment on 3d. The last reviewer disagreed with EPA's approaches (see comments below). This reviewer indicated that the modeling approaches in general were scientifically justified but provided Tier 1 comments disagreeing with EPA's decisions regarding the adversity of the immune effect (decreased antibodies) and the justification EPA provided for the use of the lower BMR. This reviewer also recommended using the rodent study by Harris and Birnbaum (1989) rather than a single epidemiological study for developmental effects.

In reference to charge question 3e, six reviewers agreed with the scientific rationale to consider deriving subchronic osRfDs but not lifetime osRfDs because of the limited quantitative information, with one of the reviewers suggesting increasing the uncertainty factor range to estimate chronic osRfDs. One reviewer commented that the Kim et al. (2019) PBPK model should have been modified for PFDA to derive subchronic osRfDs. The remaining two reviewers did not comment on 3e.

Tier 1 Necessary Revisions

Comment: A reviewer commented on the issue of confounding from PFAS coexposures and made the following recommendation: "Expand the discussion and consideration of confounding factors (especially co-exposures to PFNA) in the context of their potential quantitative impact on the derivation of the RfD for immune and developmental effects. At a minimum clarify the rationale for evaluating the effects of PFNA co-exposures as non-significant based on the analytical details of the regression models performed by the authors of Budtz-Jørgensen and Grandjean 2018 (HERO 7276745)."

EPA Response: The text in Section 3.2.2 has been revised to provide additional clarity on potential confounding by other PFAS for immune effects, as follows:

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

respectively). The authors assessed the possibility of confounding in a follow-up paper (Budtz-Jørgensen and Grandjean, 2018a) that reanalyzed data from both Grandjean et al. (2012) and (Grandjean et al., 2017) for benchmark analysis. In this reanalysis, effect estimates for PFDA were adjusted for PFOS and PFOA. Details of the analytic results were provided to EPA by the authors (Budtz-Jørgensen and Grandjean, 2018b). There was variable attenuation of the observed effect estimates across the different analyses, and PFNA (the PFAS with the strongest correlation with PFDA) was not adjusted for in these models. However, details of the regression modeling results (Budtz-Jørgensen and Grandjean, 2018b) show that PFNA was a nonsignificant predictor of either tetanus or diphtheria antibody concentrations with associations just 15% the strength of the PFDA association and thus PFNA could not have been a meaningful confounder. Further, adjustment of the PFDA association by PFOS and PFOA did not eliminate the association, so confounding by co-occurring PFAS is unlikely to fully explain the associations. The details of the effects of PFDA with, and without, control of PFOS and PFOA are shown in Appendix C.1.1 with discussion of the impact and implications of multiple confounder control. Overall, while it is not possible to rule out confounding across PFAS, the available evidence suggests that it is unlikely to explain the observed effects. Other sources of potential confounding, including possible coexposures such as PCBs, were controlled appropriately. However, Grandjean et al. (2012) showed the correlation of PCBs with PFDA in their Table 2 at age 5 years as r = 0.14; the low correlation with PFDA means that PCBs could not have been a meaningful confounder of the PFDA effect estimate."

Comment: In regard to the BMD modeling approach used to derive PODs from epidemiological studies, a reviewer commented "… From a toxicology perspective it appears unlikely that a molecule (PNDA[PFDA]) which represents only a few percent of a total mixture concentration in serum can be teased out for regulatory purposes. However, from an epidemiology perspective and a BMDL perspective this was accomplished. This needs to be explained in a lay [non-epidemiological] language. Provide the strengths and weaknesses of this methodology."

EPA Response: EPA agrees that having more mixtures-based toxicological studies may shed further light on the potential contribution of individual PFAS for different endpoints. EPA has commented in various sections on the potential impact of other PFAS coexposures, including the most highly correlated and most consistent one (PFNA), and the degree that the epidemiological evidence helps elucidate the potential impact of other PFAS on PFDA responses.

In some instances, EPA can use information about the relative effects of other measured PFAS to ascertain that a weaker effect cannot substantially confound a stronger effect (see response to previous comment).

Comment: Two reviewers commented on the selection of the BMR for immune effects. One reviewer asked for more clarity, stating "The justification for using the response level is not completely clear, and should be compared with other options, e.g., a 10% BMR, so the authors can discuss and illustrate the significance of this choice." The second reviewer disagreed with the BMR justification, indicating "… the draft assessment: (a) is not using the demonstrated

severity/adversity of the effect itself (decreased antibodies) as support for the lower BMR (i.e., ½ SD); and (b) is using an admittingly unclear definition of an adverse effect (antibodies <0.1 IU/mL)[32] in an attempt to justify/support the lower BMR of ½ SD. (a) is problematic and (b) is internally inconsistent as the draft does not scientifically support the definition of <0.1 IU/mL as the adverse cutoff. These justifications/support for the BMR should be removed (Tier 1 necessary revision)."

EPA Response: EPA defines an "adverse effect" as a "biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism or reduces an organism's ability to respond to an additional environmental challenge" (U.S. EPA, 2002). Immunosuppression is a functional impairment (IPCS, 2012). Whether or not the observed immunosuppression is eventually identified with clinical studies, it is clear that fewer antibodies reduce people's ability to respond to additional immunological challenges. Thus, EPA considers decreased antibody concentrations an appropriate endpoint for estimating an RfD. Regarding the magnitude of the benchmark response (BMR), EPA explained in Appendix C.1 that in the absence of a scientific consensus on the size of a change in antibody concentrations, the EPA technical guidance suggests the use of a 1-SD change (akin to a BMR of a 10% change), or a ½-SD change (akin to a 5% change). Thus, reviewers can compare PODs across endpoints at the ~10% or 5% BMR levels. It is noted that the practice is often to use a lower BMR (e.g., 5% or ½ SD) for effects resulting from exposure during development due to the assumption of increased susceptibility, even for similar outcomes that might warrant a higher BMR (e.g., 10% or 1 SD) if the exposure was to adults. As stated above, this practice is supported by EPA's Benchmark Dose Technical Guidance, which states "…if warranted by statistical or biological considerations, a lower or higher increment of the control [standard deviation] might be used." (U.S. EPA, 2012)

Comment: A reviewer questioned the decision to select BMDLs for the derivation of the reference dose for immune effects that did not control for PFOS and PFOA, noting that "… The results of Weisskopf et al. (2018) do not constitute reasonable doubt that for these PFDA results, the confounding from not adjusting for co-exposures to documented immunotoxicants (PFOS, PFOA) is significantly greater than the potential amplification of biases that remains undemonstrated under the same or similar circumstances. EPA should reevaluate the issues raised above in regard to implications for their draft PFDA assessment (Tier 1 necessary revision)."

EPA Response: Appendix C.1 provided explanations acknowledging the potential for confounding and confidence judgments on the resulting PODs on the basis of the uncertainty in the POD that might be attributable to potential confounding. There was a statistically significant effect of PFDA in the single-PFAS model, and when PFOS and PFOA were added to the model, there was some attenuation of the point estimate (37% smaller) and the *p*-value lost statistical significance ($p = 0.15$). Given that the multi-PFAS model included three PFAS variables that were correlated with each other by about r = 0.5, loss of statistical significance is not unexpected. There was less attenuation in the lower bound of the point estimate (18%), which is used to derive the BMDL. EPA

also noted that the cause of the difference in the effect estimates for PFDA could have been due to coexposure amplification bias and that it is not known which possible bias to assign the change in effect estimate to. EPA considered that the uncertainties in the precise value of the lower bound were small relative to the accepted uncertainty in the definition of the RfD itself. The BMDL for the single-PFAS model was 0.411 ng/mL in serum and the BMDL for the multi-PFAS was 0.497 ng/mL in serum. The candidate RfD value for PFDA from the single-PFAS model after conversion to POD_{HED} and application of the uncertainty factor was 2×10^{-9} (mg/kg-d) (see Table 5-12); had the BMDL from the multi-PFAS been advanced as a candidate RfD value, the numerical value would have been the same to within one significant figure.

The following text on potential confounding has also been added to Appendix C.1:

Additional details about other potential confounders:

- PFNA is not a significant predictor in the single-PFAS model ($\beta = -0.227$; $p = 0.16$) and the association is just 15% the strength of the PFDA association (Budtz-Jørgensen and Grandjean, 2018b), thus PFNA could not have been a meaningful confounder even though PFNA was highly correlated with PFDA (r = 0.78) Grandjean et al. (2012)).
- PCBs had a weak correlation with PFDA (r = 0.14; Grandjean et al. (2012)), meaning that PCBs could not have been a meaningful confounder of the PFDA effect estimate.

In addition, clarifying text has been added to the main document (see responses above).

Comment: The same reviewer reiterated disagreement with the hazard descriptor of "*likely*" for immune effects emphasizing the null findings on the human and laboratory animals on infectious disease (see previous comment under Section A.2.3), and asked EPA to reconsider the use of the serum antibody endpoints for quantitative risk assessment/derivation of toxicity factors. The reviewer also noted recent conclusions by the Australian government (FSANZ 2021) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2021) stating that associations between PFAS and immunological effects do not provide a suitable basis for quantitative risk assessment.

EPA Response: Vaccine immune titers are functional measures of adaptive immune response and important indicators of clinically relevant immunotoxicity; reduced antibody production is an indication of immunosuppression and may result in increased susceptibility to infectious diseases generally (i.e., not limited to those specifically studied). EPA defines an "adverse effect" as a "biochemical change, functional impairment, or pathologic lesion that *affects* the performance of the whole organism or reduces an organism's ability to respond to an additional environmental challenge" (italics added) (U.S. EPA, 2002). Reduced antibody production is thus an appropriate effect for the derivation of a candidate RfD. The majority of the external review panel (seven of nine reviewers) agreed with the scientific justification for the selection of the immune

effect used in the derivation of the lifetime RfD. Furthermore, EPA's interpretation of the immune effect was supported during the external peer review of the PFOS and PFOA Toxicological Reviews (U.S. EPA, 2022c). Moreover, reduced antibody responses were used to support a 2016 NTP conclusion that PFOS and PFOA are *presumed to be an immune hazard to humans* (NTP, 2016).

Comment: A reviewer noted the following discrepancy: "Note that in the Supplemental section Table F.3 (Page F-25) [Table F-1 of the revised Toxicological Review] is inconsistent with the text on Starling et al 2017—text says *high* confidence and table say[s] *low* confidence." This reviewer added "More broadly this information on correlation between PFASs is important for the discussion on confounding, so this summary information would be better addressed in the main document."

EPA Response: The text was incorrect and was updated to match the *low* confidence rating noted in Table F-1. Beyond the appendix, EPA discusses some of the methodological challenges related to PFAS coexposures in Section 1.2. of the main document.

Comment: A reviewer stated that "the mere 'slight' totally [totality] of the evidence for developmental effects from the over 45 different epidemiological studies included in the draft assessment should not be considered sufficient for quantitative dose-response assessment (e.g., birth weight), but rather only for potentially supportive information for hazard identification" and that "EPA should reconsider their use of these epidemiological data for quantitative risk assessment/derivation of toxicity factors."

EPA Response: Based on the hazard identification conclusion that the ***evidence indicates*** PFDA exposure is likely to cause developmental effects, and effects on fetal growth specifically, this outcome was advanced for dose-response modeling. Even though this judgment is based primarily on the available animal studies, with support from the human studies, once this judgment is made, the decision shifts to identifying the most appropriate and reliable study for deriving a quantitative estimate protective of all persons. The rationale for prioritizing the epidemiological over the animal studies is described in Section 5. This rationale includes using data from well-conducted human studies when such data exist. As noted in the evidence integration discussion in Section 3.2.8, 34 of the 45 studies across the 6 primary endpoints [fetal growth restriction (including both birth weight and length measures), gestational duration, postnatal growth, anogenital distance, birth defects, and spontaneous abortions] were either *medium* or *high* overall confidence, with the majority showing some evidence of associations for key endpoints anticipated to be measured with considerable accuracy. But, as noted, some methodological concerns (e.g., pregnancy hemodynamics in studies in which PFDA was measured during or after pregnancy) in the epidemiological database resulted in the *slight* judgment. As stated in the Toxicological Review, "the Wikström et al. (2020) study was prioritized for RfD derivation as it was a *high* confidence study that predominantly sampled maternal plasma during the first trimester thereby reducing uncertainty relating to pregnancy hemodynamics." Therefore, EPA judged that the use of data from

Wikström, 2020 for RfD derivation is reliable and sufficiently supported, with agreement from the majority of peer reviewers.

Comment: The same reviewer also stated that "Given the drastically different results across epidemiological studies and the mere 'slight' evidence of developmental effects across more than 45 such studies, EPA should reconsider use of a single epidemiological study (Wikström et al. 2020) for dose-response assessment of birth weight and RfD derivation and consider a meta-analysis and/or using the more definitive dose-response data from the mouse study for dose-response assessment of birth weight and RfD derivation."

EPA Response: As noted above, even though the hazard judgment is based primarily on the available animal studies, with support from the human studies, once this judgment is made, the decision shifts to identifying the most appropriate and reliable study for deriving a quantitative estimate protective of all persons. Thus, given the similarities in findings across evidence streams and the availability of well-conducted human studies on the endpoint of interest, EPA considered higher quality epidemiological studies for use in dose-response analysis. As per EPA guidelines and the IRIS Handbook, EPA generally considers evidence from well-conducted studies in humans to be preferable to animal studies. As stated in the Toxicological Review, "the Wikström et al. (2020) study was prioritized for RfD derivation as it was a *high* confidence study that predominantly sampled maternal plasma during the first trimester thereby reducing uncertainty relating to pregnancy hemodynamics. EPA judged that a meta-analysis would not help further elucidate the weight of evidence or improve the quantitative estimate. For example, there was some concern that pooling studies across the most common unit of exposure expression (1 ln-unit increase) would extrapolate beyond the range in most reported studies of PFDA. Overall, EPA concluded the use of data from Wikström, 2020 for RfD derivation is reliable and sufficiently supported, and this decision conclusion was upheld by the majority of peer reviewers.

Comment: The same reviewer also stated that "EPA should consider mouse data from the Harris and Birnbaum (1989) study for use as the primary basis for osRfD development based on developmental growth effects consistent with: (1) this mouse study providing the primary basis for EPA's developmental effects hazard conclusion; (2) the lack of factors that decrease certainty for fetal growth as evaluated in the mouse study (see Table 3-24 [Table 3-23 of the revised Toxicological Review]); and (3) the extensive epidemiological database merely being able to provide support for this mouse study with what amounts to 'slight' evidence for developmental effects across over 45 epidemiological studies."

EPA Response: Please see the comment response above. In addition, as stated in the Toxicological Review, the PODs from the Harris and Birnbaum, 1989 mouse study are much less sensitive (6–7 orders of magnitude) than the PODs available for developmental effects from *high* confidence human studies. Therefore, an RfD based on the mouse data (Harris and Birnbaum, 1989) would not be considered as health protective compared with using the human studies.

Comment: The same reviewer also stated the use of "the mouse data (Harris and Birnbaum 1989) instead of epidemiological study data (Wikström et al. 2020) for dose-response assessment and osRfD derivation based on developmental growth effects would make EPA's assessment somewhat more consistent with ATSDR (2021), who found the epidemiology literature inadequate for use as the basis of deriving MRLs for PFAS."

EPA Response: The Toxicological Review represents a comprehensive evaluation of the epidemiological literature and carefully considers the ability of the epidemiological studies to support dose-response decisions. The majority of the peer reviewers agreed with EPA's conclusions. Furthermore, as stated above, an RfD based on the mouse data (Harris and Birnbaum, 1989) would not be considered health protective.

Comment: The same reviewer also stated that "As the data from epidemiological studies provide only 'slight' evidence of developmental effects, including data from Wikström et al. (2020) that appears too uncertain and unsuitable for quantitative dose-response assessment, I suggest use of the mouse data (Harris and Birnbaum 1989) for dose-response assessment and osRfD derivation based on developmental growth effects."

EPA Response: Please see responses above and below.

Comment: The same reviewer noted that based on the comments above, alternative studies and effects should be considered for the derivation of the lifetime RfD and suggested "One method to begin such an evaluation may entail sorting the study data extracted by NOAEL/LOAEL, BMD or a similar criterion that would allow EPA to readily identify the next most sensitive effects based on less problematic studies that are adequate for RfD derivation. However, any newly derived toxicity factor should be subject to external expert peer review (Tier 1 necessary revision)."

EPA Response: The Toxicological Review for PFDA estimated PODs for all the identified human health hazards with adequate data for dose-response analysis for the derivation of candidate toxicity values (liver, immune, male, and female reproductive, and developmental effects) (see Table 5-10). However, for the lifetime RfD, the derivation of candidate toxicity values for liver, male reproductive, and female reproductive effects was not attempted, given the high degree of uncertainty associated with using PODs from 28-day rat studies to protect against chronic exposure; these were the only suitable studies available for these outcomes. For immune effects, the only suitable studies for dose-response analysis were human studies. For developmental effects, PODs could be derived from *high* and *medium* confidence human studies; therefore, candidate toxicity values from the human studies were prioritized for selection of the lifetime RfD, given the general preference for well-conducted human studies over animal studies described in EPA guidelines and the notably more sensitive PODs. The majority of the external review panel (seven of nine reviewers) agreed with the scientific justification for the selection of the studies/effects used in the derivation of the lifetime RfD; one reviewer did not comment in response to this charge question.

Tier 2 Suggested Revisions

Comment: Two reviewers commented on the presentation of the studies and endpoints selected for dose-response analysis of the immune effects and provided suggestions to improve clarity. One reviewer noted "This section needs a clearer presentation of the extensive analysis that EPA did on dose-response and the link to the values that are later a major focus of appendices. In particular, the presentation would be improved by providing a more detail on clinical relevance and justification for the choices made and the uncertainties associated with the determinations." The second reviewer added "Suggest providing prominent clarification in the immune section and this section which studies shown in the immune review were used to create the BDML analysis presented by Budtz-Jørgensen and Grandjean (2018). If available, it would be helpful if effect estimates and CIs for the combined cohort used for that study could be provided."

EPA Response: Additional details on the BMD analysis and results were added to Section 5.2.1 to show the analytical details from the supplemental material shared by (Budtz-Jørgensen and Grandjean, 2018a) and used by EPA to derive the BMDLs. These include two new tables highlighting the effect estimates and confidence intervals of PFDA measured perinatally and antibodies measured at age 5 years, as well as PFDA measured at age 5 years and antibodies measured at age 7 years.

Comment: A reviewer made additional suggestions to improve the toxicity value derivation section, including "Suggest addition of a sensitive endpoint figure for the summary that helps highlight data rich, moderate and poor areas" and "Suggest adding clarification to the RfD table where the values came from and how they were derived (make things easier for your readers)."

EPA Response: Table 4-1 compares hazard conclusions for PFDA and other PFAS across EPA human health assessments, highlighting major data gaps for PFDA and PFAS in general. A footnote has been added to the organ/system-specific RfD tables (see Tables 5-12 and 5-16) in the Toxicological Review draft to clarify that the details of the BMD modeling approach and results can be found in Appendix C.

Comments: A reviewer agreed with the selection of the studies for immune effects but indicated "The authors need to opine on the use of these data for extrapolation of immune suppression for a population. Is the suppression delayed or slowed or permanently suppressed? Is this an adverse response that is recognized clinically with clinical studies to back up the adverse findings? What are the problems with using a remote population of people for use in the US? Is this the first time EPA has used this endpoint for IRIS?"

EPA Response: EPA defines an "adverse effect" as a "biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism or reduces an organism's ability to respond to an additional environmental challenge" (U.S. EPA, 2002). Immunosuppression is considered a functional impairment. Whether or not the observed immunosuppression is eventually identified with clinical studies, it is clear that fewer antibodies reduce people's ability to respond to additional immunological challenges. EPA's interpretation of

the immune effect was supported during the external peer review of the PFOS and PFOA Toxicological Reviews (U.S. EPA, 2022c). Moreover, reduced antibody responses were used to support a 2016 NTP conclusion that PFOS and PFOA are *presumed to be an immune hazard to humans* NTP (2016).

Comments: In reference to the birth weight studies, a reviewer suggested that EPA "… acknowledge the broader range of PK differences associated with pregnancy that could also affect the interpretation of the human studies beyond the focused 'hemodynamic' currently discussed."

EPA Response: EPA assumes the reviewer is specifically referring to the human birth weight studies. EPA is not aware of any evidence for specific PK differences associated with pregnancy, other than those already discussed, which are likely to impact the interpretation of those data. Note that due to the long half-life of PFDA, gestational dosimetry is determined by the PK and accumulation of PFDA in the woman over the years prior to pregnancy and thus the maternal body burden during pregnancy is strongly dependent on its PK in the nonpregnant woman.

H.3.2. Public Comments on Noncancer Toxicity Value Data Selection and Modeling for the Lifetime ("Chronic") Reference Dose (RfD)

Comment: One commenter supported the use of human data as a basis for the derivation of toxicity values for PFAS citing a recent evaluation by the Health Effects Subcommittee of the New Jersey Drinking Water Quality Institute (DWQI, 2022). Another commenter disagreed with the derivation of the RfDs for immune and developmental effects, noting the following issues: "1) a failure to show clear mechanistic and quantitative links between the immunological endpoint and meaningful clinical outcome; 2) selection of critical studies based on nonrepresentative populations; 3) failure to consider the weight of evidence for reduction in birth weight in study populations; and 4) use of BMD modeling approaches that do not explicitly account for confounding by other PFAS."

EPA Response: Regarding the reviewer's concern about uncertainty in the human database for immune and developmental effects, please see responses to relevant external peer-review comments above and below.

Comment: The same commenter expanded on the rationale for not supporting the selection of the immune effects as a basis for the RfD, noting that the evidence for associations between blood PFAS serum levels and reduced antibody responses is "weak" and reductions in antibody levels do not represent a meaningful clinical outcome and are not consistent with an increase in infectious disease. The commenter cited other reviews, health agencies, and expert panels that have reached similar conclusions (Antoniou et al., 2022; Garvey et al., 2023; ATSDR, 2021; COT, 2022; DoD 2022).

EPA Response: Vaccine immune titers are functional measures of adaptive immune response and important indicators of clinically relevant immunotoxicity; reduced antibody production is an indication of immunosuppression and may result in increased susceptibility to infectious diseases generally (i.e., not limited to those specifically studied). EPA defines an "adverse effect" as a "biochemical change, functional impairment, or pathologic lesion that *affects* the

performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge" (italics added) (U.S. EPA, 2002). Reduced antibody production is thus an appropriate effect for use as the basis for an RfD. The majority of the external review panel (seven of nine reviewers) agreed with the scientific justification for the selection of the immune effect used in the derivation of the lifetime RfD. Furthermore, EPA's interpretation of the immune effect was supported during the external peer review of the PFOS and PFOA Toxicological Reviews (U.S. EPA, 2022c). Moreover, reduced antibody responses were used to support a 2016 NTP conclusion that PFOS and PFOA are *presumed to be an immune hazard to humans* NTP (2016).

Comment: The same commenter listed several limitations in the Buddtz-Jorgensen and Grandjean (2018) study that they argue make it unsuitable as a basis the derivation of the RfD for immune effects, including: 1) baseline vaccine responses are highly variable (widely distributed in the population) but confidence intervals of the responses were not documented; 2) study did not account for transfer of maternal immunoglobulin G (IgG) to children, which may have interfered with the development of vaccine antibodies; 3) factors that can influence immune health such as nutrition and lifestyle were not discussed or controlled for; 4) co-exposures to other contaminants (other than PFOS and PFOA) were not sufficiently considered or independently evaluated by EPA; 5) study is based on an isolated population on the Faroe Islands that may not be representative of the U.S. population.

EPA Response: Regression coefficients and their standard errors for the results presented in (Budtz-Jørgensen and Grandjean, 2018a) were reported in (Budtz-Jørgensen and Grandjean, 2018b) and new tables have been added in Section 5 to make these details more easily accessible to the reader. Maternal IgG would not be expected to remain in children beyond infancy and therefore would not impact tetanus and diphtheria antibody concentration in 5- or 7-year-olds. The communities living on the Faroe Islands have similar lifestyles and nutrition that would not be expected to impact their children's antibody concentration outside of the body burdens of PFAS. Additional details of the potential confounding by PFNA, which was highly correlated with PFDA, revealed that PFNA was not a significant predictor of antibody concentrations and as such would not be expected to be a confounder.

Comment: The same commenter disagreed with the BMD modeling approach for immune effects stating that it is not scientifically supported and should be revised based on the following issues: 1) selection of a benchmark dose response of 5% as a threshold of significance for which there is no clinical relevance; 2) use of BMD/BMDLs that do not control for potential confounding by PFOS and PFOA; 3) lack of reporting on whether BMD modeling approach used by Budtz-Jørgensen and Grandjean (2018) had been independently validated by EPA, and failure to describe the multivariate approach in sufficient detail for evaluation by outside reviewers.

EPA Response: A BMR of 5% was considered, but ultimately not advanced in favor of using a BMR of ½-SD change in the distribution of antibody concentrations. In the absence of a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, a default

BMR of 1-SD change from the control mean may be selected, as suggested in EPA's draft Benchmark Dose Technical Guidance Document (U.S. EPA, 2012). As noted above, a lower BMR can also be used if it can be justified on a biological and/or statistical basis. In the public external review draft, the selected BMR was ½ SD and an alternative BMR of 1 SD was shown for comparison. BMDLs were evaluated for potential confounding by PFOS and PFOA and when little potential confounding was indicated, some BMDLs that did not control for PFOS and PFOA were used as PODs. In other instances, BMDLs from models controlling for PFOS and PFOA were used as PODs. Extensive details of the regression modeling are presented in Appendix C.1. EPA did validate the BMDLs reported by Budtz-Jørgensen and Grandjean (2018) who used a BMR of 5% by checking that the regression coefficients reported in (Budtz-Jørgensen and Grandjean, 2018b) did yield the reported BMDLs; however, EPA used a different BMR of ½-SD change in the distribution of antibody concentrations as noted above.

Comment: A commenter stated that although the draft IRIS document discusses potential confounding by co-occurring PFAS, EPA did not adequately consider confounding by PFNA for the POD selected for the derivation of the RfD for immune effects. The reviewer noted in particular the strong correlations between PFDA and PFNA shown by Grandjean et al., (2012). Further, the reviewer requested clarification for considering associations with PFDA stronger than for PFNA (p. 3–58, line 6) stating "The statement about stronger associations for PFDA than PFNA may possibly be based on the fact that a doubling of serum levels represents a smaller numerical increase for PFDA than PFNA, because serum levels (at age 5 years) were lower for PFDA (geometric mean – 0.28 ng/mL) than for PFNA (geometric mean – 1.00 ng/mL) in this study." Additionally, the reviewer suggested accounting for potential confounding by PFNA in the confidence descriptor for the POD quantification for the derived immune osRfDs (see Tables 5-11 and 5-15).

EPA Response: Additional text has been added to Section 3.2.2: Details of the regression modeling results (Budtz-Jørgensen and Grandjean, 2018b) show that PFNA was a nonsignificant predictor of both tetanus and diphtheria antibody concentrations with associations just 15% the strength of the PFDA association and thus PFNA could not have been a meaningful confounder. Table 5-15 has been updated to reflect the PFDA results were not considered to be confounded by PFOS, PFOA, and PFNA with callout to Section 3.2.2 and Appendix C.1.

Comment: A commenter stated that Table 5-7 (Table 5-9 of the revised Toxicological Review) should include the numerical value (2,500 g) of the "public health definition of low birth weight."

EPA Response: The public health definition of low birth weight was added to Table 5-9.

Comment: A commenter stated that the use of the ratio of human-to-animal clearance factors for interspecies toxicokinetic extrapolation "does not appear to be appropriate for development of Human Equivalent Doses (HEDs) from PODs from short duration studies of PFDA such as the 28-day rat studies since it is only applicable at steady-state. The duration of exposure in

these studies (e.g., 28 days) is much shorter than the half-life of PFDA in the species used (e.g., male rats – 72 days; female rats – 54 days). Therefore, the serum PFDA levels are not at steady-state at the end of the dosing period in these short duration studies, since steady-state is not reached until after several half-lives of dosing (see Ito, 2011). For this reason, the serum PFDA level in rats at the POD will be lower than predicted by the equation on p. 3–22. Using this extrapolation approach when serum PFDA levels in rats have not reached steady-state would result in overprediction of the HED (i.e., result in a value higher than the actual HED). If serum PFDA data are available from short-term animal studies, it is suggested that measured serum PFDA levels (e.g., average serum levels over the course of the study, or maximum serum level at the end of the dosing period) be used to determine the POD (in benchmark dose (BMD) modeling, or as the NOAEL/LOAEL if appropriate). The human clearance factor can then be applied to the POD in terms of serum levels to determine the HED, was done for the human studies in Table 5-8 [Table 5-10 of the revised Toxicological Review]. This approach was used by the NJ DWQI to develop HEDs from laboratory animal serum data for PFOA and PFOS (DWQI, 2017; DWQI, 2018)."

EPA Response: EPA reevaluated the use of PK modeling and serum data from specific studies and has revised its approach for extrapolation of such results. Specifically, the end-of-study serum concentrations reported as part of the NTP 28-day study are now used directly via interpolation to estimate internal doses in that study and the similar (Frawley et al., 2018) 28-day rat study. PK data were not reported for the one mouse toxicity study evaluated (Harris and Birnbaum, 1989), so it was not possible to use direct interpolation similar to that used for the rat to extrapolate the mouse endpoint (decreased fetal body weight). It was concluded, however, that despite its uncertainty, EPA's PK model is more accurate than assuming steady state for mice. Hence, in response to this comment, EPA revised its analysis to avoid the assumption of steady state and used what was otherwise considered the most accurate approach possible for estimation of internal doses in rats and mice.

Comment: The same commenter also stated that "it should also be mentioned that internal (e.g., serum) PFDA levels did not reach steady-state in the 28-day studies because the half-life of PFDA in rats is much longer than 28 days" on p. 5–19, lines 11–13.

EPA Response: As noted just above, the revised approach for estimating internal doses for the 28-day rat studies (see Section 3.1.7 with supporting analyses in Appendix G.2) no longer assumes that PFDA levels in the animal reached steady state. Instead, the measured PFDA concentrations from the NTP 28-day rat study are interpolated directly to estimate the average serum concentration over the course of the study for use as the internal dose.

Comment: A commenter stated, "there was insufficient description and/or justifications for several assumptions made for PK extrapolation and for the UFs employed."

EPA Response: EPA has clarified the rationale of assumptions made for PK extrapolation in Section 3.1.7 and Appendix G.2. The application of corresponding UF_A and UF_H are straightforward: UF_A is set to 3 when a PK model or chemical-specific PK data are used and UF_H has a default value of

10 since the PK extrapolation does not account for interindividual variability. The description of the choice of UF_A in Table 5-15 has been edited to reflect the revised extrapolation approach and the aforementioned rationale is outlined in the following two paragraphs. The use of $UF_H = 10$ for a noncancer endpoint is standard practice when human variability has not been experimentally addressed, and thus a brief justification is appropriate. The choice of UF_S for duration extrapolation, including consideration of PK is discussed in considerable detail after Table 5-15 and the choice of UF_D (3) is explained in detail at the end of Section 5.2.1 and in Table 5-11.

Comment: A commenter stated that "In light of the inconsistent findings and additional uncertainty in estimating relevant exposures, it is not appropriate to rely on the epidemiological data to develop an RfD for reduced birth weight" and that EPA should reconsider using the mouse fetal body weight from the Harris and Birnbaum (1989) study to derive the developmental osRfD.

EPA Response: Please see responses to related external peer-review comments above and below.

Comment: A commenter stated that "The method of calculating human equivalent dose (HED) using human internal concentrations (i.e., HED = POD_{int} × Human Clearance) assumes that internal concentration (POD_{int}) is at steady state. Given the estimated long half-life of PFDA in humans (i.e., 4 to 12 years), as well as the fact that there is a lack of data available to determine PK of PFDA in young children, it is unlikely that this method of calculating HED is appropriate for young children. While EPA discusses the uncertainty of extrapolating this dosimetry to children, it does not discuss the implications that these uncertainties may have toward the estimation of the RfDs for young children. This omission seems particularly important given that the RfDs derived from this review are based on putative effects of PFDA exposure in infants and young children."

EPA Response: Existing data indicate that infants are born with body burdens that are within a factor of 2 of maternal levels and then receive significant exposure from lactation. Hence, the assumption is considered reasonable—children born to chronically exposed mothers may well be in a range of body burden close to steady state—and the assumption may underpredict risk from a potential large exposure through breast-milk ingestion. Further discussion on these points was added to the discussion of uncertainty in HED calculations in Section 3.1.7.

Comment: A commenter stated that "The explanation of the use of CDC (2018) data on birthweight of babies born in the U.S. and NHANES serum PFDA data for 2011–2012 to estimate the BMD and BMDL for 5% extra risk of low birth weight (Section C.1.2, starting on p. C-16) is unclear. It should be clarified so that a reader can readily understand the process and steps used in the evaluation."

EPA Response: The Appendix C.1.2 discussion on birth weight modeling lays out how the birth weight data from the CDC natality file and the NHANES serum PFDA data were used in the estimation of study-specific BMDs and BMDLs. The CDC natality file is described as the source for the mean birth weight in the general U.S. population (along with the standard deviation) used in the modeling as well as the percentage of births that fall below the clinical definition of adversity.

Equations C-3 and C-4 show how this percentage of low birth weight babies is used to calculate the BMR, and Equations C-5 through C-8 describe the calculation of the BMD and BMDL values for the Valvi et al. (2017) study (which is how all study-specific BMDs and BMDLs are calculated). Text on pages C-20 and C-21, along with Equations C-9 and C-10, clearly describe how the NHANES serum data is used to account for background exposures to PFDA in the estimation of the BMDs and BMDLs

Comment: The same commenter also stated that on page C-17, lines 14–16, "The link to CDC biomonitoring data for PFAS from 2011–12 does not work and more recent data are available. It is suggested CDC NHANES data from the same time period (2018) as the birthweight data cited earlier in the paragraph be used. CDC NHANES biomonitoring data for PFAS, including PFDA, for 2017–2018 are found at https://www.cdc.gov/exposurereport/data_tables.html."

EPA Response: The link to CDC NHANES biomonitoring data was updated (https://www.cdc.gov/exposurereport/data_tables.html). The median of serum PFDA concentrations was 0.19 ng/mL for the period 2011–2012 and 0.20 ng/mL for the period 2017–2018 in NHANES. EPA performed BMD modeling using the median of serum PFDA concentrations for the period 2017–2018. The differences of BMDLs resulting from these two different assumptions ranged from 0 ng/mL to 0.01 ng/mL, depending on studies. Given the similarity of the results, no additional revisions were deemed necessary.

Comment: A commenter stated that "The proposed RfD for reduced birth weight relies on the findings of the study by Wikström et al. (2019) which reported significant effects in the highest exposure quartile in girls in relation to maternal serum concentrations in the first trimester in a longitudinal study in Sweden. The results contrast with those from other *medium* and *high* confidence studies which raises significant question about the selection of the endpoint for derivation of the RfD." The same reviewer stated that the evidence base does not "support a link between PFDA and lower birth weight in humans and should not be relied on for the development of an RfD."

EPA Response: As discussed above, this decision was supported by the majority of peer reviewers (seven of nine panelists; one panelist did not comment on this topic).

Comment: The same commenter also stated that "Appendix F of the draft IRIS supplemental document indicates that 11 of 22 studies (including several *low* confidence studies) show evidence of associations with PFDA and mean birth weight in the overall population, and that seven of those studies indicate comparable deficits for PFNA and PFDA. EPA states that considerable uncertainty is due to potential confounding by other co-occurring PFAS in the existing literature. Nonetheless, EPA did not attempt to control for confounding with other PFAS as part of its BMD modeling approach."

EPA Response: As discussed above, given the uncertainty on the development of and interpretation of multi-PFAS models, EPA based their primary weight-of-evidence characterization on single-pollutant models since 45 studies helped inform this decision. This approach was

supported by a majority of the peer-review panel. The very few studies that did examine multi-PFAS models are detailed in Appendix F.

Comment: A commenter stated that "the potential impact of other PFAS, especially PFNA, is an important limitation for use of these dose-response data from Wikström et al. (2020) as the basis for the PFDA RfD." The same reviewer stated that this concern for confounding should be discussed in the "Confidence in quantification of the POD_{HED}" (Tables 5-11 and 5-12) and recommended that "the potential for confounding by PFNA on the quantitative relationship between serum PFDA and decreased birthweight be further considered."

EPA Response: Consistent with other endpoints, this source of uncertainty is addressed in detail in the first two sections of Table 5-11 ("Confidence in study used to derive osRfD" and "Confidence in Evidence Base supporting Hazard"), with the potential for confounding considered across multiple sections of the Toxicological Review and appendices (see responses to peer reviewers, above). Furthermore, the discussion for the "Confidence in quantification of the POD_{HED}" focuses on the methods (e.g., BMD modeling, dosimetric adjustments) used in the calculation of a POD_{HED}.

H.3.3. External Peer-Review Comments on Noncancer Toxicity Value Data Selection and Modeling for the Less-than-Lifetime ("Subchronic") Reference Dose (RfD)

Peer-Review Comment Summary

In reference to charge question 3, five reviewers agreed with the selection of the studies and effects for the derivation of the subchronic RfD for PFDA. One reviewer provided a comment for future consideration (Tier 3) that the derivation of a subchronic RfD is not strictly necessary considering that the exposure will be chronic since the chemical is highly persistent. Citing the same concerns as in response to charge question 3, one reviewer disagreed with the appropriateness of these studies to derive subchronic RfDs for immune and developmental endpoints. Another reviewer refrained from commenting and the last reviewer did not explicitly state whether they agreed with EPA's conclusions. Most reviewers noted similarities in their responses to charge question 3 with their Tier 1 and Tier 2 comments made in responses to this question (see below).

A few new comments were provided. In reference to charge question 4c, one reviewer suggested adding any existing vitro studies to Table 5-16 for organ-specific endpoints and in reference to charge question 4d, another reviewer suggested that the uncertainty factor could be increased from 1,000 to 3,000 to estimate chronic osRfDs using these studies.

Tier 1 Necessary Revisions

Comment: A reviewer made the following comment in regard to the subchronic osRfDs derived for liver, male reproductive, and female reproductive effects "… it is imperative for EPA to acknowledge whether or not the dose-response data for a species selected for osRfD derivation

(most often the most sensitive species) is known or likely to be (e.g., based on greater relevant biological similarities) the most predictive available for similar effects in humans or if dose-response data from another species might be equally relevant to humans and result in a significantly different (e.g., higher) osRfD."

EPA Response: Responses to similar comments regarding human relevance of animal data for liver and male reproductive and female reproductive effects can be found above. In short, animal data for these effects are considered relevant to humans in the absence of evidence to suggest otherwise. Language has been added to the respective hazard sections to address this issue of human relevance. For example, the following text was added to the female reproductive section (see Section 3.2.5): "These findings are interpreted as relevant to humans in the absence of evidence to the contrary. This assumption is based on *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996)."

Comment: The same reviewer stated, "As with the lifetime RfD, I do not agree with the selection of these same studies and endpoints for subchronic RfD derivation (i.e., decreased serum antibody concentrations in Grandjean et al. 2012, decreased birth weight in Wikström et al. 2020)." Instead, the reviewer suggested the use of mouse data from the Harris and Birnbaum (1989) study as a basis for the subchronic RfD for developmental effects. Additionally, the reviewer noted that alternative approaches should be considered for the derivation of the subchronic RfD arraying the available NOAEL/LOAEL or BMDLs from less problematic studies to identify the next most sensitive effects (see comments on the lifetime RfD in Section A.3.1 for more details).

EPA Response: The Toxicological Review for PFDA estimated PODs for all the identified human health hazards with adequate data for dose-response analysis for the derivation of candidate subchronic toxicity values (liver, immune, male and female reproductive, and developmental effects) (see Table 5-10). Ultimately, the candidate values for immune and developmental effects were selected for the derivation of the subchronic RfD based on considerations for sensitivity and confidence decisions about the studies, evidence base, quantification of the POD, and overall osRfD. Of the nine external peer reviewers, five agreed with EPA's scientific justification for the selection of the studies/effects used in the derivation of the subchronic RfD and one disagreed (the other three reviewers abstained or did not provide an explicit opinion).

Comment: A reviewer stated that "it is important for EPA to transparently determine whether or not the effects on estrous cyclicity may be secondary to the observed decreases in body weight because if so, these effects should not be further considered for dose-response assessment (i.e., only the body weight effects) and this should be explicitly stated in the assessment."

EPA Response: As noted above and pointed out by the respective reviewer, the Toxicological Review indicates that the PFDA-induced effects on estrous cyclicity are disproportionately more severe than changes in body weight. For example, the percentage of time spent in diestrus was statistically significantly increased by 27%–63% at ≥1.25 mg/kg-day

compared with 12%–36% decreases in body weight at the same doses. Given that the PFDA-induced estrous effects are more severe than body weight changes, these data do not provide sufficient evidence to support that the estrous findings are likely to be secondary to body weight changes. Furthermore, without time course data showing effects on estrous cyclicity and body weight at multiple intervals, it is not possible to make definitive conclusions on the possible contribution of PFDA-induced body weight changes to estrous cyclicity effects.

Tier 2 Suggested Revisions

Comment: A reviewer commented "Table 5-16 lists organ specific endpoints for subchronic RfD consideration. The table and text provide good descriptions about the evidence base supporting the toxicity, along with confidence in the POD for a human and a summary confidence statement based on these factors. If there are in vitro studies supporting the toxicity endpoints (Confidence in evidence base supporting this hazard), it is worthwhile to include."

EPA Response: The availability of in vitro studies or other mechanistic and supplemental information supportive of the identified human health effects is discussed in Section 3.2 NONCANCER HEALTH EFFECTS as part of the hazard judgments. This is also briefly mentioned in Table 5-15 under the discussion of the "Confidence in the evidence base supporting hazard" for each derived osRfD.

Comment: A reviewer agreed with the rationale for attempting the derivation of the subchronic osRfDs for liver and male and female reproductive effects but noted "… While the total composite uncertainty factor for these effects is already considerable (1,000; Table ES-1), if lifetime osRfDs based on these effects (i.e., liver, male and female reproductive effects) are desired by EPA and/or considered useful for more complete risk assessments, EPA could consider increasing the total composite uncertainty factor to the upper end of their total uncertainty factor range (3,000) to estimate chronic osRfDs (Tier 2 suggestion)."

EPA Response: As discussed in the Toxicological Review for PFDA, the available data for liver and male and female reproductive effects are limited to a 28-day study in rats (NTP, 2018). EPA judged there is too much uncertainty using PODs from a 28-day rodent study to protect against effects observed in a chronic setting and the magnitude of this uncertainty cannot be reasonably estimated without additional study (i.e., there is no information to support that increasing a UF an additional 3- or 10-fold would be expected to be health protective in a chronic setting).

H.3.4. Public Comments on Noncancer Toxicity Value Data Selection and Modeling for the Less-than-Lifetime ("Subchronic") Reference Dose (RfD)

No public comments were provided.

H.3.5. External Peer-Review Comments on Noncancer Toxicity Value Data for the Reference Concentration (RfC)

Peer-Review Comment Summary

In reference to charge question 3f, all nine reviewers agreed with EPA's decision not to derive an RfC for inhalation exposure to PFDA due to the lack of data, although one reviewer suggested that EPA explicitly state they have not identified a reliable PBPK/PK model for route-to-route extrapolation to support the decision not to derive an RfC (see below), and several reviewers provided Tier 3 future considerations (see peer-review report).

In reference to charge question 4e, seven reviewers agreed with EPA's decision not to calculate a subchronic RfC due to the lack of data, although one reviewer again suggested that EPA clearly state in the document, they have not identified a reliable PFDA PBPK/PK model from which they could perform route-to-route extrapolation (see below).

Tier 1 Necessary Revisions

Comment: A reviewer requested clarification on the availability of a PBPK/PK model for PFDA that could be used for route-route extrapolation, noting "If EPA has not identified a reliable PFDA PBPK/PK model that could be used for route-route extrapolation, then EPA's decision not to derive a subchronic or chronic RfC would be fully justified."

EPA Response: Text was added to Section 5.2.4. in response to the reviewer's comment: "Existing PBPK models for PFDA were judged insufficiently reliable for estimating human dosimetry for any route of exposure, including possible route-to-route extrapolation. Additionally, no classical PK models were identified that included inhalation dosimetry to support the derivation of an RfC."

Tier 2 Suggested Revisions

Comment: A reviewer agreed with the decision not to derive lifetime and subchronic RfCs due to the absence of data but indicated that "For PFASs with similar properties (e.g., long chain) read-across could be considered where PFAS-specific data is unlikely to be generated."

EPA Response: The IRIS Toxicological Review of PFDA is only one component of the broader PFAS strategic roadmap at EPA (https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024). Other Agency efforts are aimed at evaluating hundreds of PFAS using new approach methods and a tiered toxicity testing strategy that can inform future category grouping and read-cross decisions to fill data gaps. Although the suggested analysis is outside the scope of the Toxicological Review, text was added to the Executive Summary in the main document to highlight these broader Agency efforts.

H.3.6. Public Comments on Noncancer Toxicity Value Data for the Reference Concentration (RfC)

No public comments were provided.

H.4. CHARGE QUESTIONS 5 AND 6 – NONCANCER TOXICITY VALUE PHARMACOKINETIC EXTRAPOLATION AND UNCERTAINTY FACTORS

5. Appendix A identifies the potential for pharmacokinetic (PK) differences across species and sexes as a key science issue and lays out a hierarchy for using relevant PK data in extrapolating doses between laboratory animals and humans. Section 3.1 evaluates and synthesizes the PK data in relevant species and sexes, and among human lifestages, up to the derivation of key PK parameters used in the subsequent analysis. However, the evaluation of existing PBPK models and a one-compartment PK model found that these options were not sufficiently reliable for use. Given the information available on potential interspecies differences in PFDA PK, EPA applied a data-derived extrapolation factor (DDEF) to POD values from toxicity studies in laboratory animals to estimate corresponding human equivalent doses (HEDs) in the derivation of the respective RfDs. Similarly, the estimated human clearance (CL) was used to convert internal dose POD (POD_{int}) values from epidemiological analyses to corresponding HEDs.

   After publicly releasing the draft PFDA Toxicological Review, EPA evaluated recently published data for several other long-chain PFAS, described here (U.S. EPA, 2023, HERO ID 11181055), that are potentially relevant to evaluating PFDA dosimetry in women of childbearing age (see question 5c below).

   a) Is applying the estimated DDEF values for PFDA scientifically justified for conversion of PODs from animal toxicity studies to HEDs? If not, please provide an explanation and detail on a more appropriate approach.

   b) Is application of the human CL to estimate HEDs from POD_{int} values scientifically justified? If not, please provide an explanation and detail on a more appropriate approach.

   c) Have the uncertainties in the DDEFs and human CL been adequately evaluated and described? In answering this question, please provide an explicit recommendation on whether or not EPA should expand its adjustment for menstrual fluid loss as outlined in (U.S. EPA, 2023, HERO ID 11181055) prior to finalizing the assessment. As these newer data are from other PFAS, note that such an expansion would be based on the assumption that the pharmacokinetic effect of pregnancy and lactation on PFDA is similar to that of the other PFAS (i.e., a read-across based interpretation).

6. Do the methods used to derive toxicity values for PFDA appropriately account for uncertainties in evaluating the pharmacokinetic differences between the experimental animal data and humans? EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UF_H), interspecies differences (UF_A), database limitations (UF_D), duration (UF_S), and LOAEL-to-NOAEL extrapolation (UF_L) for PFDA.

   a) Is uncertainty in the derivation of the toxicity values scientifically justified and clearly described? Please describe and provide comments, if needed.

b) For immune effects, a UF_S of 1 and 3 were considered to account for extrapolation from less than lifetime human data; ultimately a UF_S of 1 was selected. A UFs of 10 was not considered as the developmental period is recognized as a susceptible lifestage for these types of effects and therefore exposure during this time window can be considered more relevant than exposure in adulthood (U.S. EPA, 1991). Also important is the fact that, given PFDA's long half-life and the expectation that the children and their mothers have been exposed to elevated levels of PFDA for many years, the observed effects on immune response are considered to be the result of a cumulative, prolonged exposure. Uncertainties with regards to additional susceptible lifestages (e.g., old age) are addressed as part of the UFD. Does the provided scientific rationale support this decision? Please explain.

c) For liver effects, a value of 3 is applied to extrapolate between effects in laboratory animals and in humans during the derivation of the subchronic RfD. Although PPARα dependence might support a value of $UF_A = 1$ if that were the sole pathway leading to these effects, evidence for the involvement of non-PPARα pathways is available in the PFDA database. Thus, uncertainty remains regarding the potential differences in sensitivity across species due to the involvement of both PPARα-dependent and PPARα-independent mechanisms. As such, the Toxicological Review concludes the available data are not adequate to determine if humans are likely to be equally or less sensitive than laboratory animals with respect to the observed liver effects and that a value of $UF_A = 3$ is warranted to account for the residual uncertainty in toxicodynamic differences across species. Please comment on whether the available animal and mechanistic studies support this conclusion and whether the analysis presented in the Toxicological Review is clearly documented.

d) For liver, male reproductive, and female reproductive effects, a default value of 10 is applied for the UF_S when extrapolating from 28-day animal data to a subchronic exposure. Considering the potential for some health effects (prolonged diestrus, sperm measures, and increased liver weight) to worsen with increasing duration and the large uncertainty associated with the lack of chemical-specific data to evaluate the effects of subchronic exposure on liver, male reproductive, and female reproductive outcomes, the Toxicological Review concludes that application of a UF_S of 10 is supported for the purposes of deriving the subchronic RfD from the 28-day toxicity data. Does the provided scientific rationale support this decision? Please explain.

e) Are the provided rationales for the remaining uncertainty factors (UF_L, UF_D, UF_H) scientifically justified and clearly described (to inform the UF_H, the assessment evaluates and considers the available evidence on potential susceptibility to PFDA within different populations or lifestages, including any potential impacts from early life exposure to PFDA on children's health or health later in life, although few studies on susceptibility were available)? If not, please explain.

H.4.1. External Peer-Review Comments on Noncancer Toxicity Value Pharmacokinetic Extrapolation

Peer-Review Comment Summary

- In reference to charge question 5a, six reviewers commented that the approach is scientifically justified, with two of these reviewers commenting that the presentation in the document is complicated and the justification is not readily apparent (see below) in the document text. One reviewer disagreed with the DDEF approach and encouraged EPA to pursue a PBPK model beyond what has been published by EPA authors (such as Bernstein et al., 2021) and to consider harmonization or read-across from other PFAS (see below). Two reviewers declined to comment.
- Specifically in reference to charge question 5b, two reviewers explicitly agreed with the application of human clearance values to calculate HEDs from POD_{int} values, and another reviewer agreed with the approach if a reliable value can be calculated for human clearance.
- In reference to charge question 5c, two reviewers stated the uncertainties are adequately evaluated and described, whereas three reviewers commented that further discussion of the uncertainty is needed (see below). One reviewer recommended EPA not adjust for menstrual fluid loss given the adjustment would rely on many assumptions about menstrual flow and another reviewer agreed with the notion that the adjustment requires many assumptions. Conversely, three reviewers stated EPA should expand the adjustment to account for menstrual fluid loss (see below; for Tier 3 future considerations provided on this topic, please see the peer-review report).

Tier 1 Necessary Revisions

Comment: A reviewer stated "The presentation of the complex relevant data landscape on PFDA and shortcomings of the single available PK model can be improved substantially. Given the shortcomings of the underlying data from both animals and humans the DDEF approach is justified in this case, with the caveat that there needs to be an Evidence Integration section in the Appendix AND main PFDA document that lay[s] out the logic of the choices made more clearly. Most importantly, this section needs to explicitly lay out the uncertainties as judged by the scientists who have done this extensive evaluation of the existing data."

EPA Response: EPA has revised the text throughout Section 3.1 to improve clarity. Besides presentation of the range of values for each PK parameter in each corresponding section (e.g., Section 3.1.2) an analysis of the parameters and approach used for extrapolation is provided in Section 3.1.7. Evidence synthesis summaries have been added at the end of the sections on absorption (see Section 3.1.1), distribution (see Section 3.1.2), excretion (see Section 3.1.4) and the approach for pharmacokinetic extrapolation (see Section 3.1.7). Since there is no evidence for metabolism and that section is a single paragraph, a corresponding synthesis was not needed there. The synthesis for PK extrapolation also addressed the evaluation of PBPK and PK modeling

(Section 3.1.6). Each of these new subsections addressed the corresponding results from Appendix G; thus, additional evidence integration pieces for that appendix would be duplicative.

Comment: A reviewer stated that "It was not clear from the description that assumptions are based on animal data, which introduces substantial uncertainty."

EPA Response: While the clearance of PFAS clearly differs in a substantial way between humans and animals, EPA interprets this to result from a quantitative difference rather than some fundamental difference in mechanism. Because of the much more limited PK data in humans than in experimental animals, extrapolation of features of animal PK data to humans is necessary. However, considering the long-standing knowledge from pharmacological science that most of the processes involved are similar in animals and humans, and consistent with EPA guidelines and practice in this area, EPA does not consider these assumptions (i.e., that absorption and distribution are similar) to introduce substantial or unreasonable uncertainty. Such assumptions are necessary for animal-to-human extrapolation in the absence of additional evidence.

Comment: A reviewer stated that "It is important to consider that not all women menstruate, bear children or breast feed. Those who do not would of course have lower clearance than those who do."

EPA Response: As noted elsewhere in these responses, based on reexamination of empirical data EPA is no longer assuming that menstrual fluid loss is a significant, general mechanism of clearance. Extrapolation of nondevelopmental health effects is conducted without application of this factor, i.e., for women whose clearance is no different from men and postmenopausal women. Hence, the evaluation of health effects in adult women does not presume they have children (and breastfeed).

Comment: A reviewer stated "The reasons stated why data-derived extrapolation factors (DDEFs) were used are because of the publication of a poor quality PBPK model for PFDA (Kim et al. 2019) and the inability to predicted plasma levels in rats gavage dosed with PFDA daily for 28 days (NTP 2018) using a compartment model calibrated using single dose PFDA PK data (IV and oral). I think the EPA should continue to resolve these issues and use a PBPK model beyond what has been published by EPA authors (Bernstein et al. 2021)."

EPA Response: EPA has evaluated PK data from various PFAS and reached the conclusion there are fundamental aspects of PFAS PK that are not yet understood, which are considered necessary for a proper PBPK model to be sufficiently sound for application. Considering also that the rodent data are not key drivers of this assessment, the use of more traditional PK analysis and direct evaluation of rat PK data from the NTP 28-day bioassay was deemed sufficient and retained. Further, note that the majority of panelists did not support revision of the PBPK model but indicated that a simpler PK-informed approach would be sufficient.

Comment: The same reviewer also stated "More work is needed to better understand the pharmacokinetics of PFDA for repeated animal exposures in the context of a PBPK model." The reviewer further stated "The cost of not continuing to modify the Kim et al. published PBPK model

for PFDA is problematic. To derive subchronic osRfDs a PBPK model would be superior to any other methodology because a PBPK model includes organs and tissue groups in a physiologically relevant manner and can predict internal organ dosimetry."

EPA Response: Please see above response, noting that new experimental data will likely be needed to understand the complex observations of tissue versus serum data currently available for PFDA and other PFAS.

Comment: A reviewer made a recommendation to "Edit and expand the discussion on the estimation of human clearance to state explicitly and clarify that this estimation incorporates uncertainties associated with assumptions that are based on animal data regarding the ratio of fecal/urinary clearance of PFDA." The reviewer made a recommendation to "Briefly summarize and explain the approach followed for replicating and adjusting the PBPK model of Kim et al. (2019), i.e., using the R templates provided in Bernstein et al. (2021); incorporate this information in Appendix G following the analysis of the -1 and 2-compartment PK models. (Please see Answer to Charge Question 6a for more details)."

EPA Response: EPA has revised the discussion of the human PK data to better address its uncertainty. Note that EPA has shifted its analysis to reduce reliance on quantitative animal PK data for the estimation of human clearance. However, as noted in the document, the estimate of human fecal clearance is based on biliary excretion data from only five subjects who were being treated for various diseases (Fujii et al., 2015), which EPA considers as providing an uncertain estimate of clearance in the population as a whole. Further, EPA notes Fujii et al. (2015) obtained its estimate of fecal clearance, "Assuming a volume distribution of 200 mL/kg (based on previous reported mouse experiments." This assumption was needed to estimate the extent of enterohepatic recirculation for PFOA (for which a half-life was established in humans independently), which was then assumed to also apply to PFDA. Given the overall landscape of human data for PFDA, with no time-course or controlled exposure data that could be otherwise used to independently estimate the half-life or volume of distribution, EPA considers all avenues of estimating human clearance to involve significant uncertainty. These estimates still clearly indicate a range of clearance in humans much lower than would be predicted by a $BW^{3/4}$ scaling from rats or mice, and hence are preferred, but their uncertainty is important. Thus, this uncertainty is acknowledged and discussed in multiple places in the revised Toxicological Review.

In addition, further details have been added on the attempt to replicate the results of Kim et al. (2019), also pointing the reader to the Bernstein et al. (2021) paper wherein more details are provided (see Section 3.1.6).

Comment: The same reviewer made a recommendation to "Explain (in Appendix G) why other published models (e.g., Fàbrega et al., 2015), were evaluated as not adequate for supporting pharmacokinetic calculations." The reviewer also made a recommendation to "Check and ensure that values of pharmacokinetic properties listed in the document match those reported in their

cited sources: an example is such as, e.g., the value of 215–300 days for beta- or single-phase half-life of PFDA in male rats attributed to Dzierlenga et al. (2019)."

EPA Response: An evaluation of the Fàbrega et al. (2015) model was added to Section 3.1.6 (which also includes the review of Kim et al. (2019)), noting that it too relies on a critically flawed assumption regarding tissue distribution and contains several other issues preventing its use (see Section 3.1.6).

In addition, the half-life values attributed to Dzierlenga et al. (2019) have been corrected. Other cited values were checked.

Comment: A reviewer stated that "EPA should expand its adjustment for menstrual fluid loss (as outlined in (U.S. EPA, 2023a)) prior to finalizing the assessment if EPA judges the alternative/modified adjustment to be associated with less uncertainty than that associated with not conducting such an adjustment."

EPA Response: Per previous responses, the explicit assumption on menstrual fluid loss was removed for this Toxicological Review based on NHANES data for PFDA.

Tier 2 Suggested Revisions

Comment: A reviewer stated that more discussion of the magnitude and directionality of uncertainty is needed regarding the pharmacokinetic extrapolation applied in the draft assessment. The reviewer further recommended "adding a table of uncertainties and prioritization for evidence integration."

EPA Response: A description of directionality was added when the uncertainty is clearly unbalanced in one direction. While EPA has not added a table of key uncertainties, the new evidence synthesis subsections noted above identify key uncertainties in the corresponding components of ADME and PK.

Comment: A reviewer stated the HED calculation could be strengthened by utilizing individual data on serum concentrations and immune response. The reviewer also recommended "a probabilistic type of assessment of HEDs using human serum concentrations of PFDA."

EPA Response: The HED calculation from serum concentrations is a linear conversion, multiplication by CL. Hence, performing this conversion before versus after summarizing the data would not alter the result. An established EPA process for integrating probabilistic PK and dose-response analyses does not currently exist. Further, note that the simpler PK approach was supported by the majority of panelists.

Comment: The same reviewer stated that "The big problem is that protein transporters (liver and kidney) and serum and tissue (?) protein binding are responsible for the slow whole body CL rate. A PBPK model is greatly favored over the current approach because the key biological factors can be explicitly described and used in predictions. Animals are much different than humans. The use of allometric relationships is of little or no value for across species scaling."

EPA Response: As noted above, EPA agrees that a full PBPK model that includes all these features would be preferred; however, no such adequate model exists.

Comment: The same reviewer stated that because PK data are available for PFDA, those data should be used rather than read-across. "Read-across is helpful to provide evidence of going down the right path in terms of PK."

EPA Response: EPA agrees that, to the extent possible, PFDA-specific data should be used. However, as noted above, the estimate of human fecal clearance by Fujii et al. relies on a read-across of GI resorption from PFOA (and the V_d for PFOA estimated for mice). The revised Toxicological Review now uses the fecal clearance of Fujii et al. since it is based primarily on human PK data. But while some PK data are available for PFDA in humans, there are also significant data gaps that can only be addressed by read-across, extrapolation from animals, or a combination of the two such as was done by Fujii et al. The human PK data for PFDA (i.e., that can be used to estimate PK parameters) are more limited than for other PFAS. Thus, this uncertainty is emphasized in several places throughout the revised Toxicological Review.

Comment: A reviewer made a recommendation to "Evaluate and, if feasible, apply options for alternative animal to human extrapolation approaches that do not require steady-state assumptions inconsistent with short-term study data."

EPA Response: As described above and in the revised document, EPA's revised analysis was revised to avoid the assumption of steady state for evaluation of dosimetry in the short-term rat and mouse bioassays.

Comment: A reviewer stated that "Modeling should be sure to consider enterohepatic recirculation and kidney resorption."

EPA Response: These processes are implicitly included in classical PK analysis of various data, specifically that use empirical data to identify overall clearance. In particular this is why the empirical clearance is slower than would be predicted by glomerular filtration of unbound PFDA for male rats and humans, as discussed in Section 3.1.5. As discussed above and detailed in the discussion on excretion in humans (see Section 3.1.4), the estimate of human fecal clearance is based on an estimated enterohepatic recirculation of 98%. While EPA recognizes that renal resorption is a likely factor, the available data do not allow for an estimation of dose-dependent urinary clearance in humans and EPA judged that a specific description of renal resorption as a saturable process does not improve the internal dose estimates versus the empirical estimation of total clearance obtained (see also Appendix G.1.4).

Comment: A reviewer made a recommendation "to acknowledge the finding of differences in a variety of pharmacodynamic parameters including protein binding during pregnancy with linked comment on potential for breast milk lactational pharmacokinetic considerations. Addition of references and several sentences would be suggested."

EPA Response: The following text has been added to Section 3.1.2, "Specific mechanisms known to impact the distribution of substances during pregnancy are the changes in blood volume (hemodynamics) and concentration of serum proteins. A review by Feghali et al. (2015) states that serum concentration of albumin decreases 13% by gestation week (GW) 32, but that the overall

serum volume increases by 42% by GW 38. The net impact of both these changes is then a 0.87 × 1.42 = 1.24-fold increase in the total amount of albumin in the serum, which would suggest 24% lower distribution to various tissues (since there is greater total binding in the serum), including the fetus, compared with what one would otherwise predict. However, the evaluation of fetal distribution just described is based on empirical fetal concentration data, which already depend on and therefore implicitly account for variation in maternal serum binding. Further, since we lack precise measurements of the V_d in human adults (specifically, the pregnant mother) versus the fetus and amniotic fluid, and there are no data on excretion (clearance) during pregnancy, the specific contribution of these changes in maternal blood volume and albumin concentrations to the overall empirically observed PFDA concentration changes cannot be quantified."

The discussion of lactational distribution has been given its own subheading to better emphasize that component and the following text has been added, just after the current text stating that the milk/maternal serum ratio is 0.03:1: "It should be noted that this empirically measured ratio implicitly accounts for the level of serum binding in the breastfeeding mother, that is, the extent to which that may differ from women outside of the gestational and lactational lifestages. While this low ratio indicates a rather limited level of lactational transfer to infants, the total intake by a breastfed infant will also depend on the milk ingestion rate. For an exclusively breastfed infant, the total exposure by this route could be quite large. Considering that breast milk is a key source of nutrition for infants and the lack of studies demonstrating a specific hazard from this route of exposure, versus in utero exposure and other possible routes for infants, the best option for limiting developmental exposure to PFDA is to limit maternal exposure, which will reduce both in utero and lactational exposure of the offspring."

Additional text on the significance of lactational transfer was also added to the discussion on uncertainty in HED calculations for PFDA in Section 3.1.7, per the response below.

A brief discussion of this minimal uncertainty is now included.

H.4.2. Public Comments on Noncancer Toxicity Value Pharmacokinetic Extrapolation

Comment: A commenter recommended that "the discussion of exposure to PFDA through breast milk be expanded and that the importance of exposure to PFDA in breastfed infants be given more emphasis. The draft IRIS document (p. 3–11, lines 7–10) states that the breast milk:maternal serum ratio of 0.03:1 indicates 'a rather limited level of lactational transfer to infants.' This statement may be incorrectly interpreted to mean that breast milk is not an important exposure route to infants for PFDA. However, even though only a few percent of the PFDA in maternal serum is transferred to breast milk, it is well established that infants receive substantial doses of long-chain PFAS such as PFDA through breast milk because of the bioaccumulation of these PFAS in maternal serum and the large volume of breast milk ingested by infants relative to their body weight. For example, see Goeden et al. (2019) and Post (2022). While PFDA was not specifically evaluated by Fromme et al. (2010), the data presented in this study demonstrate that serum levels of other long-chain PFAS with breast milk:maternal serum ratios similar to that of PFDA are several

fold higher in breastfed infants than in their mothers. The elevated exposure to PFDA and other long-chain PFAS in breastfed infants is important because infants are a sensitive subpopulation for the developmental and other (e.g., immune) effects of these compounds. Notably, the higher exposure of breastfed infants to long-chain perfluoroalkyl acids, including PFOA, PFOS, perfluorohexanoic acid (PFHxS, and PFNA, has been considered in the drinking water guidelines developed by several states (see Post et al., 2021) and in the draft RfDs for PFOA and PFOS presented in the recent draft EPA (2023a, b) health effects assessments of these PFAS."

EPA Response: As noted above, the potential significance of lactational transfer is now stated in the (new) subsection on human lactational distribution. Further discussion of the significance of lactational transfer was also added to the discussion on uncertainty in HED calculations for PFDA in Section 3.1.7, per the response below. However, EPA considers specific predictions of lactational transfer to be highly uncertain given the very limited data on PFAS PK in infants. Also, EPA specifically evaluated the model of (Goeden et al., 2019) and identified a mass balance error in the Excel spreadsheet used. A large bolus to the infant is still predicted when this error is corrected but not as large as shown in that publication. Given the overall uncertainty in a potential estimate of lactational transfer due to the data limitations noted in the document, EPA does not believe that further elaboration on this topic would meaningfully add to the evaluation of risk.

Comment: A commenter suggested "adding more introductory information (p. 3–7, lines 28–30) to convey the importance of exposure to PFDA during developmental lifestages and to introduce the detailed discussion that follows."

EPA Response: A detailed presentation of exposure information falls outside the scope of an IRIS Toxicological Review. The brief summaries included in the Toxicological Review are for context and are neither comprehensive nor a primary resource on these topics. Thus, additional information was not added.

Comment: "p. 3–12, lines 4–6. It is unclear why a comparison of PFDA to PFHxS is made here regarding chemical stability of PFDA. All PFAAs, short- and long-chain, are chemically stable and are not metabolized, and there is no reason to use PFHxS as a "benchmark" for chemical stability. Also, "C6" refers to PFHxA, not PFHxS."

EPA Response: The comparison is to "other shorter length PFAA chemicals" with PFHxS only mentioned parenthetically as an example: "The findings are expected since PFDA is a long-chain (C10) PFAA with chemical stability similar to that of other shorter length PFAA chemicals (e.g., perfluorohexane sulfonic acid, PFHxS)." It is not presented as a "benchmark" for chemical stability, nor is the term "benchmark" used. It is a valid example of shorter-chain PFAAs and is a C6 PFAS, so no edits were made.

Comment: "p. 3–12, lines 6–8. The sentence about potential metabolism after inhalation or dermal exposure appears to be unnecessary and potentially misleading. PFDA does not undergo

chemical reactions in the body, and there is no reason to believe that there might be metabolism after inhalation or dermal exposure."

EPA Response: Since some chemicals have different biological fates depending on the route of exposure, EPA believes the sentence is appropriate to make the point that such is not expected for PFDA.

Comment: "p. 3–12, line 15. It is important to discuss that the 'sex-specific elimination' in rats mentioned here for other PFAS is very different than for PFDA. The excretion rate of several other PFAS (e.g., PFBA, PFHpA, PFOA, PFNA, PFHxS) is faster in females than in males, and this difference is dramatic for some PFAS. For example, the rat half-life of PFOA is 4–6 days in males and 2–4 hours in females, and for PFNA, it is 30 days in males and 1–2 days in females. See Table 17-7 of the ITRC PFAS Technical and Regulatory document at https://pfas1.itrcweb.org/17-additional-information/#17_2. In contrast to these other PFAS, the rate of excretion of PFDA in rats is somewhat faster in males than in females, as indicated by the PFDA half-life in male rats that is 33% lower than in female rats (Table 3-3)."

EPA Response: EPA agrees that the sex difference observed for other PFAS does not translate to PFDA in rats. EPA's analysis indicates no significant difference between male and female rats, and the text was revised to indicate that this is in contrast to other PFAS.

Comment: "p. 3–12, lines 23–24. It is not clear if this statement about fecal excretion applies only to rats or also applies to humans and other species."

EPA Response: A paragraph on the limited human data for fecal excretion has been added.

Comment: "p. 3–12, line 24 – p, 3–13, line 2. The first two studies discussed here (Kudo et al., 2001; Vanden Heuvel et al., 1991) used high doses of PFDA (20 and 25 mg/kg) and reported much more excretion in the feces as compared with the urine. In third study (Kim et al., 2019), a much lower dose (1 mg/kg; Table 3-2) was used, and a higher percentage of excretion was in the urine. Could this difference from the other two studies have been due to the much lower dose?"

EPA Response: For most PFAAs that EPA has analyzed, excretion appears to be more rapid at higher doses and this is attributed to saturable resorption in the kidneys, leading to an expectation that urinary excretion will increase with dose, not decrease. In general, however, it is possible that the shift for PFDA is dose dependent, and a sentence indicating this hypothesis was added.

Comment: A commenter suggested "that additional information be added about infants' exposure to PFDA through breast milk" on p. 3–16, lines 22–35.

EPA Response: As IRIS Toxicological Reviews do not include detailed exposure information, the statement that PFDA transfer into breast milk is an exposure route for the breastfed child is considered sufficient.

Comment: A commenter stated "that the two compartment PK model developed by Kim et al. (2019) was abandoned prematurely" and that "The effort to update the Kim et al. model, however, could greatly reduce uncertainty in extrapolating from animals to humans."

EPA Response: Please see above responses relating to the multicompartment PBPK model of Kim et al. (2019) and EPA's evaluation and decision not to incorporate this model in the assessment.

Comment: "p. 3–24, paragraph beginning on line 29. This paragraph discusses uncertainties in the extrapolation to developmental exposure and dosimetry in children, but it does not mention exposure through breast milk. As above, breast milk is an important exposure source in infants, and this should be discussed here."

EPA Response: The discussion on uncertainty in HED calculations for PFDA in Section 3.1.7) was revised to include a discussion of PFDA exposure to children in breast milk:

"Studies evaluating the impact of breastfeeding on other PFAS have shown that it can be a significant route of exposure for the infant. For example, Koponen et al. (2018) showed a significant increase in the serum concentration of PFOS, PFOA, PFNA and PFHxS in children at 1 year of age with months of breastfeeding. A linear regression of the data estimated an approximately 3-threefold increase in PFNA and 8-eightfold increase in PFHxS concentration from 12 months of breastfeeding versus children who were not breastfed. A significant decline was then observed in serum concentrations in children at 6 and 10.5 years of age compared with 1 year (Koponen et al., 2018). Although the median ratio of PFDA concentration in breast milk to maternal serum was only 0.03 (3%) (Liu et al., 2011), breastfeeding was found to reduce PFDA serum concentrations of women who had breastfed by an average of 1.3% per month of breastfeeding (Kim et al., 2020). For comparison, the estimated average human half-life of 4.7 years corresponds to a decline of about 1.2% per month. This rate of lactational transfer is from an adult woman who has accumulated PFDA over her lifetime to a child that is 5%–10% of her body mass and thus appears to represent a significant source of exposure to the child. However, the exact extent of this transfer and the resulting time-course of PFDA in the child is unknown. The range of PFDA concentrations in breast milk found by Liu et al. (2011) was <0.001–0.070 ng/mL, over 70-fold, while the range in maternal serum was 0.052–1.271 ng/mL, about 24-fold. Two other sources of variability in the lactational transfer of PFDA to children is the source and exclusivity of breastfeeding in the child's nutrition. Not only do these results indicate wide variability in the amount of PFDA in breast milk, but in the transfer rate and efficiency from the mother by that route."

Comment: A commenter stated that "a key assumption of the DDEF approach is that male and female rats/mice are sufficiently comparable to male and female humans in their responses to PFDA. This foundation follows from guidance (USEPA 2014) that specifies that there should be a demonstrated concordance of the metabolic processes involved for the chemical between human and animal models. While the draft assessment describes the four aspects of PK (i.e., absorption, distribution, metabolism, excretion) in both animals and humans, there is no explicit discussion of similarities and differences in responses between humans and non-human animal models. Because the underlying logic of the DDEF approach relies on concordance between human and animal models, particularly in the absence of a data-based PK-model, EPA should explicitly demonstrate

that that sex-based PK patterns found in rats and mice are a reasonable choice when computing DDEF for humans."

EPA Response: In the case of PFDA, there is strong concordance between the "metabolic processes involved" in rats and humans since neither species metabolizes PFDA. There is an expected concordance between the expected response (pharmacodynamics) in rats and humans, but this concordance need not be sex specific. For example, hepatic effects observed in male rats may be predictive of hepatic effects in women, and the approach used assumes this is the case. Discussion of the general expected concordance of toxic effects between rats and humans is a component of the hazard identification, not the dosimetric extrapolation. The specific method of extrapolation does not depend on the concordance, given that the rationale is provided.

The use of sex-specific PK data or parameters for rats and mice simply accounts for the estimated PK in male versus female animals used for toxicity testing, to estimate the corresponding internal doses. Generally, when predicting the internal dose for an endpoint observed in female rats, sex-specific PK data for female rats should be used, and vice-versa for male rats. However, for PFDA, there is not a significant difference between the sexes. Thus, sex-specific parameters are not used for estimating dosimetry in humans.

H.4.3. External Peer-Review Comments on Noncancer Toxicity Value Uncertainty Factors

Peer-Review Comment Summary

- In reference to charge question 6a, four reviewers agreed that the uncertainty in the derivation of toxicity is scientifically justified and clearly described, while one reviewer commented that there were inconsistencies in the chosen uncertainty factors (see below) and that EPA should reparametrize the Kim et al. (2019) PBPK model (provided in response to question 6ai). Other reviewers declined to comment or did not specifically state their response to the question (see comments below; reviewers also provided a future consideration on this topic; see peer-review report).
- In reference to charge question 6b, five reviewers stated that the selection of an uncertainty factor of 1 to account for extrapolation from less than lifetime human data for immune effects is scientifically justified, while one reviewer thought the justification was unclear (see below) and other reviewers did not answer this question.
- In reference to charge question 6c, seven reviewers agreed with an uncertainty factor of 3 to extrapolate between effects in laboratory animals and in humans during the derivation of the subchronic RfD for liver effects, while one reviewer recommended a factor of 10 (see below).
- In reference to charge question 6d, six reviewers agreed that the justification is sufficient for selecting a UF_S of 10 when deriving a subchronic RfD for liver, male reproductive, and female reproductive effects, while one reviewer suggested use of an animal/human PBPK model and another reviewer recommended considering data from structurally related PFAS could help

inform the UF_S, which was also suggested by one of the reviewers that agreed with the justification (see below). One reviewer did not comment.

- In reference to charge question 6e, seven reviewers agreed that the remaining uncertainty factors are scientifically justified and clearly described, while an eighth reviewer suggested EPA could use language accessible to audiences without a background in epidemiology in explaining tools used to differentiate the health effects of PFDA when the chemical is part of a mixture of exposures (see below).

Tier 1 Necessary Revisions

Comment: A reviewer stated that they were "not supportive of the use of the value of 3 to account for lack of a chronic study in animals."

EPA Response: EPA assumes the reviewer is referring to the UF_D; the respective charge question was specifically focused on the UF_A. The lack of a chronic study in animals was not the reason this value was selected, although this was highlighted as a data gap. Rather, a UF_D of 3 and a UF_D of 10 were both considered due to the limited database (e.g., the lack of a two-generation reproductive toxicity study). Based on the available data for PFDA and structurally related PFAS (e.g., PFOA), the lack of a multigenerational reproductive study is not considered a major concern as developmental reproductive effects do not appear to represent a more sensitive target in the current studies. Furthermore, the database for PFDA does contain well-conducted studies on a range of health outcomes in multiple species, including sensitive evaluations of developmental and immune endpoints in humans. Remaining gaps in the database for PFDA include the lack of an evaluation of postnatal effects in animals, specific investigations of potential thyroid or neurodevelopmental effects (which have been shown to be potentially sensitive outcomes for other PFAS), and long-term studies in multiple species. Based on these residual uncertainties, a UF_D of 3 was chosen.

Comment: Two reviewers suggested that EPA consider whether data from structurally related PFAS could help inform the UF_S value for one or more of these effects with one of these reviewers further suggesting that "if they have already done so, explicitly state this in Section 5.2.3 of the draft assessment."

EPA Response: In the Toxicological Review for PFDA, toxicity data from structurally related PFAS were incorporated to partially address data gaps for PFDA as needed. For example, conclusions from structurally related PFAS were used as rationale to select a UF_D of 3. For the selection of the UF_S in the derivation of the subchronic osRfDs for liver and male and female reproductive effects, the chemical-specific data for PFDA indicate a potential to worsen with increasing duration. For example, PFDA induced a continuous state of diestrus in 100% of rats treated at the highest dose tested indicating that it is possible that PFDA-induced effects on estrous cyclicity could become more sensitive or lead to more severe downstream effects like infertility with longer exposure durations. Given that such conclusions are based on PFDA-specific data, it was

not considered appropriate to prioritize toxicity data from structurally related PFAS to inform the selection of the UF_S.

Comment: A reviewer stated that "The lack of consideration of women who are not menstruating due to amenorrhea, exercise or being on contraception should be noted in the analysis."

EPA Response: On the basis of further analysis of NHANES data, showing differences between men and women in serum levels of some PFAS but not PFDA, EPA has now concluded that menstrual fluid loss is unlikely to be a general process for PFAS clearance, although women appear to preferentially clear some PFAS more rapidly than men. The NHANES data are presumed to indicate the average case for all women, including those who do not menstruate for various reasons. Thus, this consideration no longer applies.

Tier 2 Suggested Revisions

Comment: A reviewer suggested "defining the abbreviations in Table 5-9 [Table 5-11 of the revised Toxicological Review] (this is buried and hard to find), explain why the math doesn't add up (adding uncertainty factors as shown does not produce the sum shown), and make it clear that the one to the right is the sum."

EPA Response: The definitions for the individual uncertainty factors can be found in the list of "Abbreviations and Acronyms" and throughout the text. Calculation of the composite uncertainty factor is consistent with current EPA dose-response assessment methodology as discussed in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994).

Comment: A reviewer stated that "With a long half-life PFDA may accumulate in the body over time, that is, intake exceeds excretion from the body. Thus, a lifetime exposure may result in an internal exposure that is greater than in early life, if the rate of intake exceeds the excretion rate. I recommend that a calculation be completed in this regard using an animal/human PBPK model for PFDA to gain insights into subchronic and chronic exposures. If no accumulation appears likely (quasi-steady state) then justify your UF by giving examples of chemicals that have 28-day, 90-day and 2-year toxicity information. Is a UF of 10 justified?"

EPA Response: The pharmacokinetic portion of the UF_A is based on chemical-specific PK data in animals and humans (reducing the uncertainty as compared with default extrapolations), and the UF_S was set to a value of 10 for interpretation of the rat 28-day studies to address the concern that continued exposure, even in the absence of accumulation, could lead to a larger effect due to pharmacodynamic factors.

As shown in Figure G-8, PK model predictions that are reasonably accurate indicate accumulation occurs throughout the 28-day NTP bioassay. The PK analysis now accounts for this accumulation in estimating the average concentration in rats during a 28-day study. The PK model is used to estimate the internal dose during the mouse developmental study, for which the

estimated average concentration was also used, hence accounting for accumulation. There are no 90-day or 2-year animal toxicity studies being evaluated. The estimated human half-life of 4.7 years (see Table 3-3) indicates accumulation over a number of years for humans but not for an entire lifetime. The NHANES PFDA data in Figure 3-2 indicate only modest accumulation in individuals past their mid-twenties, so for human dosimetry calculations, the assumption of steady state is deemed appropriate. Since EPA has switched from use of the DDEF to use of the PK model or interpolation of measured serum concentrations (informed by the model) for evaluation of the dosimetry in rats and mice, the revised approach addresses the lack of accumulation in the animal toxicity studies, reducing the uncertainty in extrapolation to longer durations.

Comment: A reviewer stated that "I can see the rationale, more or less, for PFDA. I am stuck with the mixture issues and the uncertainty about PFDA potency when found in a mixture. I think the epidemiologic tools used to tease out its contribution need to be more transparent, using a language that all can understand."

EPA Response: EPA details how this complex issue is addressed in the PFAS protocol (U.S. EPA, 2021b) and in Section 1.2.3: "The potential for confounding across PFAS is incorporated in individual study evaluations and assessed across studies in evidence synthesis. In most studies, it is difficult to determine the likelihood of confounding without considering additional information not typically included in individual study evaluation (e.g., associations of other PFAS with the outcome of interest and correlation profiles of PFAS within and across studies). In addition, even when this information is considered or the study authors perform analyses to adjust for other PFAS, it is often not possible to fully disentangle the associations due to high correlations. This stems from the potential for amplification bias in which bias can occur following adjustment of highly correlated PFAS (Weisskopf et al., 2018). Thus, in most studies, there may be some residual uncertainty about the risk of confounding by other PFAS. A "Good" rating for the confounding domain is reserved for situations in which concern is minimal for substantial confounding across PFAS as well as other sources of confounding. Examples of this situation include results for a PFAS that predominates in a population (such as a contamination event) or studies that demonstrate robust results following multi-PFAS adjustment, which would also indicate minimal concern for amplification bias. Because of the challenge in evaluating individual studies for confounding across PFAS, this issue is also assessed across studies during the evidence synthesis phase (as described in the systematic review protocol; Appendix A, Section 9), primarily when there is support for an association with adverse health effects in the epidemiological evidence (i.e., *moderate* or *robust* evidence in humans, as described below). Analyses used include comparing results across studies in populations with different PFAS exposure mixture profiles, considering results of multipollutant models when available, and examining strength of associations for other correlated PFAS. In situations in which there is considerable uncertainty regarding the impact of residual confounding across PFAS, it is captured as a factor that decreases the overall strength of evidence (see Appendix A.10)." In addition to the protocol text above, for each health effect with *moderate* or *robust* epidemiological

evidence (i.e., developmental and immune), there is text within the synthesis (or an accompanying appendix) that describes how these considerations were applied for that health effect. The specific approach is driven by the available data for that health effect; analyses appropriate with several studies reporting multipollutant modeling results are not appropriate when few are available. Other sections (i.e., with *slight* or *indeterminate* synthesis judgments) do not include this thorough analysis because other sources of uncertainty (e.g., inconsistency) are more substantial and it would be difficult to identify patterns related to confounding across PFAS. Lastly, EPA provided an evaluation of the potential for confounding for studies of birth weight in Appendix F. This discusses the strengths and limitations of single and multipollutant modeling and provides details on any direct evidence for confounding by comparing modeling results and examining underlying relationships between co-occurring PFAS and this endpoint.

Comment: A reviewer stated that "The justification for a UF of 1 for a sensitive lifestage was not clear."

EPA Response: The justification was revised as follows: "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 in adulthood."

H.4.4. Public Comments on Noncancer Toxicity Value Uncertainty Factors

Comment: A commenter stated "EPA asserts that the overall uncertainty in that extrapolation is less than a factor of 3, concluding that the choices involved in human clearance would offset the uncertainty of the rat dosimetry. This rationale is not clearly worded, but it appears that EPA means that the rat dosimetry would tend to result in overly high clearance rates, and that the choice to use a low fecal excretion rate in humans would offset this uncertainty. The value assumed for fecal clearance (73% of urinary clearance) is overly conservative, as noted by DOD. The review of excretion patterns in rats and humans demonstrates that fecal excretion is important for long-chain PFAS. Several sources cited in the draft report a greater proportion of excretion via the fecal route than via the urinary route for PFDA (Kudo et al.2001; Fujii et al. 2015; Kim et al. 2019). Selecting an unusually low value for fecal clearance to offset the high value of urinary clearance does not offset the uncertainty of the rat dosimetry; rather, the practice unnecessarily reduces the assumed clearance rates. Finally, it is unclear how EPA determined that the uncertainty associated with the animal-to-human extrapolation is within a factor of 3, unless EPA is asserting that fecal excretion rate has no uncertainty and thus does not contribute to the uncertainty associated with extrapolation."

EPA Response: The fecal elimination estimate of Fujii relies on animal PK data (V_d), read-across analysis from other PFAS, and data from only five human subjects, all being treated for diseases, hence is considered highly uncertain. However, based on comparisons of the results of Fujii to estimates of total versus urinary clearance of other PFAS (for which both types of estimates were available), it appears the fecal clearance estimates of Fujii are in the correct range. Therefore, the total human clearance estimate was revised to use the value for fecal clearance from Fujii.

Given the variability in the PK data and estimates, it is possible that human clearance is more than threefold higher than the value used. The estimated factor of 3 was for the possibility that clearance is lower than the value used, i.e., based on the data available, EPA considers it unlikely that the true human mean is more than threefold lower than the estimated value or that a significant part of the population has a clearance that much lower.

H.5. CHARGE QUESTIONS 7 AND 8 – CARCINOGENICITY HAZARD IDENTIFICATION AND TOXICITY VALUE DERIVATION

7. The Toxicological Review concludes there is *inadequate information to assess carcinogenic potential* for PFDA and that this descriptor applies to oral and inhalation routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies, and the analysis presented in the Toxicological Review are scientifically justified and clearly described.

8. Given the conclusion there was *inadequate information to assess carcinogenic potential* for PFDA, the Toxicological Review does not derive quantitative estimates for cancer effects for oral or inhalation exposures. Is this decision scientifically justified and clearly described?

H.5.1. External Peer-Review Comments on Carcinogenicity Hazard Identification and Toxicity Value Derivation

Peer-Review Comment Summary

In reference to charge question 7, eight reviewers agreed that there currently is inadequate information to assess the carcinogenetic potential of PFDA. However, one of the reviewers also commented on including elaboration about observations of chromosomal abnormalities and an additional reviewer agreed on these points (see below). One reviewer refrained from commenting.

In reference to charge question 8, all nine reviewers agreed that the decision not to derive quantitative estimates for cancer effects for oral or inhalation exposures was scientifically justified and clearly described.

Tier 1 Necessary Revisions

Comment: A reviewer identified an additional study by Pan et al., (2019) that reports on the association of PFDA exposure with DNA fragmentation in human sperm and stated, "A Tier 1 recommendation is given here to first determine where in the hazard identification process such DNA impacts will be reviewed and second to undertake the evaluation of such data for the additional endpoint. This reviewer would suggest to add to the supplemental information considered for reproductive impacts."

EPA Response: As stated previously, Pan et al., (2019) reported an association with increased sperm DNA fragmentation; however, the results for semen parameters most relevant for evaluation of male reproductive effects (i.e., motility, concertation, and morphology) were null,

inconsistent, or imprecise (nonsignificant). Therefore, EPA concluded that the study would not be included since it does not influence the assessment conclusions for human or mechanistic evidence or the overall hazard judgment for male reproductive effects. Text was added to Table I-1 in Appendix I to clarify EPA's disposition on the DNA fragmentation findings from the Pan et al., (2019) study.

Tier 2 Suggested Revisions

Comment: Another reviewer suggested, "To avoid readers confusing 'inadequate information' with 'not carcinogenic,' recommend that values for PFASs with similar properties (e.g., PFOA and PFOS) be noted."

EPA Response: Table 4-1 in the Toxicological Review draft compares hazard conclusions for PFDA and other PFAS (e.g., PFOS and PFOA) across published EPA human health assessments, including judgments for carcinogenicity, the latter of which includes descriptors based on EPA guidelines.

H.5.2. Public Comments on Carcinogenicity Hazard Identification and Toxicity Value Derivation

No public comments were provided.

H.6. ADDITIONAL COMMENTS

H.6.1. Additional External Peer-Review Comments

Tier 1 Necessary Revisions

Comment: A reviewer recommended "Check Table 1-1 for consistency with information currently available on online (on portals such as EPA's CompTox Chemicals Dashboard); evaluate the feasibility of expanding Table 1-1 by including values of PFDA properties that are critical for is pharmacokinetics (e.g., binding affinities to serum proteins)."

EPA Response: The physical-chemical properties listed in Table 1-1 have been updated on the basis of values from the U.S. EPA CompTox Chemicals Dashboard as of January 17, 2024. The list of properties presented in Table 1-1 is boilerplate; property values related to pharmacokinetics can be found in Section 3.

Comment: A reviewer noted that the toxicological effects of PFDA are best addressed from a mixtures perspective, since it appears unlikely that the effects of PFDA, which represents only a few percent of the total mixture, can be teased out for regulatory purposes. A section on mixtures could be added to the assessment. Additionally, the language on confounding across PFAS for epidemiological studies (page 1–11 and 1–12) under the Summary of Assessment Methods is inadequate and the strengths and weaknesses of such assumptions should be clearly articulated in nonepidemiological language.

EPA Response: This Toxicological Review is specific to PFDA and its related salts and the consideration of a potential additive effect of exposure to multiple PFAS chemicals would not be appropriate for a scientific document developed for one PFAS. The consideration of potential additive effects of exposure to multiple PFAS would be part of the risk assessment and risk management activities such as the application of this Toxicological Review (once finalized), along with other relevant assessments by risk managers addressing human exposure to multiple PFAS. Thus, this is outside of the scope of the IRIS Program. The draft human health toxicity values for PFDA are part of a broader PFAS strategic roadmap (https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024) at EPA for protecting human health and the environment from PFAS contamination. Text was added to the Executive Summary of the Toxicological Review draft to provide examples from the PFAS strategic roadmap aimed at evaluating health effects from individual PFAS and PFAS mixtures or groups. Additionally, Table 4-1 in Section 4.1 compares health effects for PFDA and other PFAS across EPA human health assessments, highlighting notable data gaps for PFDA and PFAS in general.

Tier 2 Suggested Revisions

Comment: A reviewer suggested "With the limited number of studies on PFDA some mention of the location of summary charts with all PFAS effects showing a comparison across all PFAS would be useful."

EPA Response: Table 4-1 compares hazard conclusions for PFDA and other PFAS across EPA human health assessments, highlighting major data gaps for PFDA and PFAS in general.

Comment: A reviewer commented, "A Tier 2 recommendation is for the authors to add an explanatory paragraph that states how the Computational Toxicology information, now primarily in the appendix, is or will be incorporated across endpoints and effects. Such a paragraph is needed to ensure the intended user of the current IRIS review is clear and can articulate how and when the additional information from the appendix was used and also perhaps when it was not used across endpoints."

EPA Response: Section 1.2.5 of the Toxicological Review discusses the approach for synthesizing mechanistic evidence including computational toxicology data, and more details can be found in the systematic review protocol for the PFAS Toxicological Reviews (see Appendix A, Section 9.2). Evaluation of computational toxicology data involved targeted analyses that were considered helpful to inform key decisions regarding the human and animal evidence. For example, available in vitro high-throughput screening assays from the ToxCast/Tox21 databases were compiled and analyzed to address questions of biological plausibility and human relevance of PFDA-induced liver and reproductive effects.

Comment: Three reviewers identified several references for consideration related to human and animal pharmacokinetics, in vitro and in silico studies, human exposure, human population health effects, large-scale biomonitoring programs, review articles, risk assessments, frameworks and protocols for PFDA and/other PFAS.

EPA Response: EPA has reviewed these references against the PECO criteria. Tables I-1, I-2 and I-3 in Appendix I provide the identified studies that met PECO criteria or certain supplemental evidence categories (i.e., in vivo mechanistic or MOA studies, including non-PECO routes of exposure and populations; in vitro and in silico models; and ADME and pharmacokinetic studies) and EPA's judgment on whether the studies would have a material impact on the assessment conclusions (i.e., identified hazards or toxicity values). Three ADME-related studies identified by the reviewers were incorporated into the pharmacokinetics section (see Section 3.1) of the Toxicological Review because they help address specific data gaps (Bao et al., 2022; Chen et al., 2022; Pérez et al., 2013). The results of this screening are also included on the HERO project page (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2614).

H.6.2. Additional Public Comments

Comment: One commenter noted "Given that people are exposed to PFAS mixtures and PFDA causes similar health effects at similar doses as other PFAS, EPA should consider including a section on PFAS cumulative risks.

EPA Response: This Toxicological Review is specific to PFDA and its related salts, and the consideration of a potential additive effect of exposure to multiple PFAS chemicals would not be appropriate for a scientific document developed for one PFAS. The consideration of potential additive effects of exposure to multiple PFAS would be part of the risk assessment and risk management activities such as the application of this assessment (once finalized) along with other relevant assessments by risk managers addressing human exposure to multiple PFAS. Thus, this is outside of the scope of the IRIS Program. The draft human health toxicity values for PFDA are part of a broader PFAS strategic roadmap (https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024) at EPA for protecting human health and the environment from PFAS contamination. Text was added to the Executive Summary of the Toxicological Review draft to provide examples from the PFAS strategic roadmap aimed at evaluating health effects from individual PFAS and PFAS mixtures or groups. Additionally, Table 4-1 in Section 4.1 compares health effects for PFDA and other PFAS across EPA human health assessments, highlighting notable data gaps for PFDA and PFAS in general.

APPENDIX I. EPA CHARACTERIZATION OF STUDIES IDENTIFIED AFTER PUBLIC RELEASE OF THE IRIS TOXICOLOGICAL REVIEW OF PERFLUORODECANOIC ACID (PFDA) AND RELATED SALTS

Tables I-1, I-2 and I-3 below describe literature identified during the 2023 literature search update performed after release of the public comment draft (as described in Section 1.2.1 of the IRIS perfluorodecanoic acid external review draft) or submitted in public comments received through the EPA docket[9] or during external peer review. In accordance with charge question 1, the tables show EPA's disposition on the need to incorporate these studies into the finalized Toxicological Review and the interpreted impact of these studies on key judgments in the draft Toxicological Review (i.e., identified hazards and dose-response values, or pivotal uncertainties). The panel is asked to weigh in on EPA's disposition. Supplemental study categories included here are "ADME" and "mechanistic, including non-PECO exposure route." All identified studies not meeting PECO or the aforementioned supplemental categories can be found in the HERO database.

[9]The State of New Jersey Department of Environmental Protection and the Natural Resources Defense Council (NRDC) submitted 186 studies. Of the 186 studies, 119 studies had been previously identified and can be found in the HERO database. The remaining 67 studies were screened for PECO criteria and evaluated for potential incorporation and impact on the assessment's conclusions, as stated above.

Table I-1. Human studies meeting assessment PECO criteria

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Immune effects				
Kaur et al. (2023)	Lit update	Antibody levels to SARS-COV2 in adults	Inverse but not statistically significant association (beta −0.15, 95% CI: −0.60, 0.29)	No. Findings are consistent with existing evidence and have no impact on immunosuppression conclusions (which are primarily based on studies in children) and on initial review; there are concerns for risk of bias due to lack of consideration of other COVID risk factors).
Gaylord et al. (2019)	Commenter	Asthma	No association with asthma diagnosis (OR 0.90, 95% CI: 0.69, 1.17)	No. Existing evidence on asthma is inconsistent and new studies do not change current draft judgment.
Averina et al. (2019)	Commenter	Asthma	No association with asthma, eczema, allergies	
Ammitzbøll et al. (2019)	Commenter	Multiple sclerosis	No association with multiple sclerosis overall, but an indication of interaction by sex (positive association in women, inverse association in men)	No. The evidence on potential associations with autoimmune is sparse and would likely be *indeterminate* for autoimmune effects overall.
Qu et al. (2022)	Lit update	Rheumatoid arthritis	No association with rheumatoid arthritis	
Gaylord et al. (2020)	Commenter	Celiac disease	No association with celiac disease (OR 0.89, 95% CI: 0.55, 1.46)	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Developmental effects				
Hall et al. (2022)	Lit Update	Fetal growth restriction /Gestational duration	Inverse associations for PFDA and both BWT and gestational age in males only. Associations were large (~ −200 g) for BWT and statistically significant for gestational age (~ −1.5 wk).	No. The inverse associations in males along with null results in females would not change the current draft judgment for fetal growth restriction or gestational duration.
Wang et al. (2023)	Lit update	Fetal growth restriction (birth length (BL); head circumference (HC); birthweight (BWT))	BL Male β = −0.061 (95% CI: −0.102, 0.223). Female β = −0.175 (95% CI: −0.470, 0.120). HC Male β = −0.036 (95% CI: −0.247, 0.174). Female β = −0.311 (95% CI: −0.528, −0.093). BWT Male β = 0.010 (95% CI: −0.176, 0.197). Female β = −0.010 (95% CI: −0.176, 0.197).	No. The inverse associations (BL and HC) in females along with null BWT results in females and null results in males for all three endpoints would not change the current draft judgment for fetal growth restriction.
Peterson et al. (2022)	Lit update	Fetal growth restriction	Small nonsignificant head circumference results and null associations for fetal biparietal diameter (Figure 2).	No. Mixed results for fetal biometric endpoints would not change the current draft judgment for fetal growth restriction.
Padula et al. (2023)	Lit update	Fetal growth restriction, preterm birth	GA β = −0.11 (95% CI: −0.32, 0.09) BWT_GA β = −0. 25 (95% CI: −0.37, −0.14) Term LBW OR = 2.24 (95% CI: 0.96, 5.24)	No. Null results for PTB, SGA and increased and inverse risks for Term LBW and GA and BWT-GA would not change the current draft judgment for

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
			SGA OR = 1.18 (95% CI: 0.81, 1.73) LGA OR = 0.52 (95% CI: 0.35, 0.77) PTB OR = 1.22 (95% CI: 0.80, 1.86)	either gestational duration or fetal growth restriction.
Ouidir et al. (2020)	Commenter	Fetal growth restriction	Positive associations with some fetal growth measures based on ultrasonography.	No. Study population was previously reported in a publication already in the Toxicological Review, Buck Louis et al. (2018). New results for in utero measurements would not change the current draft judgment.
Petroff et al. (2023)	Lit update	Gestational age	GA β = −0.27 ± 0.17 (p = 0.114).	No. Small nonsignificant inverse association with gestational age would not change the current draft judgment for gestational duration.
Yu et al. (2022)	Lit update	Preterm birth	OR = 1.20 (95% CI: 0.95, 1.53) per ln-unit increase OR = 1.52 (95% CI: 0.96, 2.42) per ng/mL increase	No. Small increased risks here along with the null results in Padula et al. (2023) and Liao et al. (2022b) would not change the current draft judgment for gestational duration.
Liao et al. (2022b)	Lit update	Preterm birth	PTB per unit increase (OR = 0.985; 95% CI: 0.624, 1.556) and tertiles (T3 OR = 1.066; 95% CI: 0.677, 1.679; T2 OR = 0.815; 95% CI: 0.511, 1.302).	No. The null results here and for Padula et al. (2023) above combined with small increased risks by Yu et al. (2022) above would not change the current draft judgment for gestational duration.

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Wang et al. (2016)	Commenter	Preterm birth	Similar PFDA concentrations in term and preterm births	No. This study reported only mean exposure concentrations without control for confounding so would not influence existing judgment.
Hong et al. (2022)	Lit update	Spontaneous abortion	Inverse but not statistically significant associations (OR 0.23, 95% CI: 0.03, 1.70 for IVF group, OR = 0.67; 95% CI: 0.16, 2.73 for entire clinic population	No. Updated analysis of study that is already included in the draft Toxicological Review.
Li et al. (2022a)	Lit update	Anogenital distance	No association. AGD-AF: β = 0.29 (95% CI: −0.12, 0.70) AGD-AC: β = 0.35 (95% CI: −0.16, 0.85) AGI-AF: β = 0.07 (95% CI: −0.06, 0.19) AGI-AC: β = 0.07 (95% CI: −0.12, 0.26)	No. Null findings would not change the current draft judgment.
Hepatic				
Rantakokko et al. (2015)	Commenter	Nonalcoholic fatty liver disease	Inverse association with lobular inflammation (OR 0.05, 95% CI: <0.01, 0.83 for 2–4 foci per 200× field)	Yes. Based on peer-review comments, there was some disagreement about whether the human evidence for hepatic effects was *slight* as presented in the draft or *moderate*. The new studies were added to determine whether they provided support to move to change the judgment.
Borghese et al. (2022)	Lit update	Liver enzymes	Positive but not statistically significant associations with AST and GGT, no association with ALP (ALT not analyzed	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
			with PFDA for unspecified reason)	
Liao et al. (2023)	Lit update	Liver enzymes	No association with ALT, AST, GGT, bilirubin	
Kim et al. (2023b)	Lit update	Liver enzymes	Positive but not statistically significant associations with ALT, AST, and GGT	
Yao et al. (2020)	Commenter	Liver enzymes	Positive association with ALT, AST, GGT (statistically significant for GGT)	
Salihović et al. (2019)	Commenter	Bile acid levels (liver)	Association of PFDA and bile acids examined in human plasma	No. Results would not change draft judgments.
Cancer				
Feng et al. (2022a)	Lit update	Breast cancer	No association with breast cancer (OR = 0.98, 95% CI: 0.77, 1.25) per unit increase in ln-transformed plasma PFDA levels	No. Inconsistent results across the new studies and the only other study reported nonsignificantly increased risk of breast cancer among women ≤50 yr of age; and nonsignificantly decreased risk of breast cancer among women >50 yr of age. In addition, one new breast cancer study reports on the same study population as a publication already in the Toxicological Review, Wielsøe et al. (2017). For liver cancer, the two studies are inconsistent. The weak association observed for renal cancer
Li et al. (2022d)	Lit update	Breast cancer	Increased risk for breast cancer (OR = 2.22, 95% CI: 1.55, 3.17) per SD increase in ln-transformed PFDA.	
Wielsøe et al. (2018)	Commenter	Breast cancer	Positive but not statistically significant association (OR 2.66, 95%	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
			CI: 0.64, 11.1 in high vs. low exposure for one genotype)	dissipated when controlled for other PFAS. The available epidemiological evidence on PFDA and the risk of cancer remains ***inadequate***; the new studies are not impactful.
Lee et al. (2020)	Commenter	Breast cancer	No association with mammographic density (beta −0.21, *p*-value = 0.5)	
Goodrich et al. (2022)	Lit update	Liver cancer	No association with liver cancer (OR = 0.8, 95% CI: 0.31, 2.00) for PFDA greater than the 90th% vs. less than 90th%.	
Cao et al. (2022)	External peer reviewer	Liver cancer	Positive but not statistically significant association with liver cancer (OR 1.18, 95% CI: 0.96, 1.40 per log-unit increase in PFDA)	
Shearer et al. (2021)	Commenter	Renal cancer	Positive but not statistically significant association with renal cell carcinoma (OR 1.70, 95% CI: 0.72, 4.03 in Q4 vs. Q1)	
Neurodevelopment				
Luo et al. (2022a)	Lit update	Broad neurodevelopmental scale	Inverse association with cognitive, language, and motor scores, but not social-emotional or adaptive behavior scores	No. There is considerable inconsistency in the evidence for neurodevelopmental effects, and the new studies would

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Oh et al. (2022b)	Lit update	Autism, developmental delay	No increase in odds of autism spectrum disorder, developmental delay	not influence the draft synthesis judgment of *slight* evidence.
Zhou et al. (2023)	Lit update	Broad neurodevelopmental scale	Inverse association with communication, motor, problem solving, and personal-social (latter two not statistically significant) at 6 mo but not other visits (2, 12, and 24 mo)	
Li et al. (2023c)	Lit update	Broad neurodevelopmental scale	Positive association with persistently low trajectory for gross motor ($p < 0.05$), problem solving ability, and personal-social skills, but not communication, fine motor	
Oulhote et al. (2019)	Commenter	Broad neurodevelopmental scale	No association with total scores to Boston Naming Test or Strengths and Difficulties Questionnaire	
Kim et al. (2023a)	Lit update	ADHD scale	Positive though nonmonotonic association with ADHD rating scale at 8 yr, dependent on age at exposure measurement	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Male reproductive				
Luo et al. (2022b)	Lit update	Semen parameters	Inverse but not statistically significant association with motility	No. Evidence is inconsistent and the new studies would not influence the draft conclusion.
Pan et al. (2019)	Commenter	Semen parameters	Direction of association for motility differed when exposure measured in semen vs. serum (both nonsignificant); no association with concentration or morphology; statistically significant association with increased DNA fragmentation and seminal PFDA levels	
Ma et al. (2021)	Commenter	Semen parameters	Inverse but not statistically significant association with sperm concentration and morphology; no association with motility	
Rivera-Núñez et al. (2023)	Lit update	Reproductive hormones	Positive association with free T but not T, E1, E2, or E3	No. Evidence is inconsistent in existing studies and the new studies would not influence the draft synthesis conclusion of *indeterminate* evidence.
Nian et al. (2020)	Commenter	Reproductive hormones	No association with total testosterone (beta −0.029, 95% CI: −0.09, 0.032 per ln-unit change), FSH, or LH	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Female reproductive				
Hong et al. (2022)	Lit update	In vitro fertilization outcomes	No association with oocyte maturation rate, fertilization rate, high quality embryo rate; inverse but not statistically significant association (OR = 0.81, 95% CI: 0.46–1.41) for clinical pregnancy	No. Evidence of an association with fecundity and infertility is inconsistent across new studies and was similarly inconsistent across existing studies. The conclusion of *indeterminate* evidence would remain the same; the new studies are not impactful to draft conclusions.
Cohen et al. (2023)	Lit update	Fecundity, pregnancy	Longer time to pregnancy (FR 0.90, 95% CI: 0.82, 0.98) and lower odds of clinical pregnancy (OR 0.74, 95% CI: 0.56, 0.98)	
Luo et al. (2022c)	Lit update	Fecundity, infertility	No association with reduced fecundability (FR 1.06, 95% CI: 0.92, 1.20) or infertility (OR 0.96, 95% CI: 0.74, 1.26)	
Tan et al. (2022)	Lit update	Infertility	Lower odds of infertility (nonmonotonic across quartiles and not statistically significant)	
Buck Louis et al. (2013)	Commenter	Fecundity	No association with reduced fecundability (FR 1.11, 95% CI: 0.95, 1.29)	
Whitworth et al. (2016)	Commenter	Fecundity	No association (FR 1.00, 95% CI: 0.85, 1.2)	
Ma et al. (2021)	Commenter	In vitro fertilization outcomes, pregnancy	No association with number of oocytes,	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
			zygotes, embryos, or clinical pregnancies	
Wang et al. (2019)	Commenter	Polycystic ovarian syndrome	Positive but not statistically significant association with PCOS-related infertility (OR 2.06, 95% CI: 0.63, 6.78 in 3rd vs. 1st tertile)	No. Existing evidence on gynecological conditions is inconsistent and new study does not change conclusions.
Rivera-Núñez et al. (2023)	Lit update	Reproductive hormones	Inverse association with E1 ($p < 0.05$) and T, positive association with FT ($p < 0.05$); no association with E2, E3	No. New studies would not change the current draft judgment.
Nian et al. (2020)	Commenter	Reproductive hormones	No association with total testosterone (beta −0.029, 95% CI: −0.09, 0.032 per ln-unit change), FSH, or LH	
Liu et al. (2020a)	Commenter	Reproductive hormones	Positive association with estradiol (9.1% change, 95% CI: 4.6, 13.8)	
Lin et al. (2022)	Lit update	Postpartum hemorrhage	Higher odds of postpartum hemorrhage (OR 1.16, 95% CI: 0.48, 2.82) but imprecise	No. Single study of the outcome and evidence is not strong enough to increase certainty in the evidence for female reproductive effects.
Rosen et al. (2018)	Commenter	Breastfeeding duration	Lower hazard of breastfeeding cessation with higher exposure	Yes. New section added to the Toxicological Review based on peer-review feedback.
Timmermann et al. (2017)	Commenter	Breastfeeding duration	Inverse association with duration of breastfeeding	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Pirard et al. (2020)	Commenter	Breastfeeding duration	No association with breastfeeding duration	No. Analyses designed to predict PFNA concentrations based on past breastfeeding duration without prospective measurement of exposure; high likelihood of reverse causation.
Ammitzbøll et al. (2019)	Commenter	Breastfeeding duration	Inverse association with breastfeeding duration in crude analysis	
Harris et al. (2017)	Commenter	Breastfeeding duration	Inverse association with breastfeeding duration in crude analysis	
Kim et al. (2020)	Commenter	Breastfeeding duration	Inverse association with breastfeeding duration in crude analysis	
Lee et al. (2018)	Commenter	Breastfeeding duration	Moderate positive correlation with breastfeeding duration in crude analysis	
Urinary				
Liang et al. (2023)	Lit update	Glomerular filtration rate	Positive association with GFR in women	No. Existing studies are inconsistent with considerable uncertainty due to potential reverse causation. The new studies do not inform this uncertainty.
Sood et al. (2019)	Commenter	Glomerular filtration rate	Inverse association with eGFR (beta −18.3, 95% CI: −35.3, −1.3)	
Feng et al. (2022b)	Lit update	Hyperuricemia	No association with hyperuricemia	
Yang et al. (2022b)	Lit update	Hyperuricemia	No association with hyperuricemia	
Arrebola et al. (2019)	Commenter	Hyperuricemia	No association with hyperuricemia	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Yao et al. (2020)	Commenter	Uric acid	Positive association with uric acid (beta 5.13, 95% CI: 1.92, 8.33)	
Cardiometabolic				
Maranhao Neto et al. (2022)	Lit update	Serum lipids, blood pressure, adiposity, blood glucose	Inverse associations with blood glucose, adiposity, and total cholesterol; no association with blood pressure	No. Mixed results from the new studies would not change the current draft judgment.
Haug et al. (2023)	Lit update	Serum lipids	Positive association with HDL and LDL cholesterol	
Donat-Vargas et al. (2019b)	Commenter	Serum lipids, hypertension	No association with total cholesterol, triglycerides, or hypertension	
Yao et al. (2020)	Commenter	Serum lipids, blood glucose	Positive association with total cholesterol (beta 6.29, 95% CI: 3.25, 9.53) and triglycerides, no association with blood glucose	
Sood et al. (2019)	Commenter	Blood pressure	No association with blood pressure (beta 0.4, 95% CI: −0.2, 0.9)	
Lind et al. (2018)	Commenter	Carotid artery intima-media thickness	Positive association with IMT thickness (beta 0.02, 95% CI: 0.006, 0.033)	No. These results support coherence with serum lipids but would not change the current draft judgment.
Li et al. (2023b)	Lit update	Cardiovascular disease	No association with acute coronary syndrome	No.

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
				New study contributes to existing inconsistency and would not change the current draft judgment.
Yang et al. (2022a)	Lit update	Gestational hypertension	Lower odds of gestational hypertension (OR 0.68, 95% CI: 0.47, 0.97) and lower continuous blood pressure	No. New studies contribute to existing inconsistency and would not change the current draft judgment.
Huo et al. (2020)	Lit update	Gestational hypertension	No association with gestational hypertension (OR 0.96, 9f% CI 0.63, 1.46) or preeclampsia (OR 0.95, 95% CI: 0.64, 1.41)	
Xu et al. (2022)	Lit update	Gestational diabetes	No clear association with gestational diabetes (positive but not statistically significant odds ratio in third tertile but inverse in second tertile), no association with continuous blood glucose in oral glucose tolerance test	No. Existing studies are primarily null and new studies with imprecise results and no clear dose-dependence would not change the current draft judgment.
Zhang et al. (2023)	Lit update	Gestational diabetes	No clear association with gestational diabetes (positive but not statistically significant odds ratio in third tertile but inverse in second tertile)	
Xu et al. (2020)	Lit update	Gestational diabetes	No association with gestational diabetes (OR	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
			0.76, 95% CI: 0.23, 1.91 per log-unit)	
Li et al. (2020)	Commenter	Gestational blood glucose	Positive but not statistically significant association with blood glucose in oral glucose tolerance test (beta 0.10, 95% CI: −0.05, 0.25)	
Dunder et al. (2023)	Lit update	Diabetes, blood glucose	Inverse but small and not statistically significant association (−0.01, 95% CI: −0.03, 0.006), stronger in women than men	No. Existing studies are primarily null and mixed results in new studies would not change the current draft judgment.
Christensen et al. (2016)	Commenter	Diabetes	Positive association with prediabetes (OR 2.72, 95% CI: 1.28, 6.13) but not diabetes (OR 1.00, 95% CI: 0.32, 2.19)	
Duan et al. (2021)	External peer reviewer	Diabetes	Inverse association with type 2 diabetes (OR 0.46, 95% CI: 0.25, 0.82 in T3 vs. T1)	
Donat-Vargas et al. (2019a)	Commenter	Diabetes risk, insulin resistance	No increase in diabetes risk or HOMA-IR	
Kim et al. (2015)	Commenter	Insulin resistance	Positive but not statistically significant association with HOMA (beta 0.32, 95% CI: −0.28, 0.93)	
Mehta et al. (2021)	Commenter	Insulin resistance	No association with blood glucose or HOMA-IR	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Brosset and Ngueta (2022)	Lit update	Glycemic control	No association with poor glycemic control	
Ye et al. (2021)	Commenter	Metabolic syndrome	Positive association with metabolic syndrome (OR 1.29, 95% CI: 0.96, 1.72) fasting blood glucose, triglycerides, and waist circumference	No. Studies are inconsistent and new study would not change the current draft judgment.
Schillemans et al. (2022)	Lit update	Adiposity	No association with BMI z-score	No. Mixed results in new studies would not change the current draft judgment.
Zeng et al. (2023)	Lit update	Adiposity	Positive association ($p < 0.05$) with persistent increase for BMI z-score trajectory	
Harris et al. (2017)	Commenter	Adiposity	Lower PFDA levels in obese and overweight participants (−15.6% difference, 95% CI: −28.7, −0.1 for obese vs. normal)	
Ji et al. (2012)	Commenter	Adiposity	Mean PFDA levels highest in normal weight participants (no statistical analysis)	
Pirard et al. (2020)	Commenter	Adiposity	Inverse association (beta −0.022, *p*-value 0.003) with PFDA concentration modeled as outcome	
Liu et al. (2020b)	Commenter	Adiposity	Inverse association with BMI (beta −0.01)	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Endocrine				
Jensen et al. (2022)	Lit update	Thyroid hormones	No association with free T4 or TSH	No. Existing studies are primarily null and new studies would not change the current draft judgment.
Derakhshan et al. (2022)	Lit update	Thyroid hormones	Positive association with free T4 (beta 0.27, 95% CI: 0.10, 0.45) but no association with TSH or free T3	
Li et al. (2023a)	Lit update	Thyroid hormones	Inverse but not statistically significant association with TSH in TPOAb participants, no association with free T4	
Tillaut et al. (2022)	Lit update	Thyroid hormones	Inverse association with free T3 ($p < 0.05$) and TSH ($p > 0.05$), no association with free T4	
Jain and Ducatman (2019)	Commenter	Thyroid hormones	No association with T4, T3, or TSH	
Dufour et al. (2020)	Commenter	Thyroid disease	Inverse association with hypothyroidism (OR 0.19, 95% CI: 0.05, 0.81) and hyperthyroidism (OR 0.17, 95% CI: 0.05, 0.57)	
Christensen et al. (2016)	Commenter	Thyroid disease	Inverse association with thyroid disease (OR 0.17, 95% CI: 0.01, 1.12)	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Other				
Højsager et al. (2022)	Lit update	Bone mineral density	Inverse association with bone mineral content and density ($p < 0.05$)	No. Available studies are inconsistent, and evidence would likely be *indeterminate*.
Zhao et al. (2022)	Lit update	Bone mineral density	No association with femur bone mineral density	
Colicino et al. (2020)	Lit update	Bone mineral density	No association with lumbar spine or femur density	
Shiue (2015d)	Commenter	Oral health	No association with teeth health, tooth ache, tooth loss	
Liao et al. (2022a)	Lit update	Hematology	Positive but nonmonotonic and not statistically significant association with gestational anemia (low hemoglobin) in the first and third but not second trimesters; no association with hemoglobin concentration during pregnancy	No. Inconsistent results in studies of hemoglobin. Evidence would likely be *indeterminate* overall.
Cui et al. (2022)	Lit update	Hematology	Positive association with hematocrit (2.70% change, 95% CI: 1.33, 4.1) and hemoglobin (2.50% change, 95% CI: 1.03, 3.99) during pregnancy	

Reference	Source[a]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[b]
Liu et al. (2022)	Lit update	Hematology	Inverse association with white blood cells and neutrophils	
Shiue (2015a)	Commenter	Neurologic; remembering condition	No association with difficulty remembering (RR 1.01, 95% CI: 0.54–1.90 for >3× per wk)	No. Lack of association in available studies and would likely be *indeterminate* overall.
Shiue (2015b)	Commenter	Neurologic; depression	No association with adult depression	
Shiue (2015c)	Commenter	Neurologic; hearing disturbance	No association with trouble hearing	
Gaylord et al. (2019)	Commenter	Pulmonary function	No association with FEV or FVC (FEV1 beta −0.03, 95% CI: −0.09, 0.03, FVC beta −0.03, 95% CI: −0.10, 0.04)	No. Lack of association in available study and would likely be *indeterminate* overall.

ADHD: Attention deficit hyperactive syndrome; AGD: anogenital distance; AGI: anogenital index; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aminotransferase; BL: birth length; BMI: body mass index; BWT: birth weight;E1:estrone; E2:estradiol; E3: estriol; eGFR: estimated glomerular filtration; FEV: forced expiratory volume; FSH: follicle-stimulating hormone; FVC: forced vital capacity; GFR: glomerular filtration rate; HDL: high density lipoprotein; HOMA-IR: homeostatic model assessment of insulin resistance; LBW: low birth weight; LDL: low density lipoprotein; LGA: Large for gestational age: LH: luteinizing hormone; PCOS: polycystic ovary syndrome; PTB: preterm birth; T: testosterone; T3: triiodothyronine; T4: thyroxine; TPOAb: thyroid peroxidase antibody; TSH: thyroid stimulating hormone.

[a]For literature identified by public commenters, the full comments are available here: https://www.regulations.gov/docket/EPA-HQ-ORD-2019-0287.

[b]As described in charge question 1, only studies that would notably impact the primary EPA draft judgments (i.e., the health effects identified as human health hazards and the final reference values) in the Step 4 draft will be added to the Toxicological Review by EPA prior to finalization. The panel is asked to identify (with justification) any EPA decisions on incorporation or impact that are not supported.

Table I-2. Other studies meeting assessment PECO criteria

Reference	Source[a]	Assessment topic	Results summary	EPA disposition on incorporation and characterization of impact[b]
PBPK				
Fàbrega et al. (2015)	External peer reviewer	PBPK model	This study had been previously identified as describing a PBPK model with parameters for a number of PFAS, including PFDA. However, because of the uncertainties identified early in EPA's process of developing Toxicological Reviews for multiple PFAS, the model was not considered sufficiently reliable for use in any of the reviews and discussion of the model in the draft PFDA review was inadvertently overlooked. In particular, a general issue with PBPK predictions of tissue distribution for PFAS is now described in the revised Toxicological Review: A key assumption of PFAS PBPK models common to the Fàbrega model is inconsistent with rat PK data, making the prediction of such models in humans uncertain. Further, parameters for PFDA in the Fàbrega model were nominally based on tissue concentration data from cadavers (Pérez et al., 2013), but most of the PFDA data reported by Pérez were below the limit of detection (LOD) and Fàbrega assumed the tissue concentrations were at the LOD. Then tissue/blood partition coefficients were set by comparing those values to blood levels from living human subjects from a separate study, published 6 yr prior to Pérez (Ericson et al., 2007). Comparison of model predictions to validation data was not provided. Hence, the model is considered far too uncertain for application in the Toxicological Review.	Yes. Concerns with the model and the conclusion of high uncertainty, described here, are now included in the Toxicological Review. Since the model was therefore not applied, there is no impact on the risk characterization.

[a]For literature identified by public commenters, the full comments are available here: https://www.regulations.gov/docket/EPA-HQ-ORD-2019-0287.

[b]As described in charge question 1, only studies that would notably impact the primary EPA draft judgments (i.e., the health effects identified as human health hazards and the final reference values) in the Step 4 draft will be added to the Toxicological Review by EPA prior to finalization. The panel is asked to identify (with justification) any EPA decisions on incorporation or impact that are not supported.

Table I-3. Studies meeting select categories of supplemental evidence

Reference	Source[a]	Assessment topic	Description or Results	EPA disposition on incorporation and characterization of impact[b]
ADME				
Louisse et al. (2022)	Lit update	ADME	PFDA shown to be a substrate of human renal organic anion transporter 4 (OAT4) w/ saturable kinetics.	Yes. Demonstrates mechanism of saturable resorption in the kidney, which can result in sex-dependent (due to hormonal regulation of OAT4) and dose-dependent urinary clearance (CL). Since human CL is estimated from empirical PK data, there is no quantitative impact on the assessment, but the result provides mechanistic information explaining why urinary CL in humans is much less than predicted based on the glomerular filtration rate alone.
Wang et al. (2018)	Commenter	ADME	Ratio of PFDA and other PFAS in CSF to blood serum in patients (n = 113 for PFDA).	Yes. Results will not impact HED and RfD calculations, but penetration to the brain is worth noting.
Yao et al. (2023)	Lit update	ADME	Urinary CL in infants was estimated from matched blood and urine samples.	Yes. CL for PFDA in infants (mean = 0.037 mL/kg-d, median = 0.022 mL/kg-d) in the range of values estimated in the current draft for men (0.039 mL/kg-d) and nonreproductive-age women (0.026 mL/kg-d). CL = 0.026 mL/kg-d was used in the current draft for extrapolation of (mouse) developmental effects and interpretation of human epi studies. Given the similarity in values, incorporation of these new results would not impact results presented in the draft. However, there has been significant uncertainty as to how well the ADME from adults predicts ADME in children, so incorporating these results would help inform an important uncertainty regarding whether CL is consistent across lifestages.

Reference	Source[a]	Assessment topic	Description or Results	EPA disposition on incorporation and characterization of impact[b]
			The paper also shows predicted blood concentration in the child from birth to 1 yr of age.	No. The predicted time-course from birth to age 1 appears to assume intake is constant at that estimated for a newborn and fails to account for infant growth during that time, which is significant. Hence the prediction is considered to be unreliable and not useful to add to the analysis.
Zhang et al. (2022)	Lit update	ADME, metabolomics (cord blood)	Correlation between PFAS levels in cord blood and a postnatal blood sample (median age 2.1 yr, IQR 1.3–3.8 yr) and with metabolite levels in cord blood were evaluated, along with the trend in cord blood vs. birth yr.	No. For PFDA there is only a weak correlation (r = 0.4, n = 30) between cord blood and postnatal concentration. Considering the range in age of the postnatal sample and that the extent of breastfeeding was not evaluated, hence not factored, the weak correlation is not surprising. While these observations support the assumption in the current analysis that concentrations in young children result from combined in utero and postnatal exposure, it does not change them and would have no quantitative impact on the draft HED calculations.
Blomberg et al. (2023)	Commenter	ADME	Ratio of PFAS in colostrum or breast milk to maternal serum levels.	No. PFDA was only measured in two samples (individuals) each of colostrum and breast milk in this study, compared with the 50 matched samples analyzed by Liu et al. (2011), so Blomberg does not provide any significant new data for PFDA.
Fromme et al. (2010)	Commenter	ADME	PFAS levels in maternal serum during and after pregnancy, cord blood, infant serum, and breast milk were reported.	No. Reported PFDA levels in most summary statistics were below the LOQ and correlations among or trends in matched samples were not reported, so the study does not provide any significant new data for PFDA.
Pan et al. (2017)	Commenter	ADME	Ratio of PFAS in maternal serum in each trimester to PFAS concentration in cord serum at delivery was reported and analyzed for correlations with other factors.	No. Since maternal and fetal/cord serum are likely to change during pregnancy, the nominal interpretation of the data as showing changes in transfer efficiency is highly questionable. Further, the data have no practical impact on the PK calculations used and previous data on maternal/cord PFDA concentration ratios are already presented.

Reference	Source[a]	Assessment topic	Description or Results	EPA disposition on incorporation and characterization of impact[b]
Pan et al. (2019)	Commenter	ADME	PFAS were analyzed in serum and semen. PFDA was present in semen at a level of 2% of that in serum.	Yes. The study is now described briefly in the section on ADME/distribution in humans, since there are few data on human distribution other than cord blood and placenta, but there is no quantitative impact since semen quality is not an identified endpoint.
Han et al. (2018)	Commenter	ADME	Paternal, maternal, and cord serum measurements. Cord:maternal serum ratio (medians) of 0.38.	Yes. This study is now described along with others reporting the distribution between maternal and cord serum. The ratio reported is in the range of other studies previously included, so there is no quantitative impact.
Glynn et al. (2020)	Commenter	ADME	PFDA serum concentration in school children. Boys significantly higher than girls (median 0.22 in girls vs. 0.25 in boys). Onset of menstrual bleeding was a factor for PFHxS and PFOA but not for PFDA.	Yes. This study is now discussed as part of the analysis of excretion in humans. While the difference between boys and girls may have been statistically significant, it is not large enough to have a quantitative impact and NHANES data analyzed likewise do not show a meaningful (or consistent) difference between human males and females.
Pérez et al. (2013)	External peer reviewer	ADME	Tissue concentration of PFDA in humans (n = 20). PFDA mostly below LOD in liver, bone, lung, and kidney. Interestingly 70% above LOD in brain. No serum/blood or exposure data.	Yes. The study is now cited, but because many of the measurements were below LOD, quantitative interpretation is not possible. That accumulation seems to be highest in the brain was noted. No impact on HEDs, etc.
Bao et al. (2022)	External peer reviewer	ADME	Maternal serum, placenta, and cord blood measurements of PFDA concentration.	Yes. Added to discussion of distribution during pregnancy. Only a few other studies reported levels in the placenta, so it is worth comparing these results, but they are consistent with the previously included studies. Likewise, the distribution to cord blood is consistent with previously evaluated studies. So, inclusion of these data has no impact on the HEDs.
Chen et al. (2022)	External peer reviewer	ADME	Measurement of PFDA in urine and whole blood and estimation of a urinary clearance rate.	Yes. This is only the third study evaluating urinary clearance (CL) in humans and the mean value estimated is considerably above the other two. A population-weighted mean urinary CL was calculated using results for all

Reference	Source[a]	Assessment topic	Description or Results	EPA disposition on incorporation and characterization of impact[b]
				three studies and is influenced by these results. The CL is 20% higher than if only results from the other two studies are used but the resulting mean is over 5× higher than the lowest mean, which had been selected in the previous draft. Inclusion of this study influenced the decision to calculate a weighted mean, so the overall impact is significant.
Mechanistic, including Non-PECO Routes of Exposure				
Wang et al. (2022)	Lit update	Mechanisms of PFDA-induced adipogenesis in HepG2 cells and 3-t3-Li murine white preadipocytes (liver, cardiometabolic)	PFDA induced triglyceride accumulation by modulating the NLRP3 inflammasome-mediated SREBP1 pathway in the in vitro model systems.	Yes. Although the results would not change draft judgments, they address a key data gap with respect to understanding potential PFDA-mediated liver metabolic effects in human models (available data mostly from animal models) and inform the key science issue of human relevance of liver effects observed in rodents.
Reo and Adinehzadeh (2000)	Commenter	Phospholipid metabolism (liver)	PFDA affected phosphatidylcholine and phosphatidylethanolamine biosynthesis in rat livers.	No. Results are consistent with previous findings on the mechanisms of PFDA-induced liver effects in animals but do not inform the key science issue of human relevance of liver effects observed in rodents.
Sun et al. (2023)	Lit update	PPAR-α, -β/δ, -γ (liver)	PFDA showed transcriptional activity toward the dolphin and human PPAR -α, -β, -γ isoforms in HEK cells.	
Amstutz et al. (2022)	Lit update	HepG2 cell viability and ROS formation (liver)	PFDA affected cell viability in a dose-dependent manner and increased ROS production.	
Ojo et al. (2022)	Lit Update	DNA damage and cell viability in HepG2 cells (cancer, liver)	PFDA caused a dose-dependent decrease in cell viability and a moderate increase in DNA damage.	No. Results would not change draft judgments or provide additional insights into potential mechanisms of PFDA-induced carcinogenicity or liver toxicity.

Reference	Source[a]	Assessment topic	Description or Results	EPA disposition on incorporation and characterization of impact[b]
Evans et al. (2022)	Lit update	PPAR-α and ER (liver and reproductive)	PFDA showed activity toward the human and rat PPAR-α,-γ receptors in ligand binding assays but no transcriptional activity toward the human ER in vitro.	No. Results are consistent with previous findings on the interaction of PFDA with these receptors in vitro but do not provide additional information on potential mechanisms of PFDA-induced liver or reproductive effects.
Bjerregaard-Olesen et al. (2016)	Commenter	Xenoestrogenic activity (reproductive)	The association of PFDA and xenoestrogenic activity was examined.	No. While the study may provide minimal mechanistic insight for PFDA-induced reproductive or endocrine effects, the data would not impact the hazard judgments.
Bjerregaard-Olesen et al. (2014)	Commenter	Xenoestrogenic activity (reproductive)	The association of PFDA and xenoestrogenic activity was examined.	
Running et al. (2022)	Lit update	Steroid hormone levels (reproductive, endocrine)	PFDA significantly increased progesterone and 21-hydro-progesterone levels in H295R adrenocortical carcinoma cells but had no significant effects on the levels of androgens, corticosteroids, and estrogens.	
Zhao et al. (2023)	Lit update	11β-hydroxysteroid dehydrogenase 2 activity (endocrine)	The effect of PFDA on human and rat 11β-hydroxysteroid dehydrogenase 2 activity was determined under in vitro and in silico conditions.	

Reference	Source[a]	Assessment topic	Description or Results	EPA disposition on incorporation and characterization of impact[b]
Tian et al. (2022)	Lit update	Folate levels (developmental)	The effect of PFDA on folate homeostasis was determined in adolescents.	No. While the study may provide minimal mechanistic insight on how PFDA exposure may cause developmental/neurodevelopmental effects, such information would not impact the hazard judgment.
Ding et al. (2022)	Lit update	Adipocytokines (developmental)	The association of in utero PFDA exposure and adipocytokines was examined in umbilical cord serum. Leptin levels were decreased.	
Liu et al. (2023)	Lit update	Vitamin D levels (developmental)	The association between PFDA exposure and vitamin D biomarker levels was examined in newborns.	
Zota et al. (2018)	Commenter	Inflammation and aging (immune)	The association of PFDA exposure and inflammation and cellular aging was examined during pregnancy and postpartum.	No. While the study may provide minimal mechanistic insight for PFDA-induced effects on the immune system, the data would not impact the hazard judgments.
Salo et al. (2019)	Commenter	B-cell autoimmunity and type 1 diabetes (cardiometabolic)	The association of PFDA exposure, B-cell autoimmunity, and type 1 diabetes was examined.	No. While the study may provide minimal mechanistic insight for PFDA-induced cardiometabolic effects, the data would not impact the hazard judgments.
Starling et al. (2020)	Commenter	DNA methylation (cardiometabolic)	The association of PFDA, umbilical cord blood DNA methylation, and cardiometabolic effects was examined.	
Li et al. (2022c)	Lit Update	Toxicity prediction (multiple organs)	PFAS chemicals included PFDA, identified from human serum samples, were screened for toxicity using prediction	No. While the study may provide minimal mechanistic insight for PFDA-induced effects on multiple organs, the data would not impact the hazard judgments.

Reference	Source[a]	Assessment topic	Description or Results	EPA disposition on incorporation and characterization of impact[b]
			models. PFDA was positive for multiple organ toxicities (e.g., liver).	
Li et al. (2019b)	Commenter	Metabolomics (multiple organs)	The association of PFDA exposure and the metabolome was examined.	
Jain and Ducatman (2022)	Lit update	Manganese and selenium levels (serum)	Broad analysis of associations between blood manganese and selenium levels and PFDA concentrations in adults and children (nonspecific to health effects).	No. No data relevant to draft judgments.
Bashir and Obeng-Gyasi (2022)	Lit Update	Allostatic load (general toxicity)	The association of PFDA and allostatic load was examined in adults.	

ADME: Absorption, Distribution, Metabolism and Excretion; CL: clearance; CSF: cerebrospinal fluid; HEDs: human equivalent doses; HEK: human embryonic kidney; HEPG2: human hepatocellular carcinoma; IQR: interquartile range; LOD: limit of detection; NHANES: National Health and Nutrition Examination Survey; NLRP3; Nod-like receptor family, pyrin domain containing 3; OAT4: renal human organic anion transporter 4; SREBP1: sterol regulatory element binding transcription factor 1.

[a]For literature identified by public commenters, the full comments are available here: https://www.regulations.gov/docket/EPA-HQ-ORD-2019-0287.

[b]As described in charge question 1, only studies that would notably impact the primary EPA draft judgments (i.e., the health effects identified as human health hazards and the final reference values) in the Step 4 draft will be added to the Toxicological Review by EPA prior to finalization. The panel is asked to identify (with justification) any EPA decisions on incorporation or impact that are not supported.

APPENDIX J. QUALITY ASSURANCE FOR THE IRIS TOXICOLOGICAL REVIEW OF PERFLUORODECANOIC ACID AND RELATED SALTS

This assessment is prepared under the auspices of the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) Program. The IRIS Program is housed within the Office of Research and Development (ORD) in the Center for Public Health and Environmental Assessment (CPHEA). EPA has an agency-wide quality assurance (QA) policy that is outlined in the *EPA Quality Manual for Environmental Programs* (see CIO 2105-P-01.4) and follows the specifications outlined in EPA Order CIO 2105.4.

As required by CIO 2105.4, ORD maintains a Quality Management Program, which is documented in an internal Quality Management Plan (QMP). The latest version was developed in 2013 using Guidance for Developing Quality Systems for Environmental Programs (QA/G-1). An NCEA/CPHEA-specific QMP was also developed in 2013 as an appendix to the ORD QMP. Quality assurance for products developed within CPHEA is managed under the ORD QMP and applicable appendices.

The IRIS Toxicological Review of Perfluorodecanoic acid (PFDA) is designated as Highly Influential Scientific Information (HISA)/Influential Scientific Information (ISI) and is classified as QA Category A. Category A designations require reporting of all critical QA activities, including audits. The development of IRIS assessments is done through a seven-step process. Documentation of this process is available on the IRIS website: https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#process.

Specific management of quality assurance within the IRIS Program is documented in a Programmatic Quality Assurance Project Plan (PQAPP). A PQAPP is developed using the EPA Guidance for Quality Assurance Project Plans (QA/G-5),and the latest approved version is dated June 2024. All IRIS assessments follow the IRIS PQAPP, and all assessment leads and team members are required to receive QA training on the IRIS PQAPP. During assessment development, additional QAPPs may be applied for quality assurance management. They include:

Title	Document number	Date
Program Quality Assurance Project Plan (PQAPP) for the Integrated Risk Information System (IRIS) Program	L-CPAD-0030729-QP-1-7	June 2024
Umbrella Quality Assurance Project Plan for CPHEA PFAS Toxicity Assessments	L-CPAD-0031652-QP-1-5	February 2023
An Umbrella Quality Assurance Project Plan (QAPP) for Dosimetry and Mechanism-Based Models (PBPK)	L-CPAD-0032188-QP-1-3	May 2023
Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS)	L-HEEAD-0032189-QP-1-3	June 2023

During assessment development, this project undergoes five quality audits during assessment development including:

Date	Type of audit	Major findings	Actions taken
August 2019	Technical system audit	None	None
August 2020	Technical system audit	None	None
July 2021	Technical system audit	None	None
August 2022	Technical system audit	None	None
June 2023	Technical system audit	None	None
June 2024	Technical system audit	None	None

During Step 3 and Step 6 of the IRIS process, the IRIS toxicological review was subjected to external reviews by other federal agency partners, including the Executive Offices of the White House. Comments during these IRIS process steps are available in the Docket EPA-HQ-ORD-2019-0287 on http://www.regulations.gov.

During Step 4 of assessment development, the IRIS Toxicological Review of Perfluorodecanoic acid (PFDA) undergoes public comment from April 10,2023 to June 9,2023. Following this comment period, the toxicological review undergoes external peer review by ERG from July 10,2023 to October 16, 2023. The peer-review report is available on the peer review website. All public and peer-review comments are available in the docket EPA-HQ-ORD-2019-0287.

Prior to release (Step 7 of the IRIS process), the final toxicological review is submitted to management and QA clearance. During this step the CPHEA QA Director and QA Managers review the project QA documentation and ensure that EPA QA requirements are met.

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