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IRIS Toxicological Review of Perfluorohexanoic Acid [PFHxA, CASRN 307-24-4] and Related Salts Supplemental Information

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TABLE OF CONTENTS

TABLES

FIGURES

ABBREVIATIONS AND ACRONYMS

ADME	absorption, distribution, metabolism, and excretion
AFFF	aqueous film-forming foam
A:G	albumin:globulin ratio
AIC	Akaike's information criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
C_{max}	maximum concentration
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service registry number
CBC	complete blood count
CHO	Chinese hamster ovary (cell line cells)
CI	confidence interval
CL	clearance
CL_A	clearance in animals
CL_H	clearance in humans
CPHEA	Center for Public Health and Environmental Assessment
CPN	chronic progressive nephropathy
DAF	dosimetric adjustment factor
DNA	deoxyribonucleic acid
DTXSID	DSSTox substance identifier
EPA	Environmental Protection Agency
FTOH	fluorotelomer alcohol
GD	gestation day
GGT	γ-glutamyl transferase
HAWC	Health Assessment Workplace Collaborative
HCT	hematocrit
HED	human equivalent dose
HERO	Health and Environmental Research Online
HGB	hemoglobin
HSA	human serum albumin
IQR	interquartile range
IRIS	Integrated Risk Information System
ISI	Influential Scientific Information
IUR	inhalation unit risk
i.v.	intravenous
LDH	lactate dehydrogenase
LLOQ	lower limit of quantitation
LOQ	limit of quantitation
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOEC	lowest observed effect concentration
MCH	mean cell hemoglobin
MCHC	mean cell hemoglobin concentration
MCV	mean corpuscular volume
MOA	mode of action
MW	molecular weight
NCTR	National Center for Toxicological Research
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NTP	National Toxicology Program
ORD	Office of Research and Development
OECD	Organisation for Economic Co-operation and Development
OSF	oral slope factor
osRfD	organ/system-specific oral reference dose
PBPK	physiologically based pharmacokinetic
PC	partition coefficient
PECO	populations, exposures, comparators, and outcomes
PFAA	perfluoroalkyl acids
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFCA	perfluorinated carboxylic acid
PFDA	perfluorodecanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PK	pharmacokinetic
PND	postnatal day
POD	point of departure
POD_{HED}	human equivalent dose POD
PPAR	peroxisome proliferated activated receptor
PQAPP	programmatic quality assurance project plan
PT	prothrombin time
QA	quality assurance
QAPP	quality assurance project plan
QMP	quality management plan
RBC	red blood cells

RfC	reference concentration
RfD	oral reference dose
RNA	ribonucleic acid
ROS	reactive oxygen species
RXR	retinoid X receptor
SD	standard deviation
TP	total protein
TRI	Toxics Release Inventory
TSCATS	Toxic Substances Control Act Test Submissions
TSH	thyroid stimulating hormone
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	evidence base deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor
V_d	volume of distribution

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 PFAS [per- and polyfluoroalkyl substances] 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 versions can be found at the following location: http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=345065

APPENDIX B. BENCHMARK DOSE MODELING RESULTS

As discussed in the body of the report (see Section 5), the endpoints selected for benchmark dose (BMD) modeling were hepatocellular hypertrophy from Chengelis et al. (2009a) and Loveless et al. (2009); hemoglobin and red blood cells from Chengelis et al. (2009a); Loveless et al. (2009), and Klaunig et al. (2015); postnatal body weight decreases from Loveless et al. (2009) and Iwai and Hoberman (2014); and perinatal mortality from Iwai and Hoberman (2014). The animal doses in the studies were used in the BMD modeling and then converted to human equivalent doses (HEDs) using the ratio of animal-to-human serum half-lives.

B.1. 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 benchmark response (BMR) of 1 standard deviation (SD) when no toxicological information was available to determine an adverse level of response. When toxicological information was available, the BMR was based on relative deviation, as outlined in the Benchmark Dose Technical Guidance (U.S. EPA, 2012). An adequate fit is judged on the basis of a χ^2 goodness-of-fit p-value ($p > 0.1$), scaled residuals at the data point (except the control) closest to the predefined BMR (absolute value <2.0), and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination is made as to whether the variance across dose groups is homogeneous. If a homogeneous variance model, also referred to as a "constant variance" (CV) model, is deemed appropriate based on the statistical test provided by BMDS (Test 2 for homogeneity of variance), the final BMD results are presented for the CV model. If the Test 2 p-value is significant ($p < 0.05$), the model is run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance, also referred to as "non-constant variance" (NCV). If the NCV model provides adequate fit to the variance of the data (i.e., Test 3 p-value > 0.05), the final BMD results are presented for the NCV model. If this nonhomogeneous variance model does not adequately fit the data (i.e., Test 3; $p < 0.05$), the data set is considered unsuitable for BMD modeling. In some cases, the data may be remodeled after removing one or more of the highest dose groups; if the reduced data can be modeled and results in a better fit in the low-dose region, these results will be presented with information to indicate that one or more dose groups were removed in the results table. In cases where a model with # parameters = # dose groups was fit to the data set and all parameters were estimated and no p-value was calculated that model was not considered for estimation of a point of

departure (POD). 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 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.

For body weight and relative liver weight, a BMR equal to 10% relative deviation (increase or decrease) from the control mean was used based on a biological consideration. For continuous developmental toxicity data, a BMR equal to 0.5 SD was used. The use of 1 SD for the BMR for continuous endpoints is based the observation that shifting the distribution of the control group by 1 SD results in ~10% of animals falling beyond an adversity cutoff defined at the ~1.5 percentile in the control group (Crump, 1995). This roughly 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 roughly approximates the extra risk of 5% commonly used for dichotomous developmental toxicity endpoints; similarly, the BMR for perinatal body weight is half of that for adults (5% vs. 10% relative deviation) based on biological consideration.

B.2. 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, LogLogistic, LogProbit, Multistage, Probit, Weibull, and Dichotomous Hill models available within the software were fit using a benchmark response (BMR) of 10% extra risk (5% extra risk for developmental endpoints and 1% for mortality). 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 $\chi 2$ 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. 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.

B.3. MODELING PROCEDURE FOR DICHOTOMOUS NONCANCER DEVELOPMENTAL TOXICITY DATA

For dichotomous developmental toxicity data, data for individual animals were requested from the study authors when possible. This allowed the use of the nested logistic model, which statistically accounts for intralitter similarity (the propensity of littermates to respond more like one another than pups from another litter) by estimating intralitter correlation and using litter-specific covariates. Judging model fit for this model is identical to the procedure used for regular dichotomous models. If individual animal data is available, the nested logistic model is used instead

of other models; this will be indicated in the results table and individual animal data will not be reported.

For all data types discussed in Sections B.1–B.4, the NOAEL/LOAEL approach may be used in lieu of BMD modeling to derive reference values, for example, when BMD modeling fails. The NOAEL/LOAEL approach may also be taken when a response is only observed in the highest dose group unless the response for that group is sufficiently close to the BMR, in which case BMD modeling results are used to derive values.

B.4. HEMOGLOBIN—FEMALE RATS (KLAUNIG ET AL., 2015)

Table B-1. Dose response data for hemoglobin in female rats (Klaunig et al., 2015)

Dose (mg/kg-d)	Number of animals	Mean (g/dL)	Standard deviation
0	10	15.5	0.97
5	10	15.7	0.73
30	9	15.5	0.79
200	20	14.7	0.91

Table B-2. Benchmark dose results for hemoglobin in female rats—constant variance, BMR = 1 standard deviation (Klaunig et al., 2015)

Models	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.83237671	0.7551	127.5023	182.1091	120.47632	−0.016328458
Exponential 3	0.57454386	0.7551	129.4505	189.9502	120.87504	0.002065941
Exponential 4	0.83237684	0.7551	127.5023	182.0793	120.47647	−0.015845878
Exponential 5	0.57331833	0.7551	129.4525	188.501	120.85884	−0.000961421
Hill	NA	0.7551	131.4228	42.56095	31.718073	0.000755846
Polynomial (Poly 3)	0.56681655	0.7551	129.4634	191.4936	123.01116	0.00113966
Polynomial (Poly 2)	0.56681165	0.7551	129.4634	191.4757	123.0163	0.001201719
Power	0.57425021	0.7551	129.451	190.1218	123.04966	0.002120702
Linear	**0.83402366**	**0.7551**	**127.4983**	**182.7286**	**122.7699**	**−0.014122961**

Bold row indicates the selected model and values.

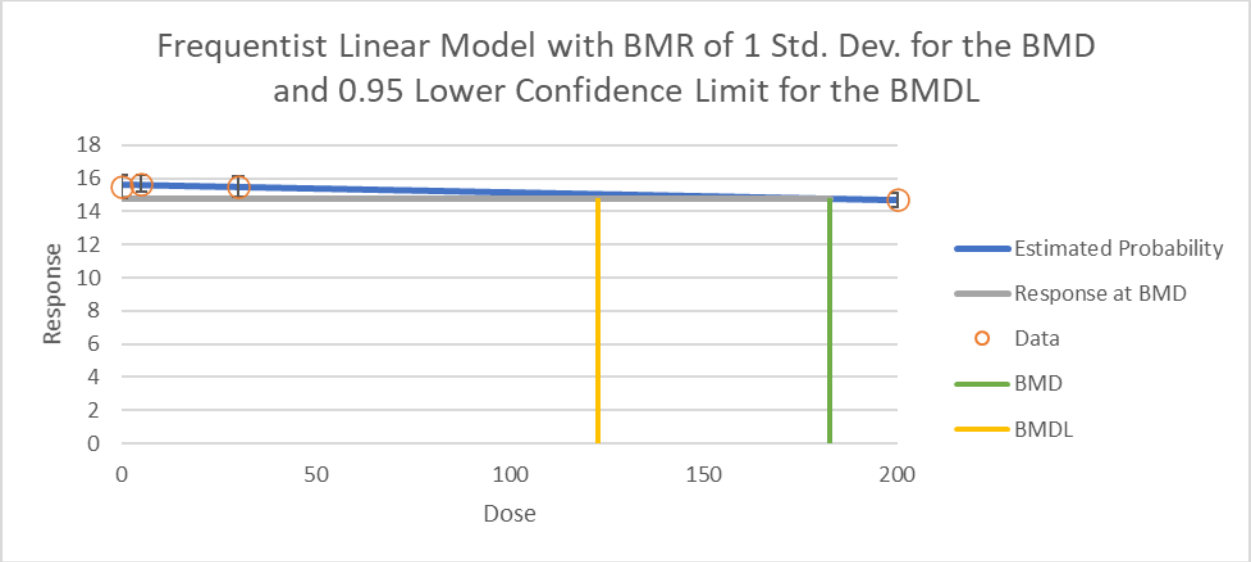

Figure B-1. Dose response curve for the Linear model fit to hemoglobin in female rats (Klaunig et al., 2015).

X-axis is dose (mg/kg-day), and y-axis is mean level of hemoglobin (g/dL).

B.5. HEMOGLOBIN—MALE RATS (CHENGELIS ET AL., 2009B)

Table B-3. Dose response data for hemoglobin in male rats (Chengelis et al., 2009b)

Dose (mg/kg-d)	Number of animals	Mean (g/dL)	Standard deviation
0	10	15.6	0.51
10	10	15.4	0.58
50	10	15.4	0.65
200	10	14.3	1.08

This data set is not considered appropriate for BMD modeling as the response in the high dose group was much larger than the BMR and there was no response in all other dose groups thus the NOAEL/LOAEL approach was applied to this endpoint.

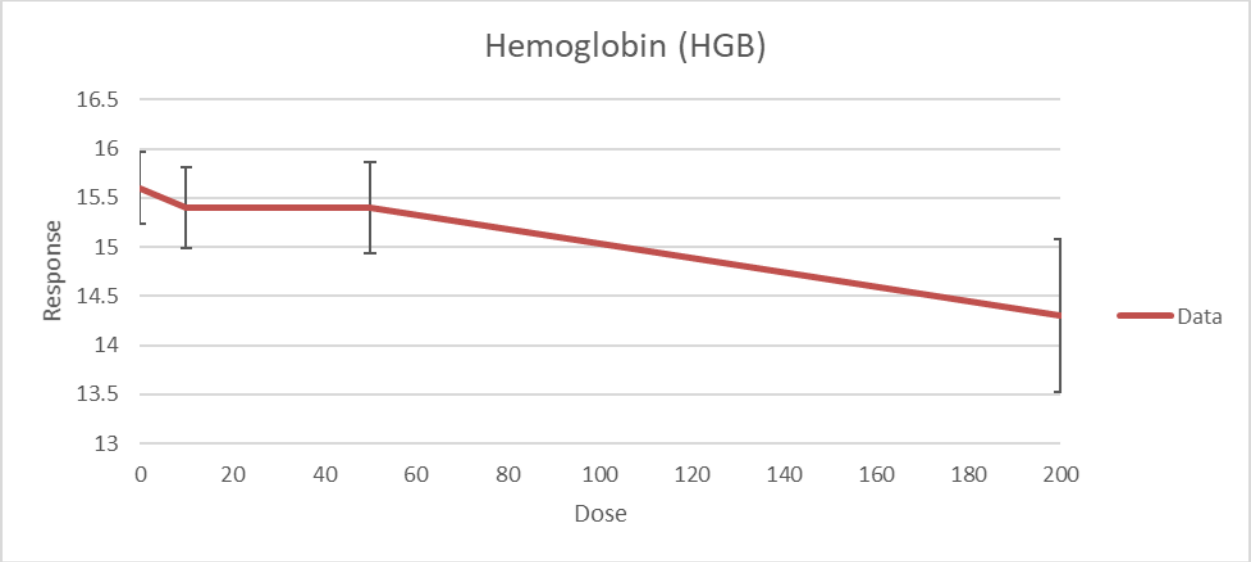

Figure B-2. Dose response data for hemoglobin in male rats (Chengelis et al., 2009b)

X-axis is dose (mg/kg-day), and y-axis is mean level of hemoglobin (g/dL).

B.6. HEMOGLOBIN—FEMALE RATS (CHENGELIS ET AL., 2009B)

Table B-4. Dose response data for hemoglobin in female rats (Chengelis et al., 2009b)

Dose (mg/kg-d)	Number of animals	Mean (g/dL)	Standard deviation
0	10	15.6	0.46
10	10	15.8	1.4
50	10	15.2	0.85
200	10	14.6	0.83

Table B-5. Benchmark dose results for hemoglobin in female rats—non-constant variance, BMR = 1 standard deviation (Chengelis et al., 2009b)

Models	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.2289302	0.0118	113.344	177.8625	106.4881	0.107636338
Exponential 3	0.2289313	0.0118	113.344	177.8107	106.4883	0.107765953
Exponential 4	0.0996002	0.0118	115.1073	145.8206	37.82113	0.036336773
Exponential 5	0.165197	0.0118	114.3214	53.45159	25.38329	0.084452285

Models	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Hill	NA	0.0118	116.3216	68.93813	40.08308	0.084913389
Polynomial (Poly 3)	0.2265515	0.0118	113.3649	179.1794	109.5823	0.105758473
Polynomial (Poly 2)	0.2265515	0.0118	113.3649	179.1817	110.5758	0.105711892
Power	0.2265515	0.0118	113.3649	179.174	109.6196	0.105830655
Linear	0.2265515	0.0118	113.3649	179.1809	110.1348	0.10575497

Both constant and nonconstant variance models failed to model the variance of the data.

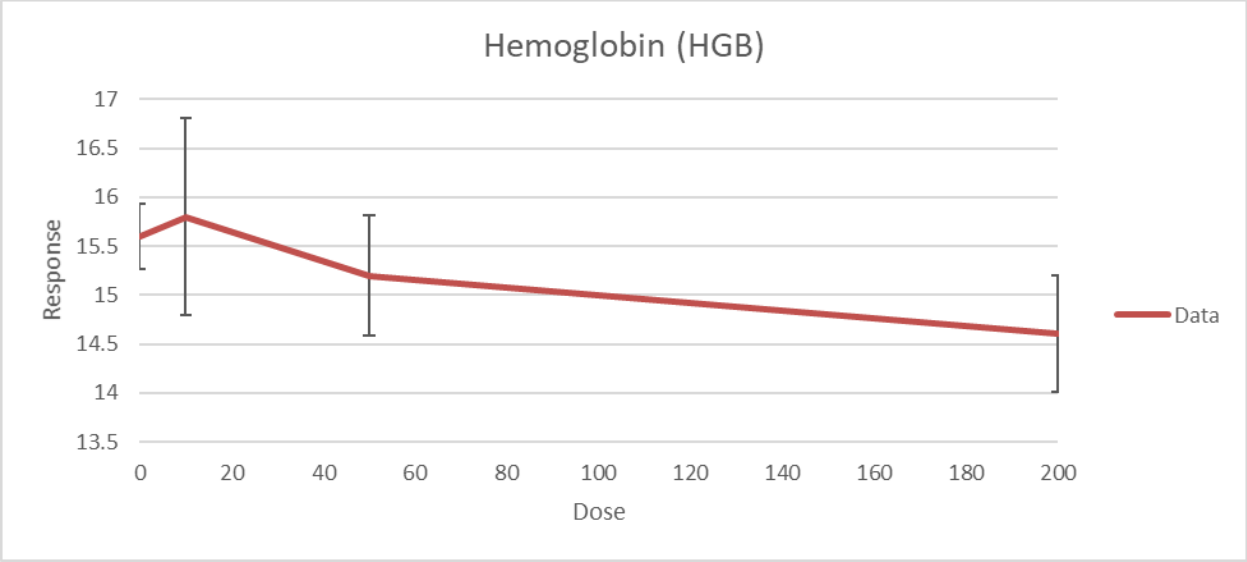

Figure B-3. Dose response data for hemoglobin in female rats (Chengelis et al., 2009b).

X-axis is dose (mg/kg-day), and y-axis is mean level of hemoglobin (g/dL).

B.7. HEMOGLOBIN—MALE RATS (LOVELESS ET AL., 2009)

Table B-6. Dose response data for hemoglobin in male rats (Loveless et al., 2009)

Dose (mg/kg-d)	Number of animals	Mean (g/dL)	Standard deviation
0	10	15.4	0.5
20	10	15.5	0.41
100	10	4.5	0.7
500	10	9.9	2.8

Table B-7. Benchmark dose results for hemoglobin in male rats—non-constant variance, BMR = 1 standard deviation (Loveless et al., 2009)

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	<0.0001	<0.0001	199.3758	9.377807	7.193218	−2.377990291
Exponential 3	<0.0001	<0.0001	239.5418	855.7401	0	0.802984088
Exponential 4	<0.0001	<0.0001	190.0784	6.631732	3.474481	−0.818335738
Exponential 5	0.071088	<0.0001	138.3961	70.68336	21.21839	−2.570261966
Hill	0.07109	<0.0001	138.3961	38.98926	21.0774	0.362134233
Polynomial (Poly 3)	<0.0001	<0.0001	239.7107	891.7542	383.4838	0.626839397
Polynomial (Poly 2)	<0.0001	<0.0001	239.7107	891.7546	383.3929	0.626839015
Power	<0.0001	<0.0001	239.7107	891.7544	383.3928	0.626839886

Both constant and nonconstant variance models failed to model the variance of the data.

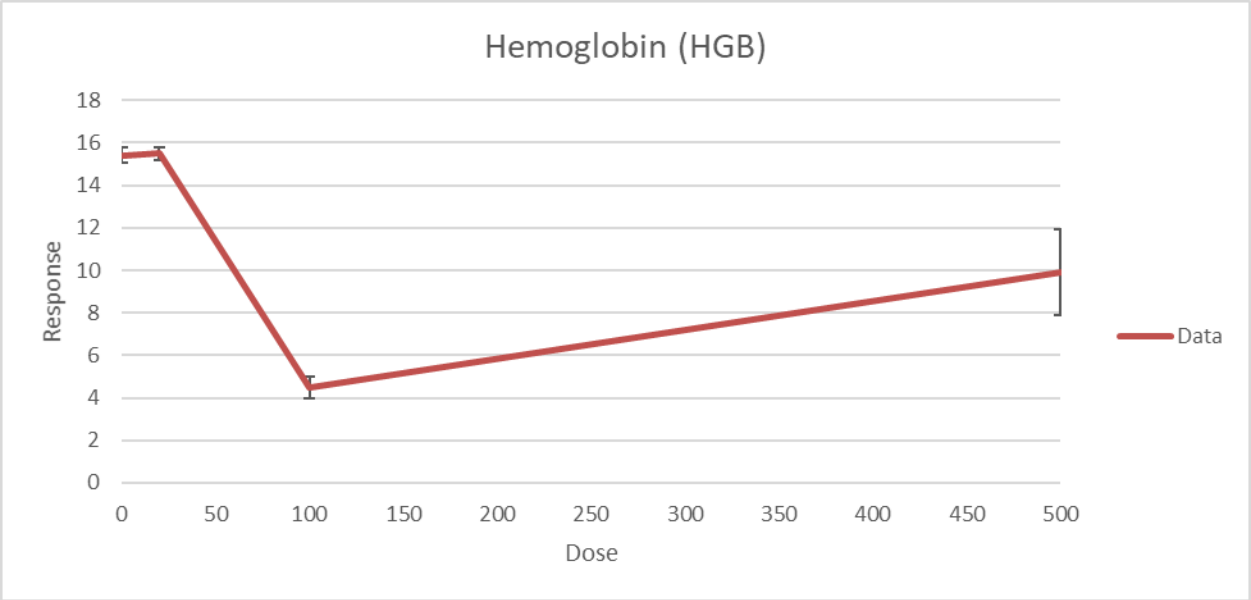

Figure B-4. Dose response data for hemoglobin in male rats (Loveless et al., 2009).

X-axis is dose (mg/kg-d), and y-axis is mean level of hemoglobin (g/dL).

B.8. HEMOGLOBIN—FEMALE RATS (LOVELESS ET AL., 2009)

Table B-8. Dose response data for hemoglobin in female rats (Loveless et al., 2009)

Dose (mg/kg-d)	Number of animals	Mean (g/dL)	Standard deviation
0	10	15.6	0.7
20	10	15.8	0.8
100	10	15.6	0.4
500	9	13.3	0.9

Table B-9. Benchmark dose results for hemoglobin in female rats—constant variance, BMR = 1 standard deviation (Loveless et al., 2009)

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.214488	0.107799	89.79631	134.0618	104.1801	1.219441036
Exponential 3	0.50507	0.107799	89.16158	264.9174	126.3561	−0.026708969
Exponential 4	0.214489	0.107799	89.7963	134.0137	104.1803	1.219907658
Exponential 5	0.505122	0.107799	89.16147	266.3836	126.3586	−0.034100913
Hill	NA	0.107799	91.14613	115.7416	102.1374	-1.06893×10^{-06}
Polynomial (Poly 3)	**0.800193**	**0.107799**	**87.16312**	**268.4412**	**127.6129**	**−0.023130227**
Polynomial (Poly 2)	0.800179	0.107799	87.16315	267.1194	127.8182	−0.018215642
Power	0.452941	0.107799	89.2806	372.7895	126.1226	0.000358346
Linear	0.259194	0.107799	89.41767	141.5272	111.6505	1.119316772

Bold row indicates the selected model and values.

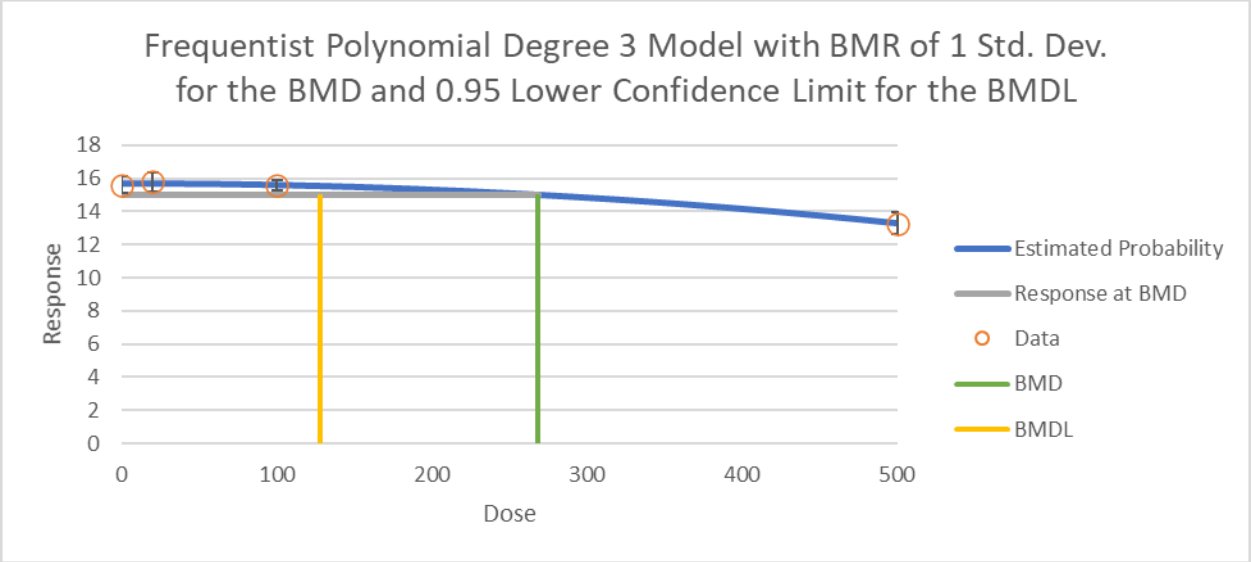

Figure B-5. Dose response curve for the Polynomial Degree 3 model fit to hemoglobin in female rats (Loveless et al., 2009).

X-axis is dose (mg/kg-d), and y-axis is mean level of hemoglobin (g/dL).

B.9. RED BLOOD CELLS—MALE RATS (KLAUNIG ET AL., 2015)

Table B-10. Dose response data for red blood cells in male rats (Klaunig et al., 2015)

Dose (mg/kg-d)	Number of animals	Mean (million/µL)	Standard deviation
0	10	9.2	0.17
2.5	10	8.8	1.52
15	9	8.66	0.92
100	19	8.8	1

Table B-11. Benchmark dose results for red blood cells in male rats—non-constant variance, BMR = 1 standard deviation (Klaunig et al., 2015)

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	<0.0001	0.863381	143.4547	808.9905	155.542	0.078851
Exponential 3	<0.0001	0.863381	143.4551	832.8003	104.7724	0.070665
Exponential 4	<0.0001	0.863381	144.0729	–9999	0	–9999
Exponential 5	<0.0001	0.863381	145.454	865.033	103.3948	0.080901

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Hill	NA	0.863381	121.2797	–9999	0	–9999
Polynomial (Poly 3)	<0.0001	0.863381	143.4552	774.4171	120.0894	0.076434
Polynomial (Poly 2)	<0.0001	0.863381	143.4552	774.9496	132.5465	0.076318
Power	<0.0001	0.863381	143.4552	775.0219	106.6368	0.076271
Linear	<0.0001	0.863381	143.4552	775.3403	153.4792	0.076151

Both constant and nonconstant variance models failed to model the data.

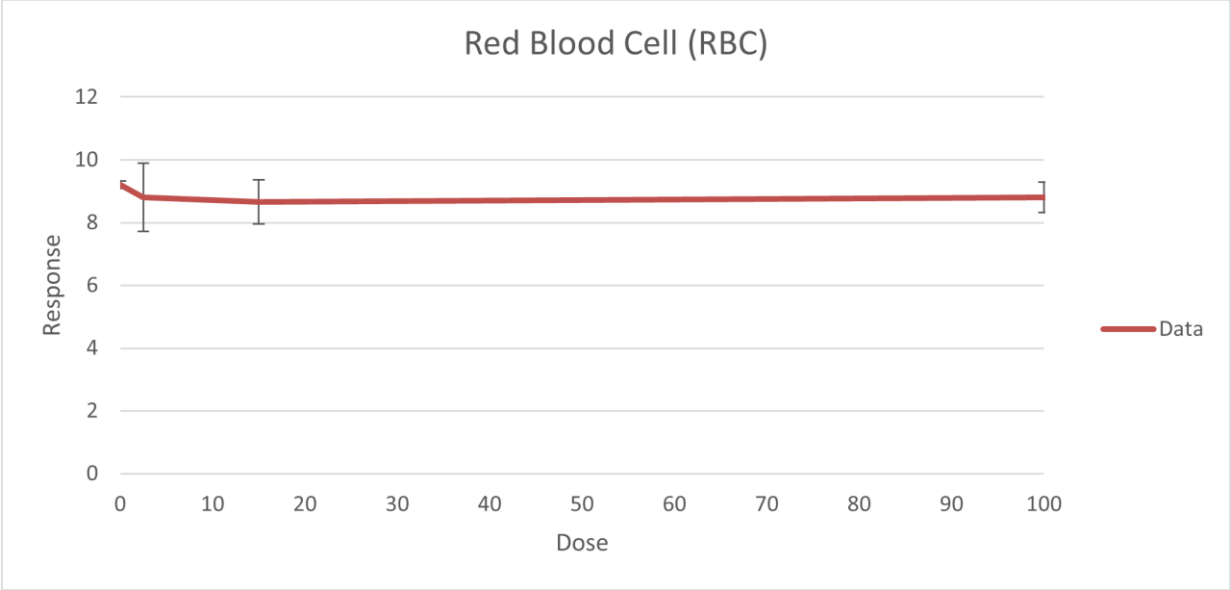

Figure B-6. Dose response data red blood cells in male rats (Klaunig et al., 2015).

X-axis is dose (mg/kg-d), and y-axis is mean level of red blood cells (million/µL).

B.10. RED BLOOD CELLS—FEMALE RATS (KLAUNIG ET AL., 2015)

Table B-12. Dose response data for red blood cells in female rats (Klaunig et al., 2015)

Dose (mg/kg-d)	Number of animals	Mean (million/µL)	Standard deviation
0	10	8.14	0.52
5	10	8.23	0.58
30	9	8.12	0.37
200	20	7.48	0.68

Table B-13. Benchmark dose results for red blood cells in female rats—constant variance, BMR = 1 standard deviation (Klaunig et al., 2015)

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.896578	0.204474	88.37423	153.772	105.8225	−0.0212
Exponential 3	0.702156	0.204474	90.30213	168.4422	106.2748	0.001301
Exponential 4	0.896578	0.204474	88.37423	153.7727	105.8231	−0.02121
Exponential 5	NA	0.204474	92.30211	168.1679	30.68698	0.001229
Hill	NA	0.204474	92.28508	40.32119	31.54188	0.000759
Polynomial (Poly 3)	0.690261	0.204474	90.3147	175.8228	109.4569	0.000354
Polynomial (Poly 2)	0.69227	0.204474	90.31254	173.1861	109.4699	0.000719
Power	0.701613	0.204474	90.3027	169.0362	109.5006	0.000962
Linear	**0.900552**	**0.204474**	**88.36539**	**155.595**	**109.1493**	**−0.01798**

Bold row indicates the selected model and values.

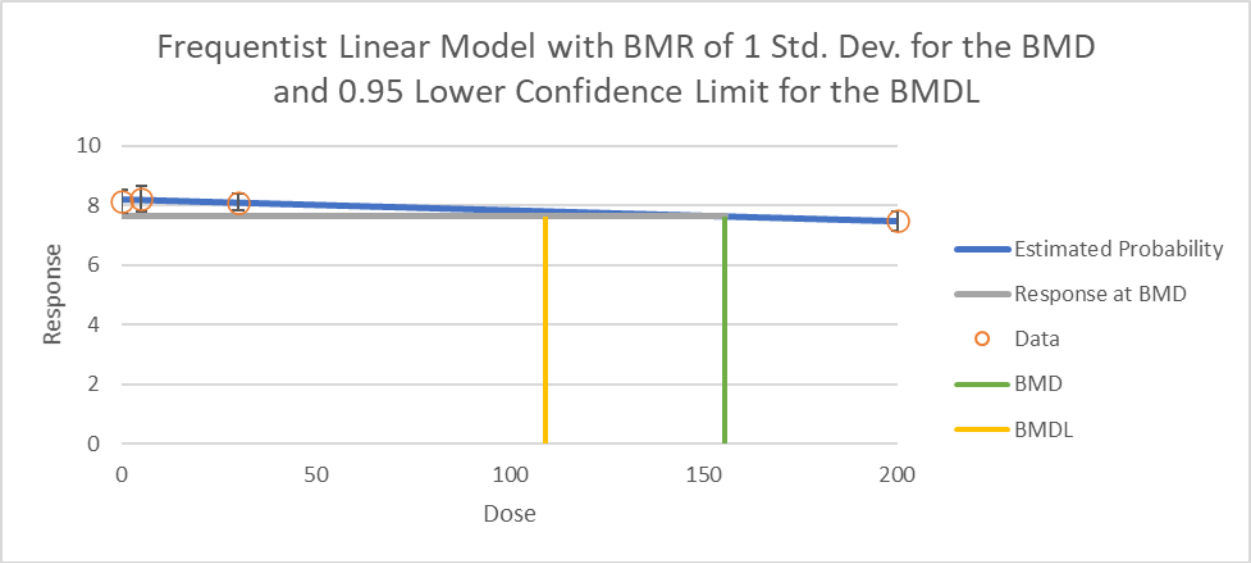

Figure B-7. Dose response curve for the Linear model fit to red blood cells in female rats (Klaunig et al., 2015).

X-axis is dose (mg/kg-d**),** and y-axis is mean level of red blood cells (million/µL).

B.11. RED BLOOD CELLS—MALE RATS (CHENGELIS ET AL., 2009B)

Table B-14. Dose response data for red blood cells in male rats (Chengelis et al., 2009b)

Dose (mg/kg-d)	Number of animals	Mean (million/µL)	Standard deviation
0	10	8.89	0.32
10	10	8.84	0.281
50	10	8.88	0.69
200	10	8.17	0.593

Table B-15. Benchmark dose results for red blood cells in male rats—non-constant variance, BMR = 1 standard deviation (Chengelis et al., 2009b)

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.211487	0.046614	60.49036	113.155	66.64235	0.93824
Exponential 3	0.211488	0.046614	60.49034	113.324	66.64082	0.936908
Exponential 4	0.112482	0.046614	61.90217	63.88893	16.43649	1.512764
Exponential 5	0.123707	0.046614	61.75292	51.86424	17.01699	1.668664
Hill	NA	0.046614	61.38555	49.50692	16.01355	1.522475
Polynomial (Poly 3)	0.208929	0.046614	60.51469	115.2832	69.56043	0.914633
Polynomial (Poly 2)	0.208929	0.046614	60.51469	115.2939	69.56068	0.914397
Power	0.208929	0.046614	60.51469	115.2866	69.56292	0.914574
Linear	0.208929	0.046614	60.51469	115.2954	69.55948	0.914492

Both constant and nonconstant variance models failed to model the variance data.

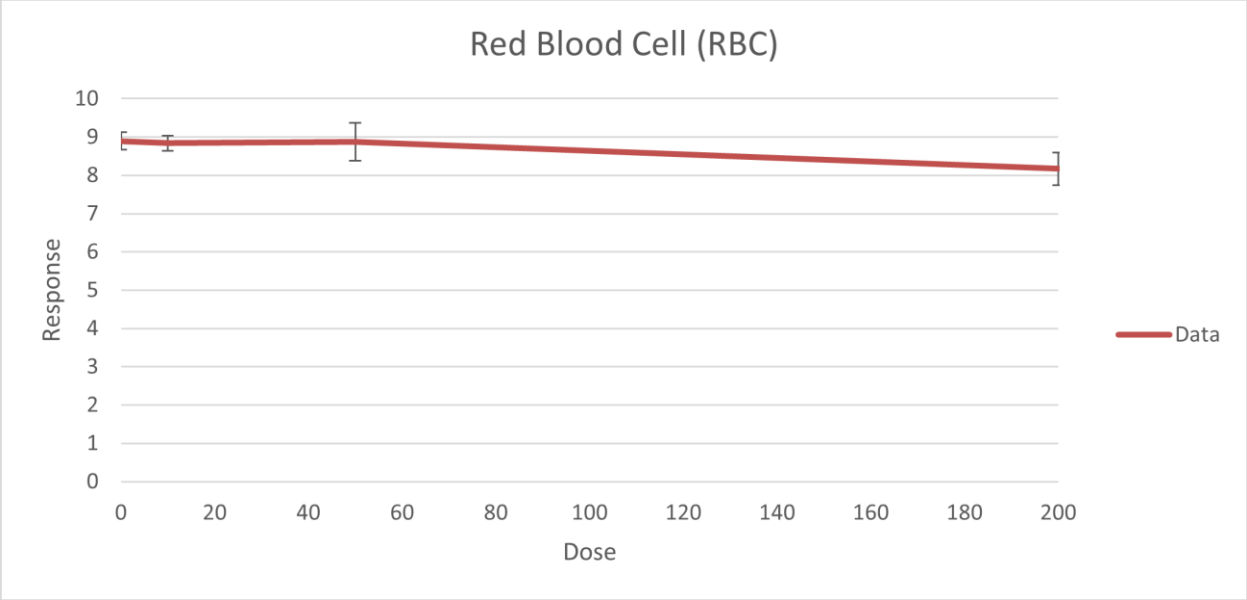

Figure B-8. Dose response data for red blood cells in male rats (Chengelis et al., 2009b).

X-axis is dose (mg/kg-d**),** and y-axis is mean level of red blood cells (million/µL).

B.12. RED BLOOD CELLS—FEMALE RATS (CHENGELIS ET AL., 2009B)

Table B-16. Dose response data for red blood cells in female rats (Chengelis et al., 2009b)

Dose (mg/kg-d)	Number of animals	Mean (million/µL)	Standard deviation
0	10	8.62	0.338
10	10	8.53	0.696
50	10	8.32	0.491
200	10	7.93	0.43

Table B-17. Benchmark dose results for red blood cells in female rats—constant variance, BMR = 1 standard deviation (Chengelis et al., 2009b)

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.819031	0.13452	61.22185	145.9541	94.47522	0.13169
Exponential 3	0.819031	0.13452	61.22185	145.9541	94.47455	0.13169

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 4	0.828537	0.13452	62.86949	112.0384	27.37312	−0.13653
Exponential 5	**0.527493**	**0.13452**	**63.2218**	**145.9511**	**16.32358**	**0.131694**
Hill	NA	0.13452	64.90674	95.16729	22.04822	0.034663
Polynomial Degree 3	0.805881	0.13452	61.25422	148.2376	97.83829	0.128637
Polynomial Degree 2	0.805881	0.13452	61.25422	148.2285	97.83846	0.128826
Power	0.805881	0.13452	61.25422	148.2268	97.80444	0.128858
Linear	0.805881	0.13452	61.25422	148.2178	97.81736	0.129033

Bold row indicates the selected model and values.

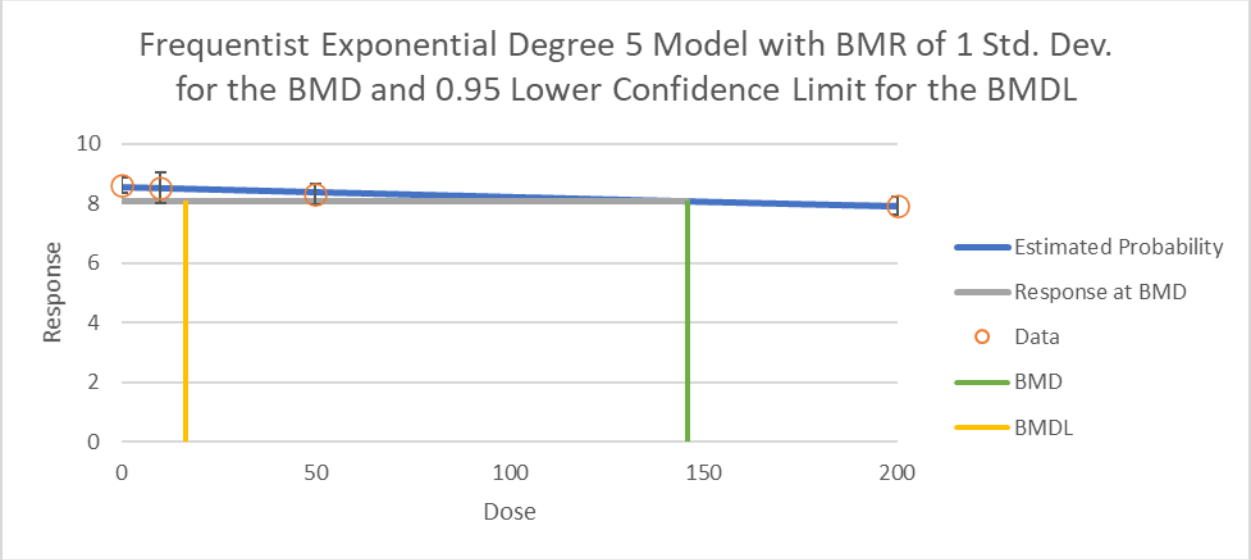

Figure B-9. Dose response curve for the Exponential 5 model fit to red blood cells in female rats (Chengelis et al., 2009b).

X-axis is dose (mg/kg-d), and y-axis is mean level of red blood cells (million/µL).

B.13. RED BLOOD CELLS—MALE RATS (LOVELESS ET AL., 2009)

Table B-18. Dose response data for red blood cells in male rats (Loveless et al., 2009)

Dose (mg/kg-d)	Number of animals	Mean (million/µL)	Standard deviation
0	10	8.89	0.36

Dose (mg/kg-d)	Number of animals	Mean (million/µL)	Standard deviation
20	10	8.95	0.34
100	10	8.46	0.41
500	10	6.09	1.27

Table B-19. Benchmark dose results for red blood cells in male rats—non-constant variance, BMR = 1 standard deviation (Loveless et al., 2009)

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.281567	0.991476	64.79171	52.64163	38.9282	0.746143
Exponential 3	0.376218	0.991476	65.03997	78.0673	43.76706	−0.23387
Exponential 4	0.281572	0.991476	64.79167	52.63495	38.92813	0.744847
Exponential 5	NA	0.991476	66.45382	91.34257	46.77432	−0.01779
Hill	NA	0.991476	66.44302	97.70618	94.33382	−0.01642
Polynomial (Poly 3)	0.291705	0.991476	65.36868	73.55976	45.76816	−0.24307
Polynomial (Poly 2)	0.291695	0.991476	65.36872	73.60792	45.76059	−0.24304
Power	0.341547	0.991476	65.16156	77.54244	46.28623	−0.27696
Linear	**0.445951**	**0.991476**	**63.87203**	**59.08585**	**44.57007**	**0.743535**

Bold row indicates the selected model and values.

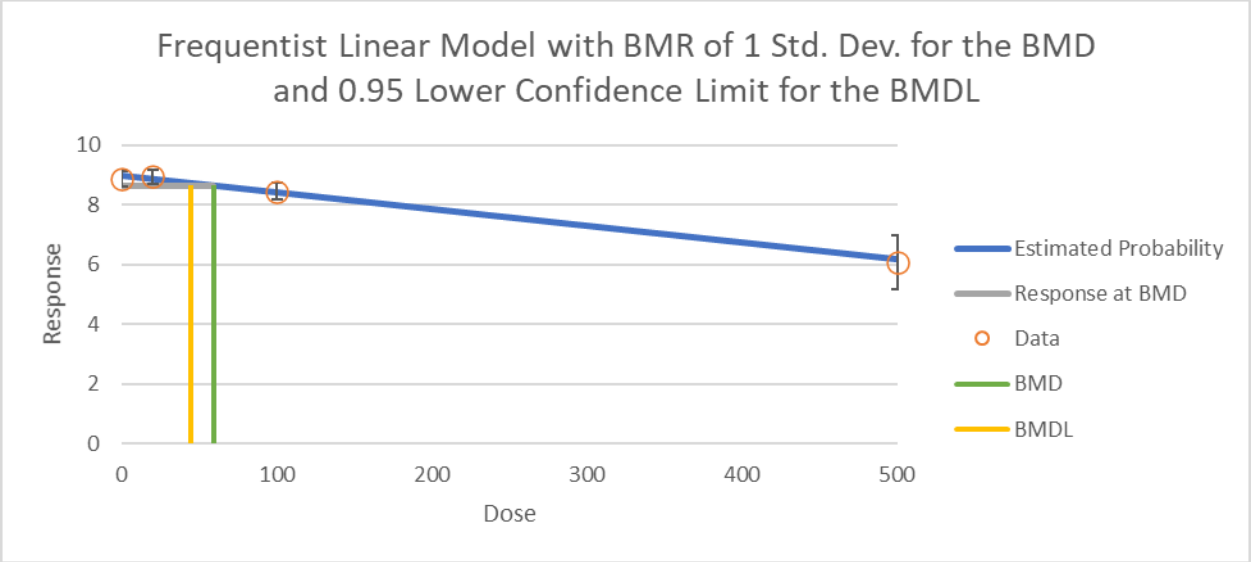

Figure B-10. Dose response curve for the Linear model fit to red blood cells in male rats (Loveless et al., 2009).

X-axis is dose (mg/kg-d), and y-axis is mean level of red blood cells (million/µL).

B.14. RED BLOOD CELLS—FEMALE RATS (LOVELESS ET AL., 2009)

Table B-20. Dose response data for red blood cells in female rats (Loveless et al., 2009)

Dose (mg/kg-d)	Number of animals	Mean (million/µL)	Standard deviation
0	10	8.34	0.43
20	10	8.53	0.52
100	10	8.32	0.27
500	9	6.85	0.63

Table B-21. Benchmark dose results for red blood cells in female rats—constant variance, BMR = 1 standard deviation (Loveless et al., 2009)

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.21884	0.087567	57.58768	133.0328	102.9324	1.002642
Exponential 3	0.331861	0.087567	57.49047	238.0109	116.9504	−0.08346
Exponential 4	0.21884	0.087567	57.58768	133.0037	102.9322	1.002848

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 5	0.331861	0.087567	57.49047	238.0095	116.9523	−0.08345
Hill	NA	0.087567	59.42671	113.2878	101.18	-2.4×10^{-06}
Polynomial Degree 3	0.320732	0.087567	57.53481	261.7164	122.0763	−0.18275
Polynomial Degree 2	0.320735	0.087567	57.5348	261.8718	122.0761	−0.1845
Power	0.330478	0.087567	57.49587	243.0686	122.3971	−0.09028
Linear	**0.268591**	**0.087567**	**57.17798**	**142.5548**	**112.3638**	**0.87655**

Bold row indicates the selected model and values.

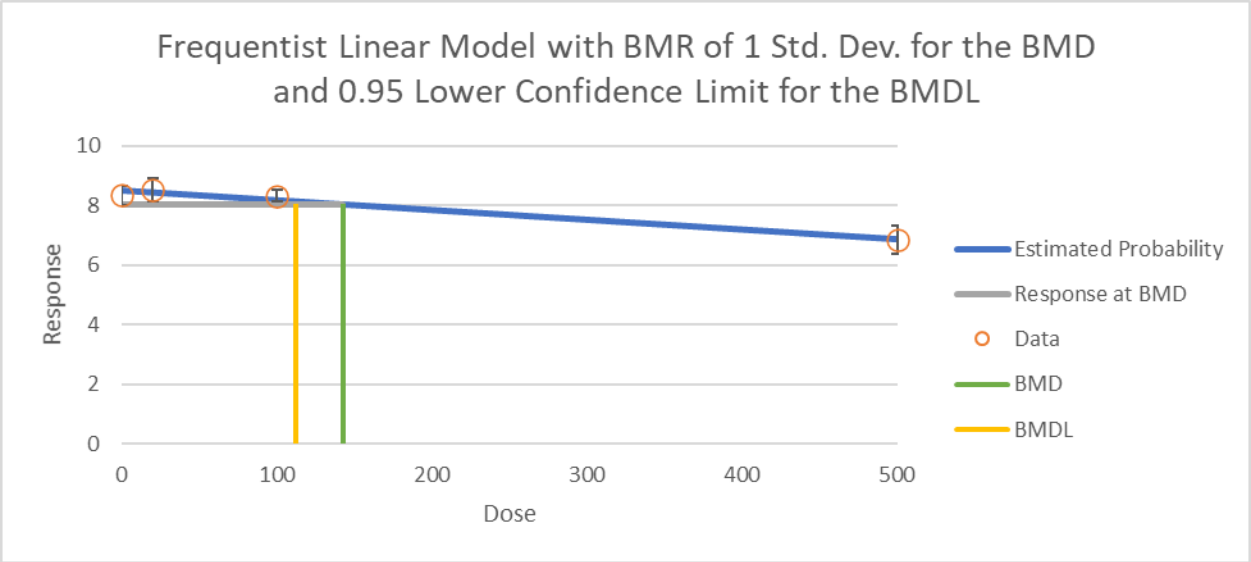

Figure B-11. Dose response curve for the Linear model fit to red blood cells in female rats (Loveless et al., 2009).

X-axis is dose (mg/kg-d), and y-axis is mean level of red blood cells (million/µL).

B.15. HEPATOCELLULAR NECROSIS—FEMALE RATS (KLAUNIG ET AL., 2015)

Table B-22. Dose response data for hepatocellular necrosis in female rats (Klaunig et al., 2015)

Dose (mg/kg-d)	Number of animals	Incidence	Percentage of incidence
0	60	2	3

Dose (mg/kg-d)	Number of animals	Incidence	Percentage of incidence
10	60	0	0
50	60	3	5
200	70	12	17

The NOAEL was selected over the modeled data. This was based on dose spacing (5, 10, and 50 mg/kg-d) that was closer to 0 than the dose at which an effect was observed (LOAEL = 200 mg/kg-d). The NOAEL of 50 mg/kg-day is more health protective than the BMDL of ~100 mg//kg/d and therefore chosen over modeled data.

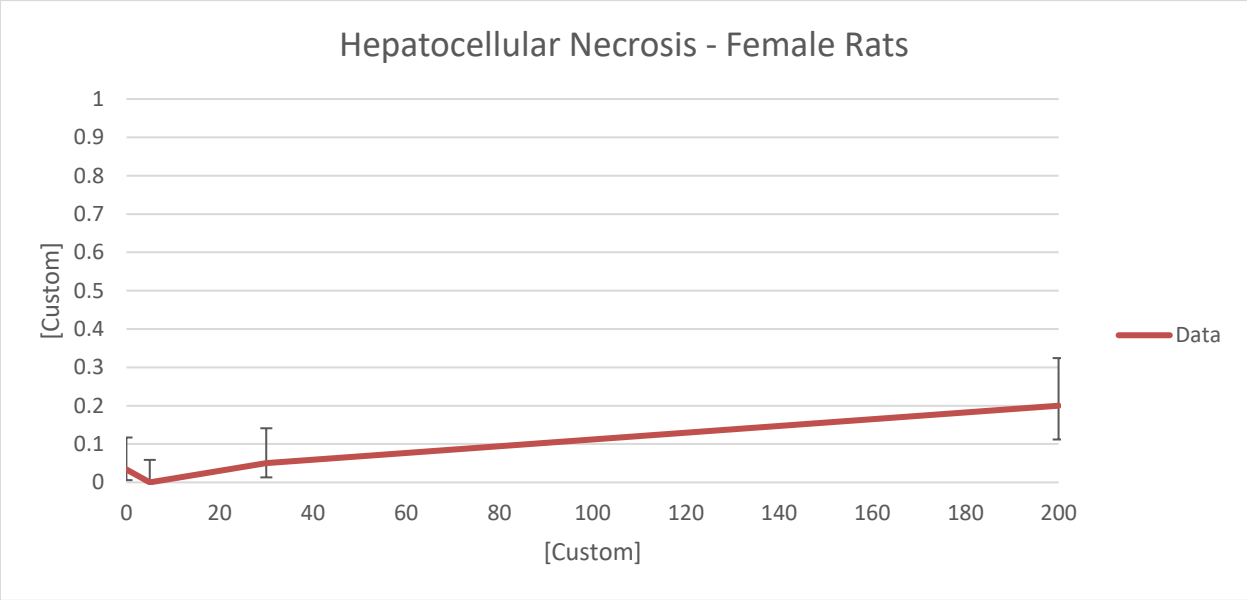

Figure B-12. Dose response data for hepatocellular necrosis in female rats (Klaunig et al., 2015).

X-axis is dose (mg/kg-d), and y-axis is percent incidence.

B.16. HEPATOCELLULAR HYPERTROPHY—FEMALE RATS (LOVELESS ET AL., 2009)

Table B-23. Dose response data for hepatocellular hypertrophy in female rats (Loveless et al., 2009)

Dose (mg/kg-d)	Number of animals	Incidence	Percentage of incidence
0	10	0	0
20	10	0	0
100	11	0	0
500	10	5	50

This data set is not considered appropriate for BMD modeling. The response in the high dose group (50%) is much larger than the BMR and there was no response in all other dose groups.

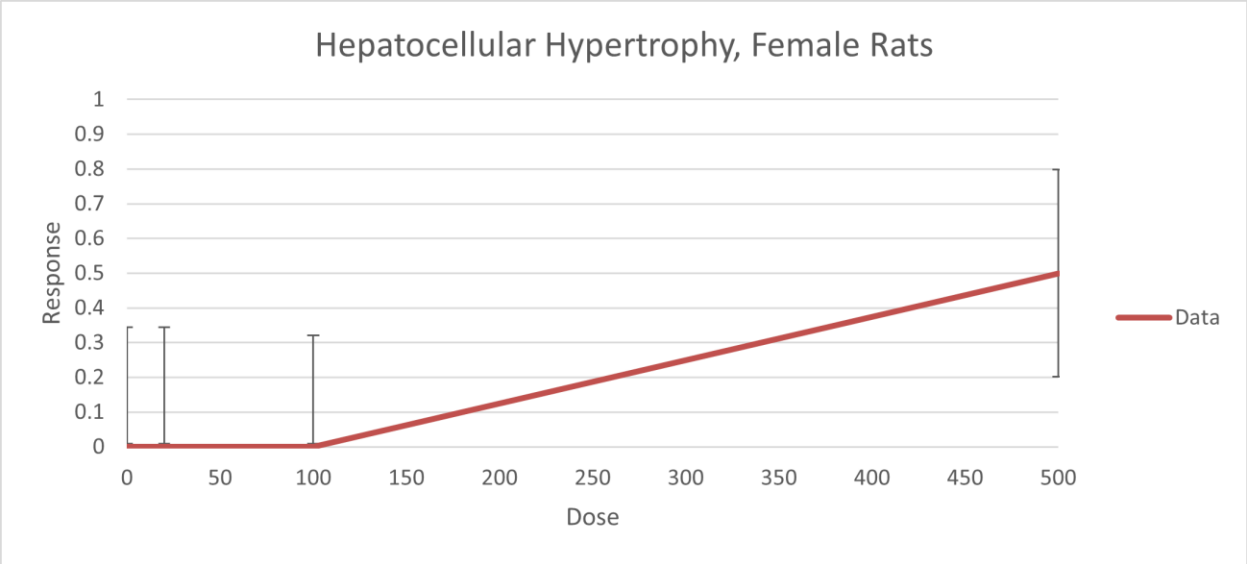

Figure B-13. Dose response data for hepatocellular hypertrophy in female rats (Loveless et al., 2009).

X-axis is dose (mg/kg-d), and y-axis is percent incidence.

B.17. HEPATOCELLULAR HYPERTROPHY—MALE RATS (LOVELESS ET AL., 2009)

Table B-24. Dose response data for hepatocellular hypertrophy in male rats (Loveless et al., 2009)

Dose (mg/kg-d)	Number of animals	Incidence	Percentage of incidence
0	10	0	0
20	10	0	0
100	10	4	40
500	10	10	100

Table B-25. Benchmark dose results for hepatocellular hypertrophy in male rats—nested model BMR = 10% extra risk (Loveless et al., 2009)

Model	Goodness of fit (*p*-value)	AIC	10% Extra risk		Scaled residual for dose group near BMD
			BMD	BMDL	
Dichotomous Hill	1	17.46023	85.47371	28.3855	-1.1×10^{-06}
Gamma	0.999944	17.46046	70.57884	20.71965	0.00025
Log-Logistic	1	15.46023	85.49796	28.38513	2.91×10^{-07}
Multistage Degree 3	0.997823	15.54154	59.28867	16.83509	−0.20131
Multistage Degree 2	0.902071	17.8587	46.58058	16.60448	−0.44287
Multistage Degree 1	**0.391117**	**20.9779**	**18.16542**	**10.6581**	**−1.10904**
Weibull	0.987025	17.51174	62.10697	19.73504	0.025832
Logistic	0.999997	17.46024	89.81641	41.88635	4.78×10^{-05}
Log-Probit	1	17.46023	78.71963	26.71976	-7.5×10^{-12}
Probit	0.999765	15.47853	71.58692	37.71366	0.012573

Bold row indicates the selected model and values.

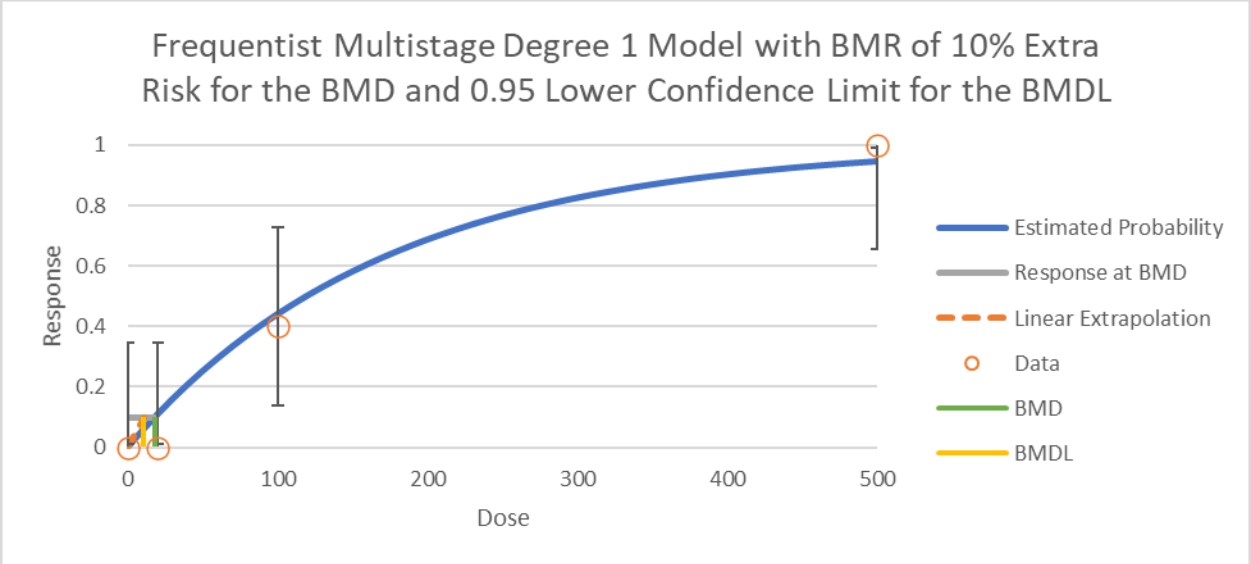

Figure B-14. Dose response curve for the Multistage Degree 1 model fit to hepatocellular hypertrophy in male rats (Loveless et al., 2009).

X-axis is dose (mg/kg-d), and y-axis is percent incidence.

B.18. POSTNATAL (F_1) COMBINED RAT BODY WEIGHT ON PND 0 (LOVELESS ET AL., 2009)

Table B-26. Dose response data for postnatal (F_1) combined rat body weight on PND 0 (Loveless et al., 2009)

Dose (mg/kg-d)	Number of animals	Mean (g)	Standard deviation
0	20	7.1	0.9
20	20	6.8	0.6
100	20	6.3	0.4
500	20	5.8	0.4

Table B-27. Benchmark dose results for postnatal (F_1) combined rat body weight on PND 0—non-constant variance, BMR = 5% relative deviation (Loveless et al., 2009)

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.000613	0.257697	150.4828	154.17	126.6598	−2.10808
Exponential 3	0.000613	0.257697	150.4828	154.2311	126.6606	−2.10618
Exponential 4	0.417875	0.257697	138.3442	28.86879	18.04413	−0.30628
Exponential 5	0.417869	0.257697	138.3442	28.89287	18.02549	−0.3069
Hill	**0.721731**	**0.257697**	**137.8148**	**20.37779**	**10.61916**	**0.013748**
Polynomial (Poly 3)	0.000461	0.257697	151.0527	164.7639	137.82	−2.14368
Polynomial (Poly 2)	0.000461	0.257697	151.0527	164.763	137.8213	−2.144
Power	0.000461	0.257697	151.0527	164.7277	137.8381	−2.14471
Linear	0.000461	0.257697	151.0527	164.7482	137.8256	−2.14516

Bold row indicates the selected model and values.

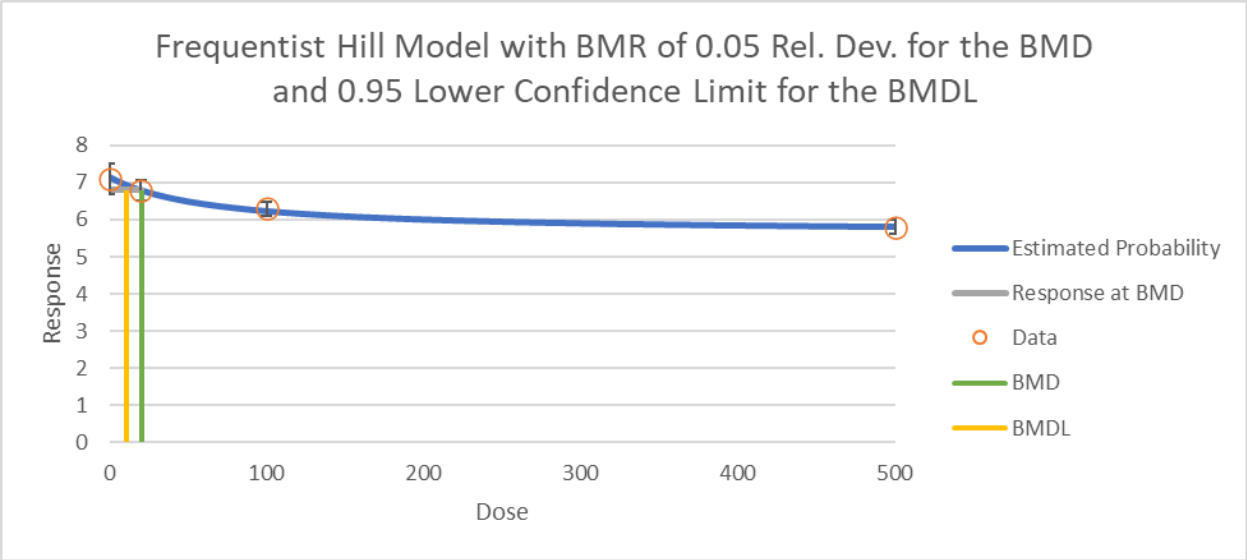

Figure B-15. Dose response curve for the Hill model fit to postnatal (F_1) combined rat body weight on PND 0 (Loveless et al., 2009).

X-axis is dose (mg/kg-d), and y-axis is mean body weight (g).

B.19. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASE 2) ON PND 0 (IWAI AND HOBERMAN, 2014)

Table B-28. Dose response data for postnatal (F_1) combined mouse body weight (phase 2) on PND 0 (Iwai and Hoberman, 2014)

Dose (mg/kg-d)	Number of litters	Mean (g)	Standard deviation
0	20	1.562	0.120
7	17	1.561	0.119
35	19	1.579	0.115
175	20	1.447	0.180

Table B-29. Benchmark dose results for postnatal (F_1) combined mouse body weight (phase 2) on PND 0—constant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	5% Relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.5476652	0.11356	−83.22986065	110.1988	72.6152	−0.18098
Exponential 3	0.6427413	0.11356	−82.21886799	162.9802	78.154	−0.00108

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	5% Relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 4	0.5476662	0.11356	−83.22986423	110.2315	72.6152	−0.18210
Exponential 5	0.6427936	0.11356	−82.21893566	163.6378	78.15859	−0.00024
Hill	NA	0.11356	−80.21900716	80.26504	36.86639	0.37613
Polynomial (Poly 3)	**0.971011**	**0.11356**	**−86.19475647**	**151.5619**	**80.06441**	**−0.00309**
Polynomial (Poly 2)	0.8402282	0.11356	−84.08587922	140.6661	79.398	−0.018554
Power	0.6428503	0.11356	−82.21900901	172.0405	121.5756	2.26045×10^{-05}
Linear	0.5601161	0.11356	−83.27482038	111.6004	75.16344	−0.16700

Bold row indicates the selected model and values.

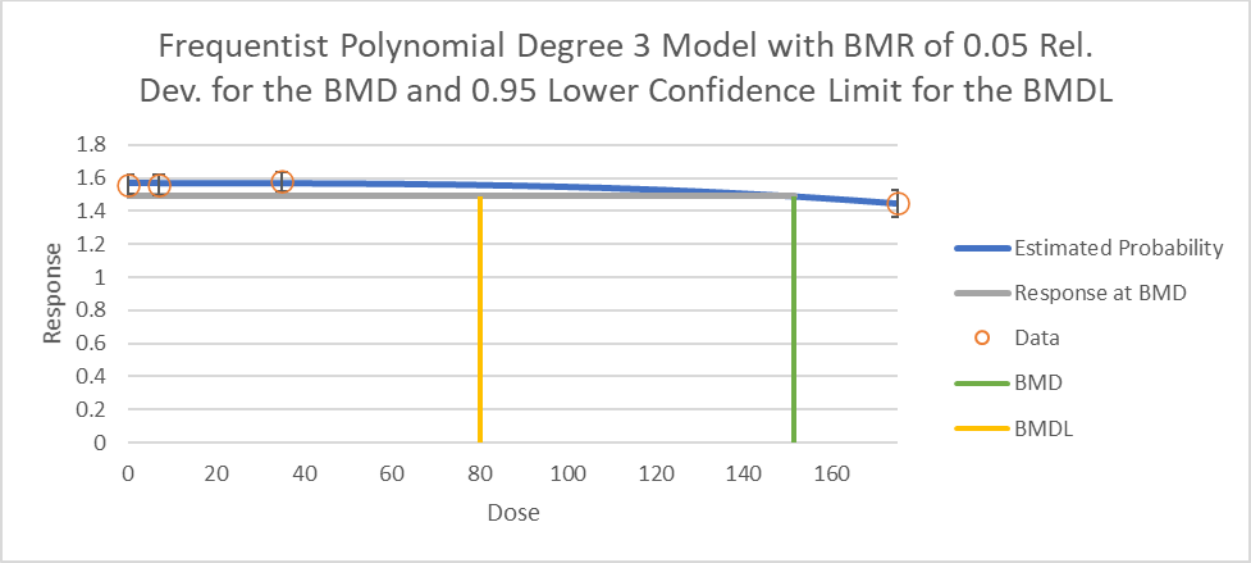

Figure B-16. Dose response curve for the Polynomial Degree 3 model fit to postnatal (F_1) combined rat body weight (phase 2) on PND 0 (Iwai and Hoberman, 2014).

X-axis is dose (mg/kg-d), and y-axis is mean body weight (g).

B.20. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASE 1) ON PND 0 (IWAI AND HOBERMAN, 2014)

Table B-30. Dose response data for postnatal (F_1) combined mouse body weight (phase 1) on PND 0 (Iwai and Hoberman, 2014)

Dose (mg/kg-d)	Number of litters	Mean (g)	Standard deviation
0	19	1.597	0.166
100	19	1.484	0.100
350	19	1.365	0.237
500	13	1.396	0.187

Table B-31. Benchmark dose results for postnatal (F_1) combined mouse body weight (phase 1) on PND 0—non-constant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.1454254	0.01314	−38.99028302	153.0166	106.9649	−0.9649
Exponential 3	0.1454276	0.01314	−38.99031401	152.9732	106.9641	−0.9649
Exponential 4	0.0502847	0.01314	−37.01452559	152.2536	22.05564	−0.9633
Exponential 5	NA	0.01314	−38.08812965	101.2731	78.25327	−0.6831
Hill	NA	0.01314	−38.08803429	100.2818	93.46723	−0.6814
Polynomial (Poly 3)	0.1237777	0.01314	−38.66793064	163.1923	116.9646	−0.9923
Polynomial (Poly 2)	0.1237777	0.01314	−38.66793064	163.1927	116.9612	−0.9923
Power	0.1237777	0.01314	−38.66793064	163.1924	116.9832	−0.9923
Linear	0.1237777	0.01314	−38.66793064	163.1923	117.1098	−0.9923

Both constant and nonconstant models failed to model the variance data.

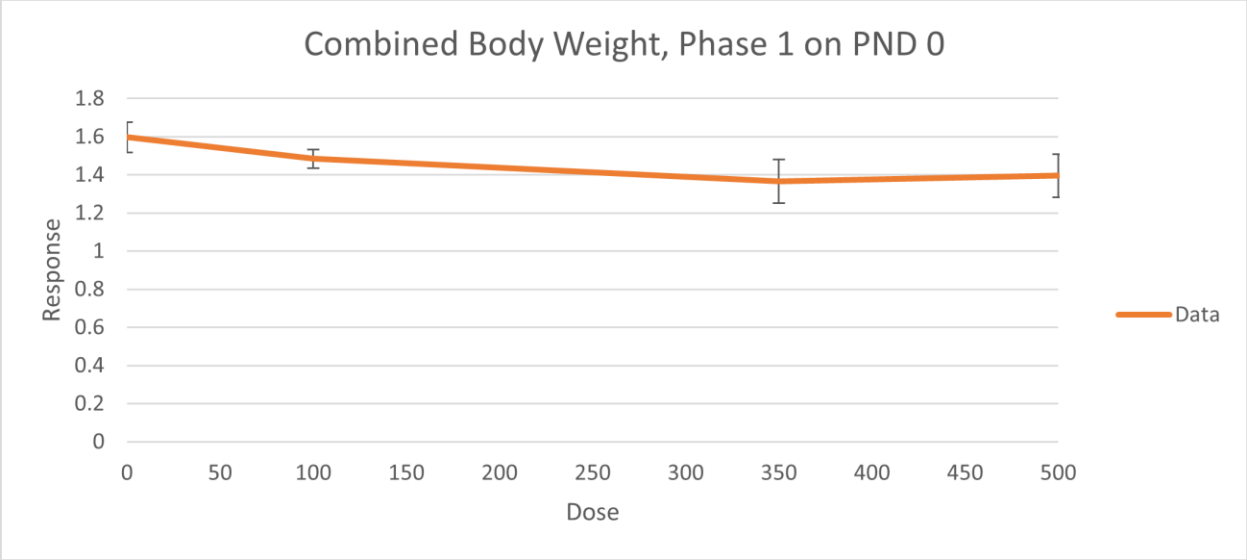

Figure B-17. Dose response data for postnatal (F1) combined rat body weight (phase 1) on PND 0 (Iwai and Hoberman, 2014).

X-axis is dose (mg/kg-d), and y-axis is mean body weight (g).

B.21. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASES 1 AND 2) ON PND 0 (IWAI AND HOBERMAN, 2014)

Table B-32. Dose response data for postnatal (F_1) combined mouse body weight (phases 1 and 2) on PND 0 (Iwai and Hoberman, 2014)

Dose (mg/kg-d)	Number of litters	Mean (g)	Standard deviation
0	27	1.577	0.154
7	17	1.561	0.119
35	19	1.579	0.115
100	19	1.484	0.1
175	20	1.447	0.18
350	19	1.365	0.237
500	13	1.396	0.187

Table B-33. Benchmark dose results for postnatal (F_1) combined mouse body weight (phases 1 and 2) on PND 0—non-constant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential2	<0.0001	<0.0001	−113.7079045	142.9071	106.0466	−0.7543
Exponential3	<0.0001	<0.0001	−113.7083052	143.1479	105.978	−0.7565
Exponential4	<0.0001	<0.0001	−113.841077	96.60292	54.95459	−0.4165
Exponential5	<0.0001	<0.0001	−114.9537952	124.9714	78.92795	−1.0491
Hill	<0.0001	<0.0001	−114.828486	120.2128	87.33994	−0.9886
Polynomial (Poly 6)	<0.0001	<0.0001	−113.1738885	151.8416	120.7674	−0.8470
Polynomial (Poly 5)	<0.0001	<0.0001	−113.1738881	151.8497	118.5011	−0.8469
Polynomial (Poly 4)	<0.0001	<0.0001	−113.1738881	151.8707	114.3476	−0.8473
Polynomial (Poly 3)	<0.0001	<0.0001	−113.1738834	124.918	89.12952	−0.8474
Polynomial (Poly 2)	<0.0001	<0.0001	−113.1738827	124.9218	89.12984	−0.8475
Power	<0.0001	<0.0001	−113.1738835	124.9172	89.12881	−0.8474
Linear	<0.0001	<0.0001	−113.1738818	124.9413	89.12913	−0.8478

Both constant and nonconstant models failed to model the variance data.

Figure B-18. Dose response data for postnatal (F1) combined rat body weight (phases 1 and 2) on PND 0 (Iwai and Hoberman, 2014).

X-axis is dose (mg/kg-d), and y-axis is mean body weight (g).

B.22. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASE 2) ON PND 4 (IWAI AND HOBERMAN, 2014)

Table B-34. Dose response data for postnatal (F_1) combined mouse body weight (phase 2) on PND 4 (Iwai and Hoberman, 2014)

Dose (mg/kg-d)	Number of litters	Mean (g)	Standard deviation
0	20	2.844	0.307
7	16	2.850	0.320
35	19	2.976	0.335
175	20	2.726	0.442

Table B-35. Benchmark dose results for postnatal (F_1) combined mouse body weight (phase 2) on PND 4—constant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential2	0.2719642	0.3216	62.91273812	169.116	79.86226	−0.259556406
Exponential3	0.1915765	0.3216	64.01402032	171.7121	88.6223	0.000775748
Exponential4	0.2719642	0.3216	62.91273812	169.1154	79.86194	−0.259556232
Exponential5	0.1915772	0.3216	64.01401491	171.7578	88.62246	0.000965695
Hill	0.191581	0.3216	64.01398562	90.59589	37.60928	1.049750693
Polynomial (poly 3)	**0.6280973**	**0.3216**	**60.04847961**	**167.4876**	**89.7897**	**−0.008262428**
Polynomial (poly 2)	0.3908438	0.3216	62.1874628	164.7988	88.29103	−0.042810195
Power	0.1915812	0.3216	64.01398451	174.0668	106.2633	4.3382×10^{-06}
Linear	0.2746896	0.3216	62.89279543	168.0092	81.96061	−0.247433913

Bold row indicates the selected model and values.

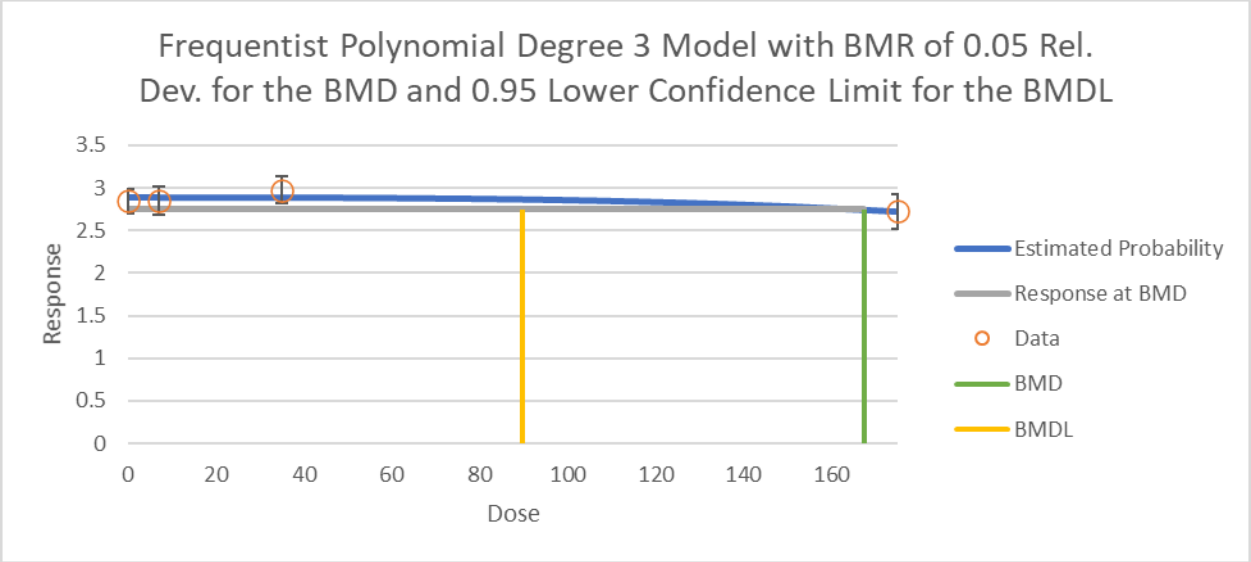

Figure B-19. Dose response curve for the Polynomial model (poly 3) fit to postnatal (F1) combined rat body weight (phase 2) on PND 4 (Iwai and Hoberman, 2014).

X-axis is dose (mg/kg-d), and y-axis is mean body weight (g).

B.23. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASE 1) ON PND 4 (IWAI AND HOBERMAN, 2014)

Table B-36. Dose response data for postnatal (F_1) combined mouse body weight (phase 1) on PND 4 (Iwai and Hoberman, 2014)

Dose (mg/kg-d)	Number of litters	Mean (g)	Standard deviation
0	18	2.966	0.460
100	19	2.771	0.248
350	17	2.256	0.650
500	11	2.382	0.482

Table B-37. Benchmark dose results for postnatal (F_1) combined mouse body weight (phase 1) on PND 4—non-constant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential2	0.0805287	0.01040	90.85546713	84.46765	60.74916	−0.0400

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential3	0.0805258	0.01040	90.85553976	84.40685	60.76206	−0.0404
Exponential4	0.0358662	0.01040	92.22063699	59.93938	28.42237	0.3237
Exponential5	NA	0.01040	90.99580479	113.1456	55.45824	−0.6168
Hill	NA	0.01040	90.99580314	102.7811	95.12445	−0.6171
Polynomial (poly 3)	0.064938	0.01040	91.28582701	94.9049	70.88712	−0.1219
Polynomial (poly 2)	0.064938	0.01040	91.28582701	94.90514	70.88731	−0.1219
Power	0.064938	0.01040	91.28582692	94.90368	70.88898	−0.1219
Linear	0.064938	0.01040	91.28582698	94.90395	70.88785	−0.1220

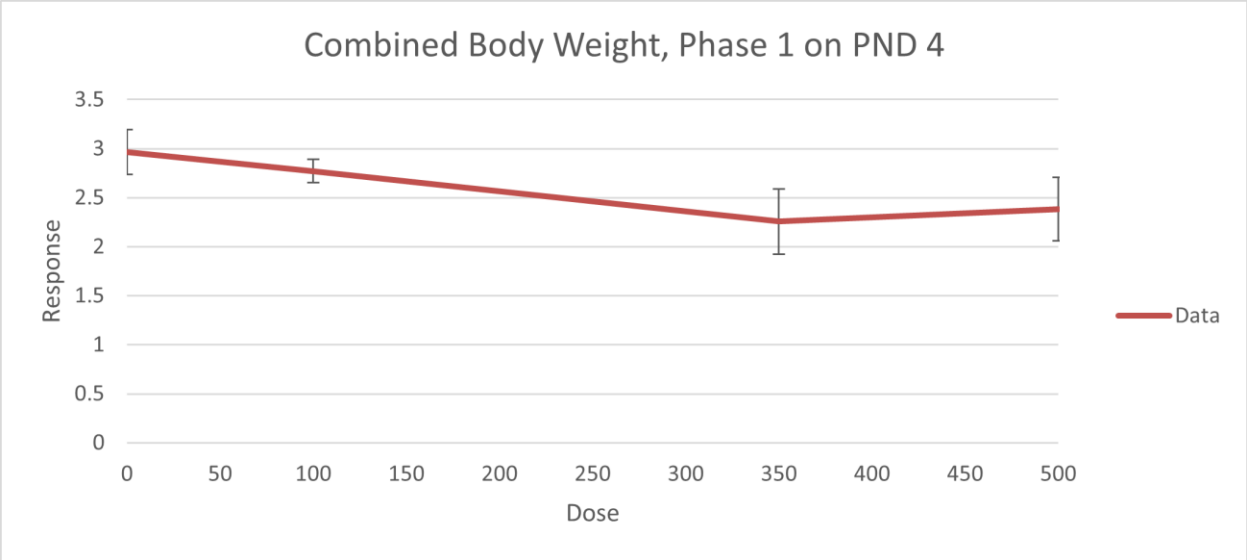

Figure B-20. Dose response data for postnatal (F1) combined rat body weight (phase 1) on PND 4 (Iwai and Hoberman, 2014).

X-axis is dose (mg/kg-d), and y-axis is mean body weight (g).

B.24. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASES 1 AND 2) ON PND 4 (IWAI AND HOBERMAN, 2014)

Table B-38. Dose response data for postnatal (F_1) combined mouse body weight (phase 1) on PND 4 (Iwai and Hoberman, 2014)

Dose (mg/kg-d)	Number of litters	Mean (g)	Standard deviation
0	38	2.902	0.387

Dose (mg/kg-d)	Number of litters	Mean (g)	Standard deviation
7	16	2.85	0.320
35	19	2.976	0.335
100	19	2.771	0.248
175	20	2.726	0.442
350	17	2.256	0.650
500	11	2.382	0.482

Table B-39. Benchmark dose results for postnatal (F_1) combined mouse body weight (phases 1 and 2) on PND 4—non-constant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Model	Goodness of fit (*p*-value)	Test 3 (*p*-value)	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential2	0.1128908	0.2000	147.7836654	96.26436	71.43879	−0.0186
Exponential3	0.0802807	0.2000	149.2060003	120.9626	73.66277	−0.3003
Exponential4	0.1128924	0.2000	147.7836603	96.26532	71.43823	−0.0189
Exponential5	**0.4079141**	**0.2000**	**145.7744096**	**155.2176**	**102.9449**	**0.4071**
Hill	0.3365496	0.2000	146.2553381	167.5058	144.5742	0.2848
Polynomial (poly 6)	0.110707	0.2000	147.8372526	103.2872	78.92902	−0.0985
Polynomial (poly 5)	0.110707	0.2000	147.8372543	103.3039	78.96729	−0.0986
Polynomial (poly 4)	0.110707	0.2000	147.8372526	103.2829	78.85834	−0.0984
Polynomial (poly 3)	0.110707	0.2000	147.8372498	103.2662	78.84649	−0.0983
Polynomial (poly 2)	0.110707	0.2000	147.8372526	103.2867	78.85151	−0.0985
Power	0.0672452	0.2000	149.6433133	118.4167	79.55287	−0.2561
Linear	0.110707	0.2000	147.8372526	103.2844	78.84044	−0.0985

Bold row indicates the selected model and values.

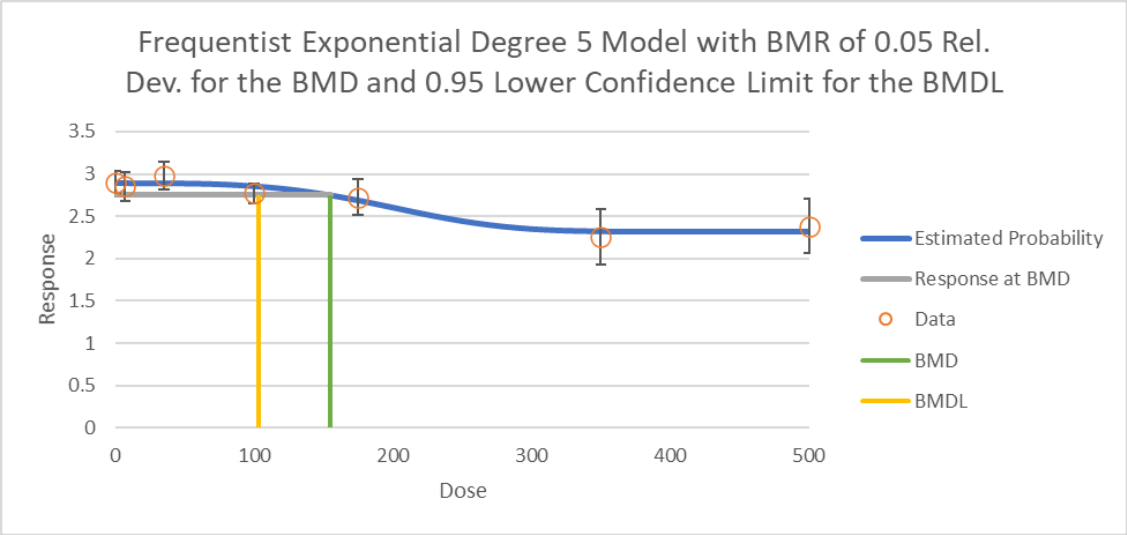

Figure B-21. Dose response curve for the Exponential 5 model fit to postnatal (F1) combined mouse body weight (phases 1 and 2) on PND 4 (Iwai and Hoberman, 2014).

X-axis is dose (mg/kg-d), and y-axis is mean body weight (g).

B.25. PERINATAL MORTALITY (PHASE 2) ON PND 0–21 (IWAI AND HOBERMAN, 2014)

Table B-40. Nested model summary for perinatal mortality (phase 2) on PND 0–21, BMR = 1% extra risk (Iwai and Hoberman, 2014)

Model type	Litter-specific covariate	Intralitter correlation	Goodness of fit (*p*-value)	AIC	BMD	BMDL
Nested Logistic	Yes	Yes	0.223	145.10	150.6	24.50
	Yes	No	0.0037	158.29	157.6	39.26
	No	**Yes**	**0.2113**	**141.20**	**151.4**	**24.77**
	No	No	0.003	154.50	158.0	39.03

Nested Logistic model with intralitter correlation is selected as the best model based on the lowest AIC value. Bold row indicates the selected model and values.

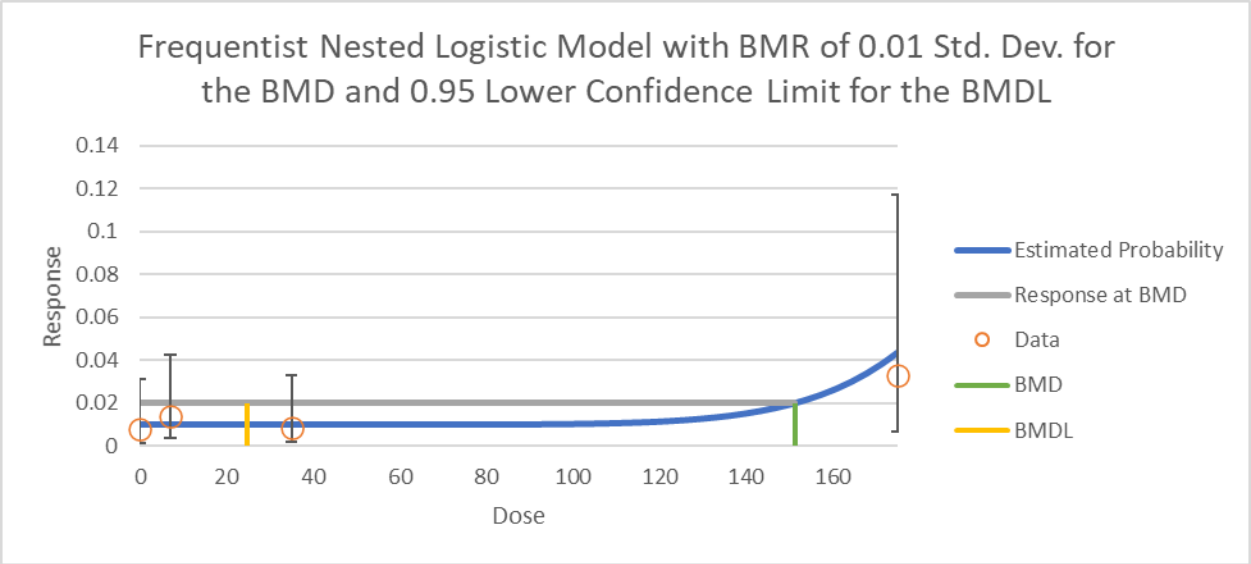

Figure B-22. Dose response curve for the Nested National Center for Toxicological Research model fit to perinatal mortality (phase 2) on PND 0–21(Iwai and Hoberman, 2014).

X-axis is dose (mg/kg-d), and y-axis is mortality.

B.26. PERINATAL MORTALITY (PHASE 1) ON PND 0–21 (IWAI AND HOBERMAN, 2014)

Table B-41. Nested model summary for perinatal mortality (phase 1) on PND 0–21, BMR = 1% extra risk (Iwai and Hoberman, 2014)

Model type	Litter-specific covariate	Intralitter correlation	Goodness of fit (*p*-value)	AIC	BMD	BMDL
Nested Logistic	Yes	Yes	0.053	356.23	206.1	105.8
	Yes	No	<0.0001	478.37	238.9	177.2
	No	Yes	0.0593	353.33	201.7	98.61
	No	No	<0.0001	477.04	233.1	162.7

The means of the data cannot be modeled (all goodness of fit *p*-value > 0.1) for phase 1 data; the data is not amenable to BMD modeling.

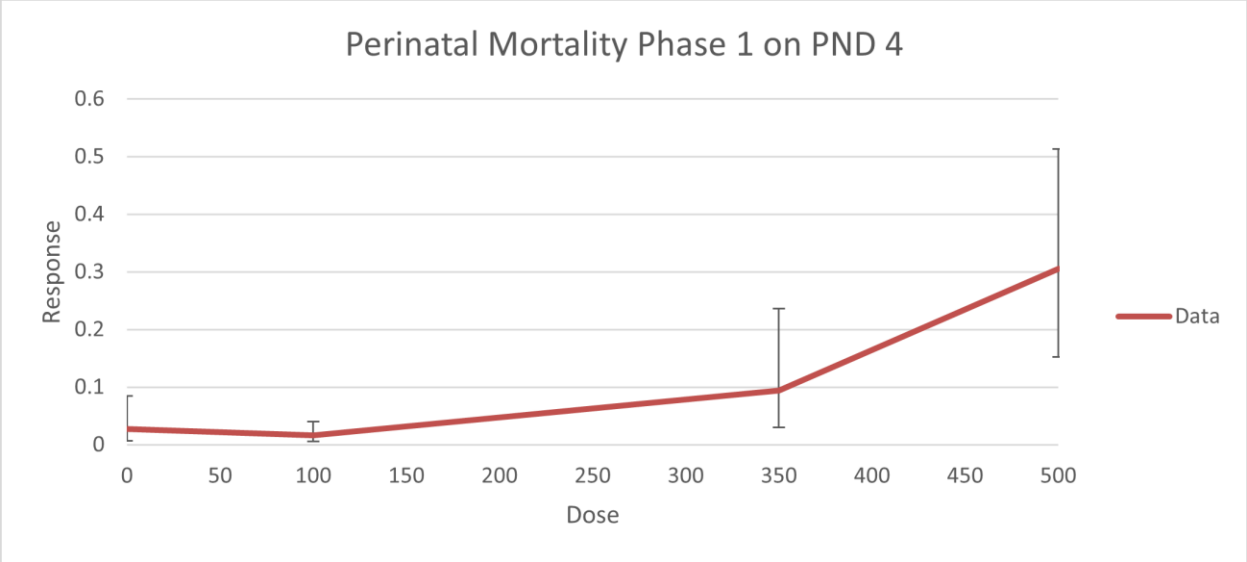

Figure B-23. Dose response data perinatal mortality (phase 1) on PND 4(Iwai and Hoberman, 2014).

X-axis is dose (mg/kg-d), and y-axis is percent incidence.

B.27. PERINATAL MORTALITY (PHASES 1 AND 2) ON PND 0–21 (IWAI AND HOBERMAN, 2014)

Table B-42. Nested model summary for perinatal mortality (phases 1 and 2) on PND 0–21, BMR = 1% extra risk (Iwai and Hoberman, 2014)

Model type	Litter-specific covariate	Intralitter correlation	Goodness of fit (*p*-value)	AIC	BMD	BMDL
Nested Logistic	Yes	Yes	0.024	495.44	150.9	85.15
	Yes	No	<0.0001	632.14	199.7	138.2
	No	Yes	0.029	491.80	147.7	83.59
	No	No	<0.0001	629.52	195.2	134.0

The means of the data cannot be modeled (all goodness of fit *p*-value > 0.1) for phases 1and 2 data; the data is not amenable to BMD modeling.

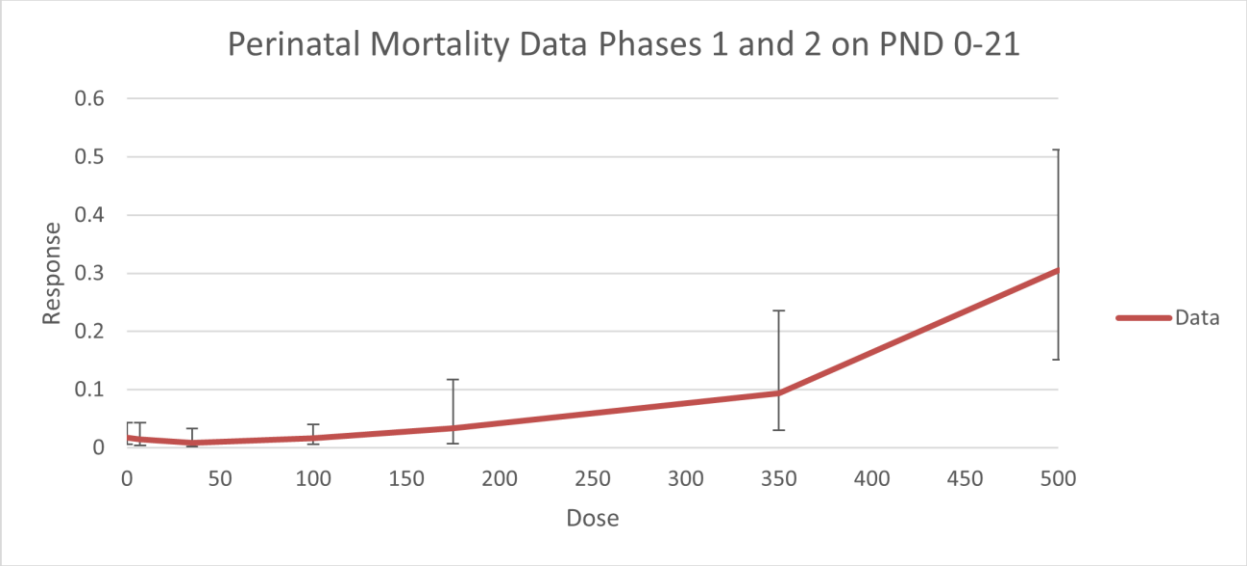

Figure B-24. Dose response data perinatal mortality (phases 1 and 2) on PND 0–21 (Iwai and Hoberman, 2014).

X-axis is dose (mg/kg-d), and y-axis is percent incidence.

B.28. TOTAL THYROXINE (T4) IN MALE RATS - (NTP, 2018)

Table B-43. Dose response data for thyroxine (T4) in male rats (NTP, 2018)

Dose (mg/kg-d)	Number of animals	Mean (g)	Standard deviation
0	10	4.26	0.461692538
62.5	10	3.4	0.730486139
125	9	2.933	0.483
250	10	2.9	0.521775814
500	10	2.37	0.322552321
1,000	10	1.77	0.547074035

Table B-44. Benchmark dose results for total thyroxine (T4) in male rats—constant variance, BMR = 1 standard deviation (NTP, 2018)

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential2	0.001553	0.2402	17.8629446	145.2153	266.23938	−0.59715

Model	Goodness of fit (*p*-value)	Test 2 (*p*-value)	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential3	0.001553	0.2402	19.8639886	145.2153	266.23938	−0.59715
Exponential4	0.028228	0.2402	17.8629446	43.1832	131.51922	−1.09556
Exponential5	0.028228	0.2402	15.9850469	43.1832	131.51922	−1.09556
Hill	**0.121780**	**0.2402**	**16.4752017**	**25.96669**	**92.61432**	**−0.73657**
Polynomial (poly 6)	0.000100	0.2402	18.9269281	261.7194	390.80563	−1.01211
Polynomial (poly 5)	0.000100	0.2402	17.8629446	240.1021	390.82343	−1.01211
Polynomial (poly 4)	0.000100	0.2402	15.8629568	240.1021	390.79722	−1.01211
Polynomial (poly 3)	0.000100	0.2402	17.8629446	240.1022	390.61877	−1.01211
Polynomial (poly 2)	0.000100	0.2402	17.8629436	240.1086	390.56516	−1.01211
Power	0.000100	0.2402	17.8629446	240.1066	390.5741	−1.01211
Linear	0.001553	0.2402	19.8639886	145.2153	266.23938	−0.59715

Bold row indicates the selected model and values.

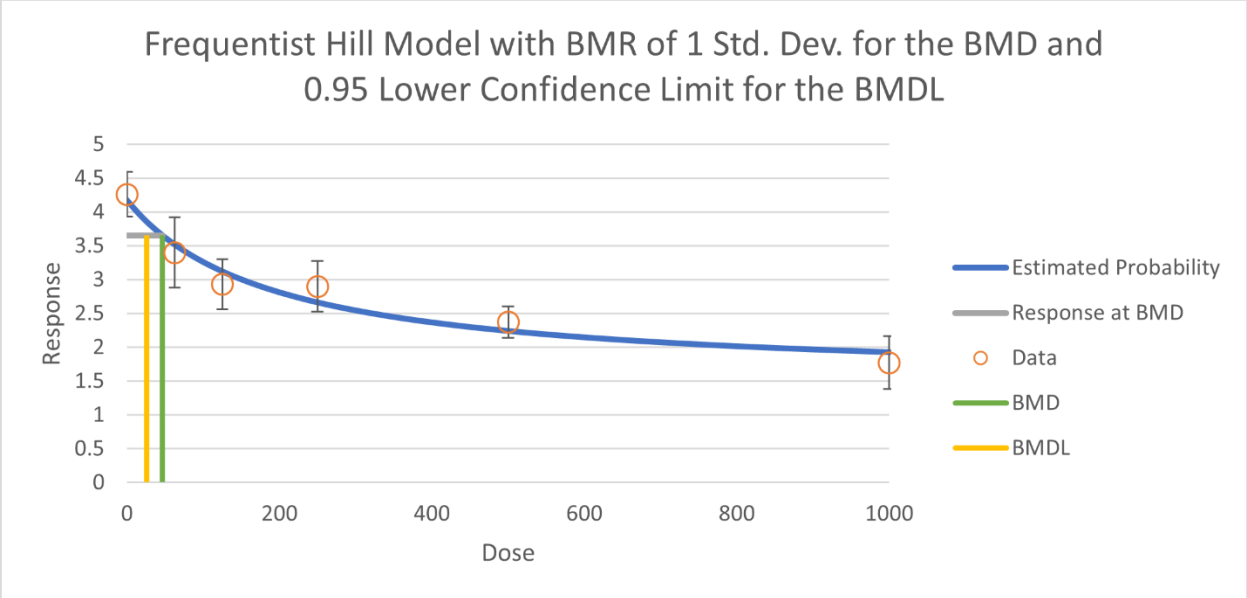

Figure B-25. Dose response curve for the Hill model fit to thyroxine (T4) in male rats (NTP, 2018).

X-axis is dose (mg/kg-d), and y-axis is mean level of thyroxine (T4) (µg/dL).

APPENDIX C. EVALUATION OF PFHXA ELIMINATION

C.1. EVALUATION OF PFHXA ELIMINATION IN RATS AND MICE

Pharmacokinetic parameters were estimated separately for male and female rats and mice using a hierarchical, Bayesian framework to allow for the partial pooling of time-course concentration data across multiple studies. Data extracted from the studies described above were fit to the following model formulation, which describes the absorption (when necessary), distribution, and elimination phase of PFHxA through a two-compartment pharmacokinetic model:

$$C_i = abs_{flag,i}(-A_i - B_i)e^{-k_{abs,i}t} + A_i e^{-\alpha_i t} + B_i e^{-\beta_i t} \quad \text{(C-1)}$$

Here, *i* represents the *ith* compartment for PFHxA measurement (e.g., plasma, liver, kidney). A_i and B_i represent the ratio of chemical mass going to each empirical compartment, normalized by the central compartment volume, resulting in units of PFHxA concentration. For PFHxA concentrations measured in the plasma (i.e., central compartment) following intravenous (i.v.) exposure, $abs_{flag,i}$ is set to zero to remove the absorption term.

Conventionally, each compartment with pharmacokinetic data is fit independently to equation C-1 and tissue-specific half-lives for each species and sex are derived from the estimated β, i.e., $t_{1/2,l} = \ln(2)/\beta_i$. However, when a compound is in the elimination phase, β should be constant across all tissues. To determine this PFHxA-specific β and use the time-course concentration data from every study across multiple compartments, a partial pooling of data in a hierarchical Bayesian framework assumes that, although β_i differs for each tissue, they are all sampled from a common group distribution. Following completion of the Markov-chain Monte Carlo analysis, the top-level posterior distribution of β is used to determine the median PFHxA half-life, with uncertainty, for each species/sex. The remaining study-level coefficients are used to estimate the additional pharmacokinetic values, for example, area under the curve (AUC_{inf}), clearance (CL), volume of distribution ($V_{d\beta}$).

Along with the half-life analysis, a separate distribution of CL = dose/AUC_{inf} and $V_{d\beta}$ = CL/β is generated for each experiment (study/route/dose/sex), where AUC_{inf} is obtained by integrating equation (C-1) from time = 0 to infinity, to yield

$$AUC_{inf,i} = \frac{A_i}{\alpha_i} + \frac{B_i}{\beta_i} - \frac{abs_{flag,i}(A_i + B_i)}{k_{abs,i}} \quad \text{(C-2)}$$

Median and 5th and 95th percentiles of the distributions for $t_{1/2,l}$, CL_i *and* $V_{d\beta}$ are then pooled across each study/route/dose to calculate the species- and sex-dependent values.

C.1.1. Mice

Data for male and female mice were obtained from Gannon et al. (2011) who evaluated the pharmacokinetics after single oral doses of 2 and 100 mg/kg. Original data files were provided by e-mail from Shawn Gannon, The Chemours Company, Wilmington, Delaware to Paul Schlosser, U.S. EPA, Durham, North Carolina on January 23, 2020. Although the data for the 2 mg/kg dose appeared appropriately censored below the dose-specific limit of quantification (LOQ), the 100 mg/kg data appeared to reach a plateau just above the corresponding LOQ (~0.25 μg/g plasma), in a concentration range for which clearance after the 2 mg/kg dose was quite rapid. EPA interpreted this result as indicating an interfering background signal. For this reason, only data with measured concentration >0.5 μg/g plasma were used for the 100 mg/kg dose. The resulting statistics for the elimination half-lives (90% confidence interval) are 2.8 hours (1.0−7.0 hours) and 6.7 hours (2.2−16 hours) for females and males, respectively.

Female mouse data were from Daikin Industries (2010), who exposed groups of mice to 35, 175, or 350 mg/kg PFHxA by oral gavage and measured serum concentration at time-points up to 24 hours. Because three separate mice were analyzed at each time point, means and standard deviations were calculated and used for statistical modeling. Data at the first time points with concentrations below the lower limit of quantification (LLOQ) were assigned a value of LLOQ/$\sqrt{2}$ for the purpose of computing the means. Specifically, for the 24-hour time point, two of three animals in the 175 and 350 mg/kg dose groups had results <LLOQ, so the substitution was made for those animals prior to calculating the time-point mean. In the 35 mg/kg group, one animal had results <LLOQ at 6 hours so the substitution was made prior to calculating the mean. Because all animals in this group were below LLOQ at 8 hours, the value for that time point was treated as equal to LLOQ/$\sqrt{2}$, and the results for the 24-hour time-point were censored. Finally, in the 175 mg/kg group, 1 animal in the 8-hour group had a reported concentration 15 times higher than the other 2 animals in that group, 80% higher than the average concentration for that dose at 6 hours and higher than any animal in the 350 mg/kg group at the same time point (8 hours). Therefore, this measurement was censored.

Fits of the pharmacokinetic (PK) model curves to the data sets are shown below. Median and 5th, and 95th percentile values for each parameter are provided in Table 5-3 (see Section 5.2.1, Approach for Animal-Human Extrapolation of PFHxA Dosimetry).

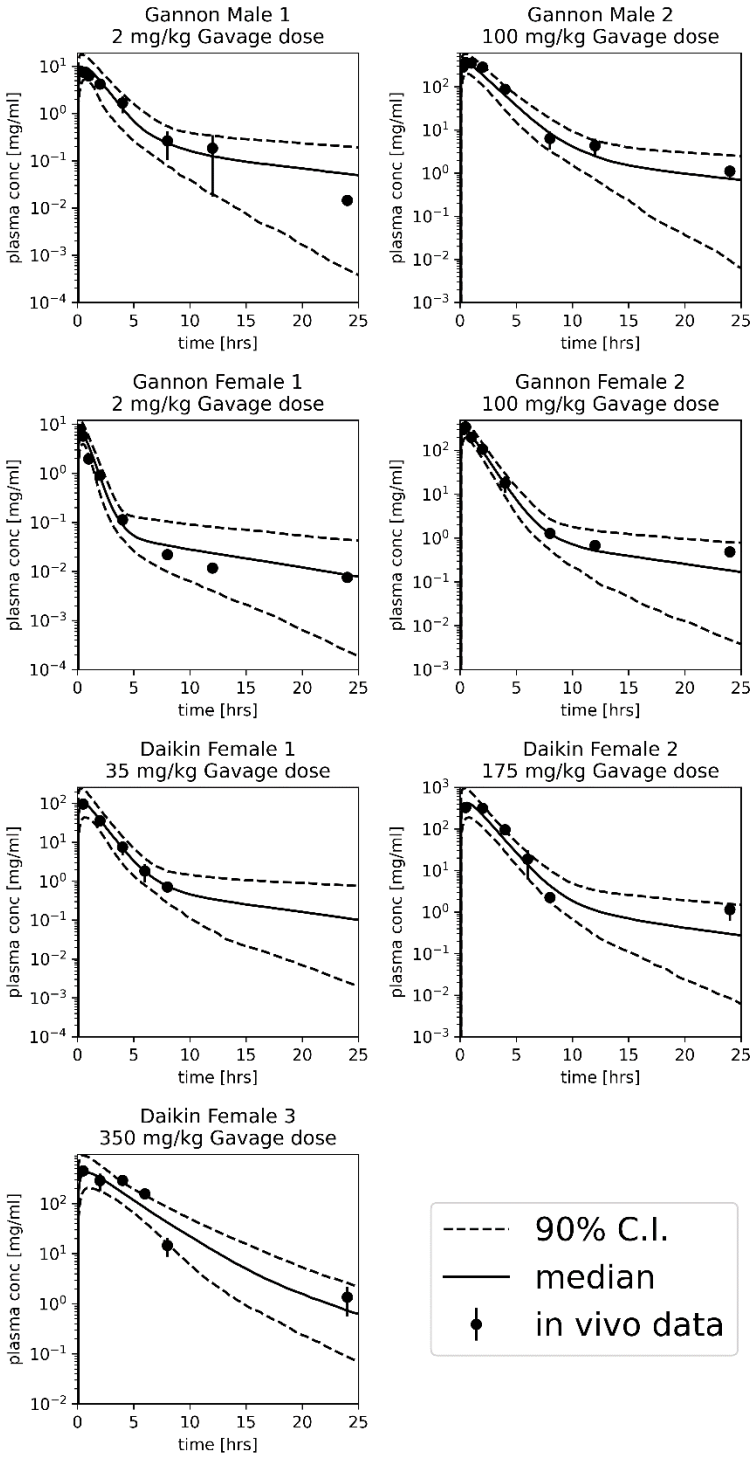

Figure C-1. Fits of population pharmacokinetic model to data for male (top row) and female (remaining rows) mice following 2–350 mg/kg oral exposure PFHxA.

Source: Data from Gannon et al. (2011) and Daikin Industries (2010).

C.1.2. Rats

PFHxA the following PK data for male and female rats were evaluated:

- Chengelis et al. (2009a): male and female Sprague-Dawley rats exposed once by intravenous injection (i.v.; 10 mg/kg) or by single-day or Day 25 of repeated gavage (50, 150, or 300 mg/kg). (i.v. data for males and females and oral data for males are provided in published tables. Oral data for females were obtained by digitizing the plot of single-day exposure data. The 25-day female rat data, however, were not digitized or used because the digitization process has some uncertainty; reported dose-specific half-lives for females were quite similar for the single- and 25-day studies, and results for males were similar with and without the 25-day data.)

- Dzierlenga et al. (2019): male and female Sprague-Dawley rats exposed by i.v. (40 mg/kg) or by gavage (40, 80, or 160 mg/kg; data from National Toxicology Program website).

- Gannon et al. (2011): male and female Sprague-Dawley rats exposed by gavage (2 or 100 mg/kg; data from study authors).

- Iwabuchi et al. (2017): male Wistar rats exposed by gavage (0.1 mg/kg; data from published tables or digitized from figures).

The resulting statistics for the elimination half-lives, clearance values, and volumes of distribution (with 90% confidence intervals) are listed in Table 5-3 (see Section 5.2.1, Approach for Animal-Human Extrapolation of PFHxA Dosimetry).

C.2. EVALUATION OF PFHXA ELIMINATION IN HUMANS

Data for human PFHxA analysis were extracted from Nilsson et al. (2013) where PFHxA concentrations were measured in the blood of ski wax technicians exposed to PFAS compounds over the course of multiple ski seasons. Because timing of the initial PFHxA exposure and the resulting absorption kinetics are unknown for this population, EPA fit a one-compartment infusion pharmacokinetic model to the reported time-course data:

$$C_i = \begin{cases} \frac{A_i}{t_{inf,i}\beta_i}\left(1 - e^{-\beta_i t}\right) & if\ t \le t_{inf,i} \\ \frac{A_i}{t_{inf,i}\beta_i}\left(1 - e^{-\beta_i t_{inf,i}}\right)\left(1 - e^{-\beta_i (t - t_{inf,i})}\right) & if\ t > t_{inf,i} \end{cases} \quad \text{(C-3)}$$

Here, i represents the *ith* ski wax technician and $t_{inf,i}$ represents the time at which exposure to PFHxA ends. All other model parameters are the same as described above for the rat and mouse fits. Briefly, this model assumes a constant exposure to PFHxA throughout the ski season when time is less than t_{inf}. Once t_{inf} is reached, PFHxA is eliminated under a first order elimination assumption.

Similar to the methods described for the rat and mouse, β_i for each ski wax technician is sampled hierarchically from a population distribution while all other parameters in the model are fit only to the individual technician. Finally, to use limit of detection (LOD) data reported in this

study, we implemented a left-censored likelihood function in the Bayesian inference model for samples reported below the LOD (<0.05 ng/mL). This ensured that the likelihood function for these data were sampled only from a probability distribution with an upper bound at the LOD.

Results for each ski wax technician are shown below following sampling of the technician-specific posterior distributions. Technician half-lives (90% credible interval) are presented in the panel for each technician with the population half-life determined to be 11.45 (6.06 – 21.21) days. Technicians 1–8 represent data from the 2007–2008 ski season, when samples were taken late enough in the spring to allow quantification of post-exposure clearance.

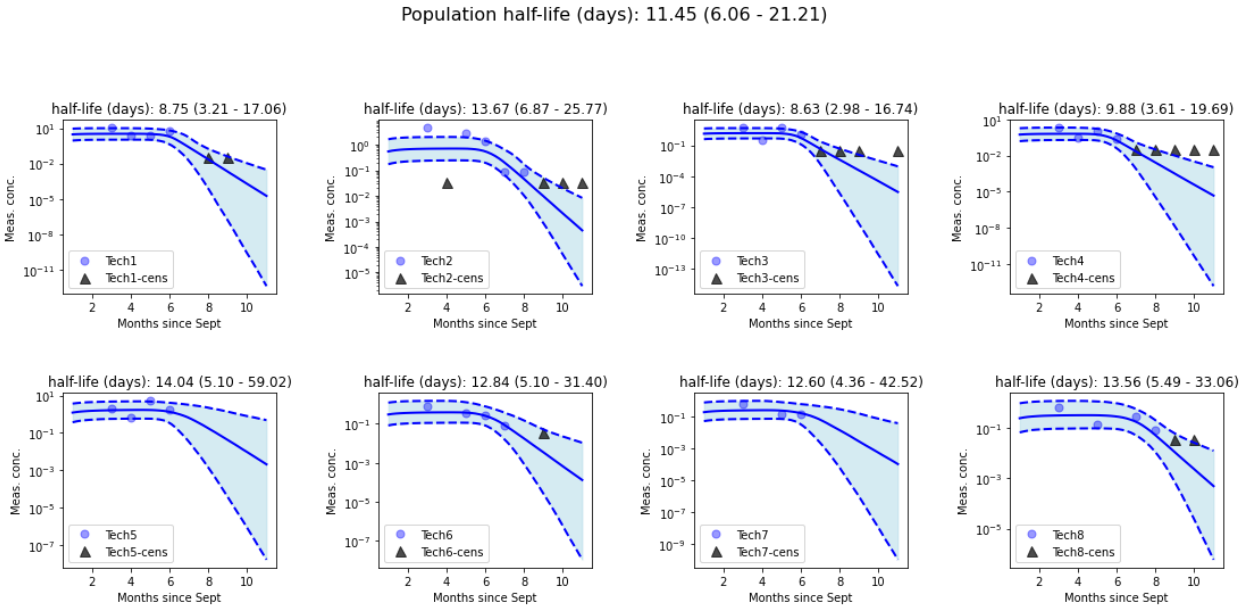

Figure C-2. Fits of human PFHxA data from ski-wax technician blood samples.

Blue circles represent data above LOD while black triangles are data samples reported at the LOD (<0.05 ng/mL). 90% credible intervals are illustrated with the light blue bands and dashed lines.

APPENDIX D. QUALITY ASSURANCE FOR THE IRIS TOXICOLOGICAL REVIEW OF PFHXA

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 outlined in the *EPA Quality Manual for Environmental Programs* (see CIO 2105-P-01.1) and follows the specifications outlined in EPA Order CIO 2105.1.

As required by CIO 2105.1, 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). A National Center for Environmental Assessment (NCEA)/CPHEA-specific QMP also was 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 PFHxA is designated as 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 PFAS assessments 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 October 2021. All PFAS assessments follow the PFAS PQAPP, and all assessment leads and team members are required to receive QA training on the PFAS 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 PFAS Assessments	L-CPAD-0031652-QP-1-5	February 2023
Program Quality Assurance Project Plan (PQAPP) for the Integrated Risk Information System (IRIS) Program	L-CPAD-0030729-QP-1-5	June 2022
An Umbrella Quality Assurance Project Plan (QAPP) for Dosimetry	L-CPAD-0032188-QP-1-2	December 2020

Title	Document number	Date
and Mechanism-Based Models (PBPK)		
Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS)	L-HEEAD-0032189-QP-1-2	October 2020
ICF-General Support of CPHEA Human Health Assessment Activities QAPP	L-CPAD-0031961-QP-1-2	April 2021

During assessment development, this project undergoes 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

During Step 3 and Step 6 of the IRIS process, the IRIS toxicological review is 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-2021-0561 on http://www.regulations.gov.

During Step 4 assessment development, the *IRIS Toxicological Review of Perfluorohexanoic Acid and Related Salts* underwent public comment from February 2, 2022, to April 4, 2022. Following this comment period, the toxicological review underwent external peer review by a contractor-led panel performed by ERG from April 5,2022 to August 25,2022. 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-2021-0561.

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.

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

The Toxicological Review of Perfluorohexanoic Acid and Related Salts was released for public comment in February 2022. Public comments on the assessment were submitted to the U.S. Environmental Protection Agency (EPA) by April 4, 2022. The Toxicological Review has also 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 May 16 and 17, 2022, which included another 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. A summary of comments made by the external peer reviewers and public commenters, as well as EPA's responses to these comments, are arranged by charge question. In many cases, the comments of the individual reviewers have been synthesized and paraphrased for brevity (please consult the final peer review report for the full text of the panel's comments: Peer Review Report). 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 PFHxA charge questions to the peer reviewers, which can be found in the public EPA docket (EPA-HQ-ORD-2021-0561):

- 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 PFHxA 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 PFHxA 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 PFHxA Toxicological Review, they could inform future reviews or research efforts.

Appendix E lists all Tier 1 recommendations and Tier 2 Suggestions from the external peer reviewers organized by charge question. For Tier 3 Considerations, please refer to the external peer review report linked above. Where public comments were made on topics raised by the external peer reviewers, they are noted along with the external peer review comments. All Tier 1

recommendations were implemented in this revised assessment, either through revision or addition to the peer reviewed analyses or text. 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 in raising the suggestion, and the level of effort to implement. For this assessment, all Tier 2 suggestions deemed to be impactful to the toxicity value conclusions were implemented in this revised assessment. Additional public comments not raised by the peer reviewers are included in a separate section at the end of each charge question section. In many cases, the public comments have been synthesized and paraphrased for brevity, both in this Appendix and in the summary, document provided as a courtesy to the external peer review panel. Please see docket (EPA-HQ-ORD-2021-0561) for both this summary document and the full text of the submitted public comments.

External peer reviewer 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.

E.1. CHARGE QUESTIONS 1 AND 2 – SYSTEMATIC REVIEW AND DOCUMENTATION

1) The Toxicological Review for PFHxA 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 (Literature Searching and Screening) and in full detail in Appendix A (Systematic Review Protocol for the PFAS IRIS Assessments). Please comment on whether the search strategy and screening criteria for PFHxA literature are clearly described. If applicable, please identify additional peer-reviewed studies of PFHxA that the assessment should incorporate[1].

2) The Toxicological Review provides an overview of individual study evaluations and the results of those evaluations are made available in the Health Assessment Workplace Collaborative linked HAWC. Note that a "HAWC FAQ for assessment readers" document is available (scroll to the bottom of the page, and the document is available for download under "attachments") and is intended to help the reviewer navigate this on-line resource. Data from studies considered informative to the assessment are synthesized in the relevant health effect-specific sections, and study data are available in HAWC.

    a. Please comment on whether the study confidence conclusions for the PFHxA studies are scientifically justified and clearly described, considering the important methodological features of the assessed outcomes. Please indicate any study confidence conclusions that are not justified and explain any alternative study evaluation decisions.

[1]Newly identified studies (i.e., studies identified by EPA or the public that meet PECO criteria but were not addressed in the external review draft, for example due to recent publication) will be characterized by EPA and presented to the peer review panel. This 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. The peer review panel is asked to review EPA's characterization and provide tiered recommendations to EPA regarding which studies, if any, to incorporate into the assessment before finalizing.

b. Results from individual PFHxA studies are presented and synthesized in the health system specific sections. Please comment on 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.

E.1.1. External Peer Reviewer Comments on Systematic Review and Documentation

For charge question 1, "all reviewers agreed that the search strategy and criteria were appropriate and clearly described. One reviewer noted how inherently challenging it is to identify pertinent studies with the increasing interest in PFAS, which has led to an increasing rate of new publications. Several reviewers provided references to additional studies for EPA's consideration." For charge question 2, "six of the seven reviewers agreed that the confidence conclusions for the PFHxA studies were scientifically justified and clearly described. For example, one reviewer noted that the visual presentation of the evaluation results for the animal studies was very effective and found the use of interactive graphics to be very convenient." The seventh reviewer provided a Tier 1 Recommendation to improve the presentation. The report also noted that "reviewers generally found the presentation and analysis of the study results as they appear in the health system-specific sections to be clear but recommended several Tier 1 and Tier 2 revisions to improve the clarity and accuracy of the presentation." These comments are described below.

Tier 1 Recommendations

Comment: EPA should add text describing the major reasons for excluding the 194 articles during the screening process, as shown in Figure 2-1.

EPA Response: Studies are excluded if they do not meet all PECO criteria. 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. In these instances, tags were added (if not already present) to indicate the type(s) of potentially relevant supplemental information and can be visualized using the interactive HAWC literature tag tree available by clicking the following link: https://hawc.epa.gov/lit/assessment/100500070/references/visualization/. 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: EPA should add several sentences to Section 1 that describe the in-press paper EHP (DOI 10.1289/EHP 10343) shown in EPA's slides during the May 16, 2022, peer review. In particular, the reviewer noted that the evidence maps illustrating how EPA is going to synthesize evidence across the PFAS compounds would be a good addition to the text.

EPA Response: A brief description of the EHP paper was added to Section 4.1, before Table 4.1 that provides an overview of health effects that have been described for several other recent EPA PFAS assessments.

Comment: EPA should update HAWC for PFHxA to include assessments/evaluations of recent studies that will be considered in finalizing this Toxicological Review.

EPA Response: The date of the last literature search used for the Toxicological Review (April 2022) was added to Section 2.1 and in HAWC. Updates to the literature incorporated into the assessment are reflected in a separate document posted to the docket ("EPA-HQ-ORD-2021-0561-0019") and provided to the peer reviewers. This document describes the consideration of the studies deemed relevant based on the methods laid out in the protocol and documents the justification for the subset of those incorporated into the revised assessment.

Comment: EPA should expand the discussion in Section 1.2.4 (or an additional section) on the use of low confidence studies to support mechanistic evidence when the mechanistic evidence is used across health effects.

EPA Response: Additional text was added to Section 1.2.4 on the use of low confidence studies to support coherence of mechanistic findings.

Comment: In Table 3-28, EPA should include the results of two high confidence studies that did not report significant changes to histopathology as the inclusion of only the one study with significant effects is being highlighted, paints an incomplete picture.

EPA Response: The thyroid histopathology data from NTP (2018) and Klaunig et al. (2015) were added to the relevant table in Section 3.2.5.

Comment: To clarify how decisions were made for each health endpoint, EPA should add a brief section on the considerations used in evaluating study quality and summarize the basis for assignments. Inclusion of this information solely within the HAWC template does not enable the reader to readily identify the basis for judgments about individual studies or the rationale behind the assignments.

EPA Response: Additional text was added to Section 1.2.2 that describes the study evaluation for the epidemiology and animal toxicology studies. Readers are referred to the Protocol for a detailed description of the study evaluation approach for both human epidemiology and animal toxicology studies (Appendix A, Sections 6.2 and 6.3, respectively).

Comment: EPA should enumerate the adaptations made to the structured evaluation considerations first introduced by Hill (1965).

EPA Response: Additional text was added to Section 1.2.4 that describes the specific modified Hill considerations that are applied for IRIS Assessments. Readers are referred to the Protocol (Appendix A, Section 9) for detailed descriptions of the considerations and the application during evidence synthesis and integration.

Tier 2 Suggestions

Comment: EPA should summarize key points for other EPA PFAS reviews so a user of the IRIS materials could see similarities and differences in this family of related chemicals. The reviewer noted that users of the IRIS documents will usually be addressing mixtures of these

compounds in the field, therefore, a common summary in one place would help the user community coordinate the information.

EPA Response: Table 4-1 has been added to the assessment (see Section 4.1) to facilitate comparisons of toxicity hazard conclusions across EPA PFAS assessments.

Comment: In the systematic review protocol in Appendix A (Table 5-2), EPA should clarify why dam health (e.g., weight gain, food consumption) was only considered in "Developmental" and not in "Reproductive" or tied to the specific effect on dam health observed (e.g., weight gain as an endpoint).

EPA Response: Although effects on dam body weight were not specified as endpoint grouping categories for animal toxicology studies in the PFAS protocol (Appendix A, Table 5-2), these data were synthesized in the female reproductive health effects section (Section 3.2.7) as well as considered when interpreting the developmental health effects (Section 3.2.2) in the toxicological review. As stated on pg 5–3, lines 7–11, the endpoint groupings outlined in Tables 5-1 and 5-2: "are meant to serve as a starting place for consistency in presentation and analysis across studies and assessments, although assessment-specific deviations are possible (e.g., depending on the assessment-specific database of endpoints in the available studies or PFAS-specific understanding of mechanistic relationships across outcomes)."

Comment: EPA should consider including a list of documents relevant to PFHxA risk characterization that have been developed by state and international regulatory agencies in the literature searches and in resulting databases.

EPA Response: State and international regulatory agency documents related to PFAS are included in EPA literature searches and managed in HERO. EPA does not generally include a description of non-EPA judgements in EPA assessments but does use these documents as a resource for the identification of key science issues and potentially relevant studies that may have been missed by a database search at early stages of draft assessment development.

Comment: EPA should consider incorporating recently published studies in the Toxicological Review.

EPA Response: Additional studies were considered for incorporation into the toxicological review for PFHxA. Of the studies that were considered, a subset was prioritized for inclusion depending on whether they were expected to inform critical data gaps. Additional details regarding the studies that were prioritized for inclusion can be found in the docket (see EPA-HQ-ORD-2021-0561-0019).

Comment: For increased transparency and ease of reference, EPA should consider adding the HAWC animal toxicity study evaluation figure to the main document in addition to including it in the HAWC.

EPA Response: A copy of animal study evaluation heat map in HAWC has been added to Section 2.2 of the toxicological review.

Comment: For hepatic effects (Table 3-11), EPA should consider several revisions: 1) Consider additional tables and/or figures to help readers visualize the coherence of liver histopathology with liver weight effects since these results are only presented in separate tables in the document; 2) reconsider whether to include decreases in bilirubin amongst the serum biomarkers of hepatic injury cited in Table 3-11 based on the Loveless et al. (2009) and Hall et al. (2012) studies; and 3) in characterizing the strength of this evidence, reconfirm that the significant variability of responses across studies and sexes was considered and weighed, as well as the magnitude (frequently modest) and direction of change in the cases where there was a change in one of the serum enzyme biomarkers (in many cases there were decreases).

EPA Response: The hepatic evidence is discussed considering the Hall et al. (2012) criteria in Section 3.2.1 under the subheading "Considerations for Potentially Adaptive Versus Adverse Responses". The criteria for considering the adversity of hepatic effects according to Hall is listed and a summary of the hepatic findings are included below the Hall criteria where decreases in bilirubin, globulin, and total protein are also summarized. The serum enzyme findings were clarified to indicate the magnitude of change and a statement summarizing the different clearance rates of PFHxA in rats (faster clearance in females than males) may underlie sex-specific differences.

Comment: For developmental effects, EPA should consider revisions to further characterize the mouse dose-response for decreases in postnatal body weight.

EPA Response: Additional text was added to Section 3.2.2 clarify that the data from the mouse study by Iwai and Hoberman (2014) represent two separate experimental cohorts with overlapping dose ranges. Although, in general, similar effects are observed across the two cohorts there is some variability in the dose response pattern which, as now discussed in the assessment, could be explained by normal variation across the control body weights in two experiments or a survivor bias at the higher doses (e.g., higher mortality among low body weight pups in the higher doses).

Comment: For hematopoietic effects, EPA should consider revisions to: 1) add a table and/or figure to help readers visualize the coherence of these effects since these results are presented in separate figures and tables in the document; and 2) add information on the results of several chronic studies which are an important exception to the cited "consistent treatment related effect on platelet levels."

EPA Response: Findings from the chronic study are made available in the draft on hematological effects for all hematological findings that are described throughout **Section 3.2.4**. The reviewer may have been referring to time points beyond 52 weeks of age that were not considered based on as quantitative measures of hematology measures beyond 52 weeks may be complicated by natural diseases occurring in rodents and test variability leading to decreased sensitivity and increasing variability with the results (AACC, 1992). The collection of blood findings are summarized in a visualization available in HAWC (linked here:

https://hawc.epa.gov/summary/data-pivot/assessment/100500070/pfhxa-animal-toxicology-hematology-effects-eryth/) as well as in the evidence integration section to help readers understand the coherence of effects.

E.1.2. Public Comments on Systematic Review and Documentation

Comment: Several public commenters noted a lack of clarity regarding the literature search and screening results, including inconsistencies in the screening results shown in HERO, HAWC, and within the Toxicological Review, lack of clarity on how potentially relevant supplemental information and newly identified studies would be incorporated in the Toxicological Review. Some public commenters provided specific references or additional data that were not included in the public comment draft of the PFHxA Toxicological Review.

EPA Response: EPA has taken several steps to clarify the literature search and screening results for PFHxA that are now resolved and are available for viewing in HAWC and are available using the following link:
https://hawc.epa.gov/lit/assessment/100500070/references/visualization/

E.2. CHARGE QUESTION 3: NONCANCER HAZARD IDENTIFICATION

3) For each health effect considered in the assessment and outlined below, please comment on whether the available data have been clearly and appropriately synthesized to describe the strengths and limitations. For each, please also comment on whether the weight-of-evidence decisions for hazard identification are scientifically justified and clearly described.

   a. For hepatic effects, the Toxicological Review concludes the available evidence indicates PFHxA likely causes hepatic effects in humans under relevant exposure circumstances. This conclusion is based on studies of rats showing increased liver weight, hepatocellular hypertrophy, increased serum enzymes, and decreased serum globulins. The hepatic findings for PFHxA were similar for other PFAS and determined to be adverse and relevant to humans.

      i) Additional considerations influenced the hepatic 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 PFHxA 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 hepatic effects. The Toxicological Review ultimately concludes evidence from in vivo (including genetic mouse models) and in vitro studies support a potential role for multiple pathways operant in the induction of hepatic effects from PFHxA exposure, but those pathways cannot be specifically determined. Please comment on whether the conclusions regarding the available animal and mechanistic studies are scientifically justified and clearly described. The hepatic findings for PFHxA were similar for other PFAS and determined to be adverse and relevant to humans.

b. For developmental effects, the Toxicological Review concludes the available evidence indicates PFHxA likely causes developmental effects in humans under relevant exposure circumstances. This judgment is based primarily on gestational exposure experiments in mice, with supportive findings in rats exposed throughout gestation and lactation, showing increased perinatal mortality, decreased offspring body weight, and delayed eye opening. These effects are similar to those observed for other PFAS following developmental exposure and were determined to be adverse and relevant to humans.

c. For hematopoietic effects, the Toxicological Review concludes the available evidence indicates PFHxA likely causes hematopoietic effects in humans under relevant exposure circumstances. This judgment is based on consistent findings, including decreased red blood cells [RBCs], hematocrit, and hemoglobin, across study designs that, when interpreted together, signifies PFHxA-related hematological effects such as anemia. These findings were determined to be adverse and relevant to humans.

d. For endocrine effects, the Toxicological Review concludes the available evidence suggests, but is not sufficient to infer, that PFHxA may cause endocrine effects in humans under relevant exposure circumstances. This conclusion is based on some evidence of thyroid effects based on hormone and histopathological changes in two rat studies; however, the data is limited, lacking consistency across studies, and histopathological changes may be explained by non-thyroid related effects

e. For all other potential health effects (i.e., renal, male and female reproductive, immune, and nervous system), the Toxicological Review concluded the available evidence is inadequate to assess whether PFHxA may cause effects in humans under relevant exposure circumstances. In general, these conclusions were driven by sparse evidence bases or data that were largely null.

E.2.1. External Peer Review Comments on Hepatic Effects

All seven reviewers were in agreement with the draft Toxicological Review that the data has been "clearly and appropriately synthesized in order to describe the strengths and limitations of the data" and that the weight-of evidence decisions used for hazard identification were "scientifically justified and clearly described" for the hepatic effects from PFHxA. One reviewer also noted, "importantly, recommendations of the Hall et al. (2012) paper were considered by the EPA in assessing the adversity of observed hepatic effects," while another reviewer applauded inclusion of this discussion and outcome stating that it was, "a compelling narrative, which compares point by point the PFHxA responses against this guide concludes that these responses are adverse, human relevant and of concern for such biological effects of necrosis". One reviewer noted that, "the IRIS draft report included a section that discussed "evidence from other PFAS" …was especially important for interpreting the PFHxA results and by structural analogy that PFHxA would also work via both PPAR alpha and non PPAR alpha response pathways. These comparisons showed that the involvement of other non PPAR alpha receptors in the response to PFAS and by structural relationship relevance for PFHxA." Several Tier 2 Suggestions are described below.

Tier 1 Recommendations

Reviewers had no Tier 1 recommendations.

Tier 2 Suggestions

Comment: To improve clarity, EPA should revise the text (page 2–3) stating, "All outcomes rated low confidence or higher were used for evidence synthesis and integration." The reviewer commented that it may be unclear how this statement can be consistent with the statement on page 1-12 that "no low confidence studies were used in the evidence syntheses for PFHxA included in the narrative," since low confidence studies may presumably have outcomes that would also be rated as low confidence, which might be assumed to be included in evidence synthesis and integration based on the first sentence cited.

EPA Response: The text in Section 1.2.4 has been edited. It now correctly states that "all studies meeting PECO criteria were used for evidence synthesis and included in the narrative."

Comment: EPA should consider utilizing information on other PFAS compounds (e.g., PFBA) to supplement and bolster the evidence consistent with the adversity of PFHxA-induced hepatic effects.

EPA Response: In addition to the text in the Hepatic outcome Section (Section 3.2.1), the assessment now includes an additional section in Section 4 including narrative description and table summary (Table 4-1) comparing the hazard conclusions across published EPA PFAS Assessments.

Comment: A reviewer noted an inconsistency in discussions of necrosis in rats and suggested that EPA revise the wording to be consistent.

EPA Response: The text has been edited to correct the inconsistency. The synthesis in Section 3.2.1 now correctly states that necrosis was observed in females but not males.

Comment: In the "Evidence from other PFAS" section, EPA should consider emphasizing that the observations of PPARα independent and dependent pathways from the four other PFAS are consistent for both short-chain (e.g., PFBA) and long-chain (e.g., PFNA) substances, increasing the plausibility that it also applies to PFHxA.

EPA Response: Although there is no evidence specifically challenging the role of PPARα in PFHxA-mediated hepatotoxicity, based on PFHxA structural similarity with other PFAS, most notably PFBA, it is reasonable to infer that PFHxA exposure in genetic mouse model systems would elicit similar effects as structurally similar PFAS. Therefore, text was added to Section 3.2.1 specifically stating evidence from structurally similar PFAS, including PFBA, suggest PPARα independent and dependent pathways also apply to PFHxA (Evidence from other PFAS subheading).

Comment: A reviewer commented that while the interpretation of both epidemiologic studies is reasonable, it is not clear why the potential for confounding is considered to be so substantial without some indication of the rationale for expecting that serum PFHxA levels are

associated with the confounding factors. EPA should consider including stronger reasoning as to why such confounding would be expected. This comment applies to all health effect sections.

EPA Response: Text was added to human studies sections to further explain the concerns for confounding. While not explicitly described in each section, studies were rated as "critically deficient" for confounding and "uninformative" overall when there was no consideration (e.g., adjustment, exclusion, stratification) for potential confounders in heterogeneous populations. There is particular concern with PFAS that lack of adjustment for age and sex would lead to substantial bias given that these variables are associated with both PFAS exposure and most of the outcomes of interest.

E.2.2. Public Comments on Hepatic Effects

Comment: Public comments on EPA's conclusions made regarding hepatic effects in the toxicological review were mixed. One commenter agreed with the overall conclusion and supported EPA's position that the hepatic effects are adverse and relevant to humans. In contrast, two public commenters expressed concerns about the human relevance of mechanistic support for hepatic effects via PPARα mediated pathways and the adversity of the observed hepatic effects in animal toxicity studies. Based on these concerns the commenters felt that the hepatic effects should be considered to be inadequate to assess whether PFHxA may cause hepatic effects in humans.

EPA Response: The conclusions in the draft Toxicological Review regarding hepatic effects were supported by the external peer review committee who provided tier 2 recommendations that were addressed in the assessment. The text includes evidence from other PFAS (short and long chain) in models challenging the role of PPARα in PFHxA-mediated hepatotoxicity indicating roles for PPARα dependent and independent pathways. The evidence is considered relevant to PFHxA considering the PFAS evaluated are of similar carbon chain length and structure. The conclusions were also supported by supplemental mechanistic evidence indicating the human PPARα binds and is activated by PFHxA at similar or lower concentrations than rodent PPARα. Further, evaluation of the available evidence was considered in the context of the Hall et al. (2012) criteria. While PFHxA exposure does not clearly lead to cancer there is evidence for hepatic toxicity rather than adaptation in rodents. The overall evidence is considered to be adverse and relevant to humans.

E.2.3. External Peer Review Comments on Developmental Effects

All seven reviewers agreed with the assessment conclusions for developmental effects. One reviewer stated that, "The integration of available animal data, based on two high quality animal studies (with three experiments) and on plausibility for human relevance, supports the finding that PFHxA likely causes developmental effects in humans." Another reviewer stated that "The Agency's logic was clear and transparent, and their conclusions scientifically justified." Several Tier 2 Suggestions are described below.

Tier 1 Recommendations

Reviewers had no Tier 1 recommendations.

Tier 2 Suggestions

Comment: EPA should consider improving the discussion of human relevance such as by adding information on the conserved biological processes or similarities in anatomy and physiology between rodents and humans that EPA considers relevant to the observed developmental effects, or whether rodents (particularly the mouse) have been shown to be good laboratory animal models for assessing potential human developmental effects.

EPA Response: The text has been edited in Section 3.2.2. "These findings are interpreted as relevant to humans in the absence of evidence to the contrary. This assumption is based on Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991)." The assumption in the EPA Guidelines is based on data for known developmental toxicants which have shown that animal models are largely predictive of effects in humans.

E.2.4. Public Comments on Developmental Effects

Comment: Public comments on EPA's conclusions made regarding developmental effects in the draft toxicological review were mixed. One commenter agreed with the overall conclusion that PFHxA is likely to cause developmental effects in humans. In contrast, two public commenters expressed concerns about strength of the evidence base to support the conclusion, specifically the small evidence base (two animal toxicology studies) to inform developmental effects of PFHxA. Concern about the adversity of decreased offspring body weight and these effects may be secondary to maternal toxicity rather than a direct effect on development. Based on these concerns these commenters felt that EPA should reconsider the conclusions for this health effect.

EPA Response: The conclusions in the draft Toxicological Review regarding developmental effects were supported by the external peer review committee and retained in the revised assessment. The evidence integration narrative in 3.2.2 discusses the potential impacts of maternal toxicity on the interpretation of the animal evidence based and the rationale for why maternal toxicity was not expected to be a primary driver of the observed developmental effects.

E.2.5. External Peer Review Comments on Hematopoietic Effects

Six of seven reviewers supported the overall conclusions of the hematopoietic effects section, while one reviewer recommended clarifying, and possibly strengthening, the animal evidence synthesis judgment (see Tier 1 Recommendation below). One reviewer commented that "the weight-of evidence decisions used for hazard identification were scientifically justified and clearly described and that when the rat studies are examined as a collective of study results, they provide compelling evidence for PFHxA causing macrocytic anemia (low hemoglobin and large RBC) and could be expected to cause serious harm in humans". While one reviewer stated that "the findings are consistent with similar effects for multiple other PFAS and are reasonably determined

to be adverse and relevant to humans" a separate reviewer suggested "EPA should consider adding additional information supporting the human relevance of hematopoietic effects observed in rats." (see Tier 2 Suggestion below).

Tier 1 Recommendations

Comment: EPA should clarify why the animal evidence is "moderate" rather than "robust" given that all four animal studies were assessed high confidence and there was agreement across study findings and doses. The reviewer noted that this clarification would provide context for what drives the "moderate" decision, and it will help to align with the conclusion that "the currently available evidence indicates that PFHxA likely causes hematopoietic effects in humans."

EPA Response: Based on external peer review input and further review of the evidence, it was determined that there is *robust* animal evidence for hematopoietic effects and the judgment was changed in the assessment. This did not change the overall evidence integration judgement based on based on identification of only one uninformative human study and uncertainty around the human relevance of the rodent findings. Specifically, rodent hematological parameters differ from humas by smaller erythrocytes, higher percentage of circulating reticulocytes (or polychromasia), physiologic splenic hematopoiesis and iron storage, and more numerous and shorter-lived erythrocytes and platelets (O'Connell et al., 2015). These differences could explain the possible regenerative response in the spleen and bone and the increase in reticulocytes (i.e., erythrogenesis and RBC turnover more rapid in rodent vs. human). Therefore, the currently available ***evidence indicates*** that PFHxA likely causes hematopoietic effects in humans given sufficient exposure conditions.

Tier 2 Suggestions

Comment: EPA should consider improving the discussion of human relevance such as by adding information on the conserved biological processes between rats and humans that EPA considers relevant to the observed hematopoietic effects, or whether rodents (particularly the mouse) have been shown to be good laboratory animal models for assessing potential human hematopoietic effects.

EPA Response: A discussion of the human relevance of hematopoietic effects (Section 3.1.4) in rodents was added to the integration narrative. This additional discussion included background information on the rodent model strain and origin (all animal models were obtained from the same outbred population and supplier). Additional text also included a comparison between murine and human hematological parameters. Specifically, rodent hematological parameters differ from humas by smaller erythrocytes, higher percentage of circulating reticulocytes (or polychromasia), physiologic splenic hematopoiesis and iron storage, and more numerous and shorter-lived erythrocytes and platelets (O'Connell et al., 2015). These differences could explain the possible regenerative response in the spleen and bone and the increase in reticulocytes (i.e., erythrogenesis and RBC turnover more rapid in rodent vs. human).

E.2.6. Public Comments on Hematopoietic Effects

Comment: There were mixed responses to the conclusions made regarding hematopoietic effects in the toxicological review. One commenter agreed with the overall conclusion and recommended additional text be drafted discussing similarity of effects observed across related PFAS. In contrast, two public commenters expressed concerns about strength of the evidence base to support the conclusion, specifically citing the lack of mechanistic and informative human data and questioning the adversity and biological significance of findings in animals. Based on these concerns these commenters felt that EPA should reconsider the conclusions for this health effect.

EPA Response: EPA added additional discussion on the human relevance of the hematologic effects (Section 3.2.4) observed in rodents and determined that, while there is *robust* evidence available from the animal data, there is indeterminate human evidence and some residual uncertainty around the human relevance of the observed effects; therefore, the ***evidence indicates*** PFHxA likely causes hematopoietic effects, a judgement that was supported by external peer review. Please see the responses above to peer reviewer comments.

E.2.7. External Peer Review Comments on Endocrine Effects

There were mixed responses from the committee on the conclusions made regarding endocrine effects in the toxicological review. Three reviewers agreed with the conclusion that "the currently available evidence suggests, but is not sufficient to infer, that PFHxA might cause endocrine effects in humans under relevant exposure circumstances." One reviewer stated, "Overall, the critical available data on endocrine effects are clearly and appropriately synthesized to describe the strengths and limitations. In this reviewer's opinion, the weight-of-evidence decision for endocrine effects is scientifically justified." In contrast, three reviewers recommended EPA reconsider the conclusion on endocrine effects and their specifics comments are outlined in the Tier 1 Recommendations below. One reviewer did not comment on the overall conclusions or provide other specific recommendations or suggestions in response to this charge question.

Tier 1 Recommendations

Comment: Two reviewers recommended that EPA strengthen the evidence integration judgment and conclude that the available ***evidence indicates*** that PFHxA exposure is likely to cause thyroid toxicity in humans given relevant exposure circumstances, primarily based on short-term studies in rats reporting a consistent and coherent pattern of effects on thyroid hormones following PFHxA exposure, but also drawing from the consistency of effects when considering evidence from structurally related PFAS. A third reviewer recommended EPA re-examine the part of the statement that says, "but is not sufficient to infer" that PFHxA could cause endocrine effects in humans.

EPA Response: Based on the Tier 1 recommendation from the external peer review committee to reconsider the endocrine effects evidence in light of information on thyroid hormone biology provided by the committee and findings for related PFAS, the overall evidence integration judgement for endocrine effects was changed from ***evidence suggests but is not sufficient to infer***

to ***evidence indicates (likely)***. The evidence synthesis and integration text in Section 3.2.5 has also been updated to reflect newly identified mechanistic evidence. Consistent with the recommendations from the external peer review committee, decreased serum total T4 from the 28-day rat study by NTP (2018) was advanced for dose response analysis.

Comment: One reviewer recommended EPA delete or provide better justification for the statement, "some of these inconsistencies could be explained by differences in the test article (i.e., PFHxA vs. PFHxA salts)" since both the acids and salts will dissociate at biologically relevant pH to form the identical anion.

EPA Response: This statement was deleted from the text.

Tier 2 Suggestions

Reviewers had no Tier 2 suggestions.

E.2.8. Public Comments on Endocrine Effects

Comment: One commenter disagreed with the conclusion on Endocrine effects, suggesting that the integration judgement should be changed to evidence is inadequate, citing a weak evidence base (i.e., *inadequate* human evidence and *slight* animal evidence).

EPA Response: For the reasons described in the EPA response to the tier 1 recommendation from external peer reviewers above, EPA strengthened the overall evidence integration judgment from evidence suggests to evidence indicates based on concluding that the animal evidence is moderate rather than slight.

E.2.9. External Peer Review Comments on All Other Potential Health Effects

All seven reviewers agreed with the conclusions of the draft Toxicological review for all other potential health effects in the assessment. One reviewer stated they "agreed that the data has been "clearly and appropriately synthesized in order to describe the strengths and limitations of the data" and would in general agree with the comment that these endpoints did not have adequate data to determine impact or not." Another reviewer commented that, "The Agency clearly characterized both strength and weaknesses of these studies and the conclusion that there is inadequate information to assess whether PFHxA affects these physiological domains is scientifically justified." Their Tier 1 Recommendations and Tier 2 Suggestions are provided below.

Tier 1 Recommendations

Comment: EPA should improve transparency by including observations across other PFAS compounds for the broad list of potential endpoints in this section, either by each endpoint listed in charge question 3(e) or by providing an overall summary table of input from evaluation of other PFOS compounds for these endpoints.

EPA Response: Table 4-1 has been added to the assessment (see Section 4.1) to facilitate comparisons of toxicity hazard conclusions across EPA PFAS assessments.

Comment: For renal effects, EPA should note reverse causality as a concern in the Seo et al. (2018) study and provide a clearer justification for considering Zhang et al. (2019)as "uninformative."

EPA Response: The evidence synthesis text was edited in Section 3.2.3 to clarify the rationale underlying the study evaluation judgments for these studies. The potential for reverse causality was added as a factor that decreases certainty to the evidence integration table.

Tier 2 Suggestions

Comment: EPA should consider re-examining the respiratory effects observed in the 28-day NTP (2018) study and the 90-day Loveless et al. (2009) study for potential incorporation in the Toxicological Review.

EPA Response: The nasal lesions described in the 28-day NTP (2018) study and the 90-day Loveless et al. (2009) study were presumed to be driven by irritation stemming from inadvertent aspiration of the gavage dose. On this basis, the results were considered by EPA to have unclear toxicological relevance and not prioritized for synthesis and integration, however the results are summarized in the animal literature inventory. This rationale is described and a link to the animal literature inventory is provided in Section 3.2 of the Toxicological Review.

Comment: For renal effects, EPA should consider several revisions to Table 3-19: 1) Consider noting the potential for reverse causality as a factor that decreases certainty for the association of PFHxA with decrease in estimated eGFR; 2) consider adding "weak, no, or inconsistent dose-response" as a factor that decreases certainty for organ weight; 3) as a factor that decreases certainty, consider adding that "blood biomarkers of renal function were inconsistent"; and 4) as another factor that decreases certainty, consider adding difficulty in interpreting the observed effects as adverse or non-adverse.

EPA Response: Edits were made to Table 3-19 (Renal profile table for PFHxA) to reflect these suggestions.

Comment: For immune effects, EPA could improve clarity by moving asthma to its own Pulmonary Effects section, since the one human asthma study examined was mostly of non-immune mediated outcomes.

EPA Response: Asthma can be driven by both immune and respiratory effects. Since there is no respiratory effects section in the PFHxA Toxicological Review and the study included evaluation of immune related markers of asthma, the data from this study are retained in the immune effects section.

Comment: In Table 3-37 in the nervous system effects section, EPA should indicate the "preferred metric" for brain weight is absolute brain weight to be consistent with Table 3-31.

EPA Response: This text has been added to Table 3-37 in the nervous system effects section.

Comment: For nervous system effects, zebrafish studies are common for PFAS and should be considered as useful supplemental data to inform evaluations. The reviewer also commented

that this section could benefit from discussion of known impacts of other PFAS that might inform design of future studies

EPA Response: Mechanistic information from newly identified studies, including two early life stage zebrafish studies, have been added to the nervous system effects synthesis and integration in section 3.2.9. While these data did not change the conclusions of the assessment, they were included on the basis that they help to inform critical data gaps for nervous system effects.

E.2.10. Public Comments on All Other Potential Health Effects

Comment: One commenter disagreed with the conclusion that the evidence is inadequate for renal effects. They suggested that histopathological changes observed in the kidney (i.e., papillary necrosis and tubular degeneration) in the chronic rat study by Klaunig (2015) are adverse and should be used as the basis for derivation of the RfD. One commenter agreed with the conclusions for immune and nervous system effects.

EPA Response: The conclusions in the draft Toxicological Review regarding other effects were supported by the external peer review committee. The EPR also supported the conclusions in the draft regarding the renal effects, although there were Tier 1 and 2 comments that were addressed above. The EPA is aware of the report prepared by Luz et al. (2019), and the author conclusion for an RfD based on papillary necrosis in female rats exposed to 200 mg/kg-d PFHxA from the chronic study (Klaunig, 2015, 2850075). While the histopathological renal effects observed by Klaunig et al. (2015) in females were the most significant effect, there were inconsistencies across studies at similar observations times and doses and lack of coherence with other renal findings. Therefore, the judgment of *slight* animal evidence was retained in the revised assessment. The decision in the draft assessment that overall the renal evidence is ***inadequate*** is similarly retained and renal endpoints were not advanced for RfD derivation.

E.3. CHARGE QUESTIONS 4 AND 5: NONCANCER TOXICITY VALUES DATA SELECTION

4) For PFHxA, no RfC was derived. The study chosen for use in deriving the RfD is the Loveless et al. (2009) one-generation reproductive toxicity study based on decreased offspring body weight in rats exposed continuously throughout gestation and lactation to PFHxA sodium salt via the dam. Is the selection of this study and these effects for use in deriving the RfD for PFHxA scientifically justified and clearly described?

   a. If yes, please provide an explanation.

   b. If no, please provide an alternative study(ies) or effect(s) that should be used to support the derivation of the RfD and detail the rationale for use of such an alternative.

   c. As part of the responses in "a" or "b" above, please comment on whether the effects selected are appropriate for use in deriving the RfD, including considerations regarding

adversity (or appropriateness in representing an adverse change) and the scientific support for their selection.

d. Given the lack of studies on inhalation exposure to PFHxA, no reference concentration (RfC) is derived. Please comment on this decision.

5) In addition, for PFHxA, an RfD for less-than-lifetime ("subchronic") exposures is derived. No "subchronic" RfC was derived. The same study and outcome were chosen for use in deriving the RfD. Is the selection of this study and these effects for the derivation of the subchronic RfD for PFHxA scientifically justified and clearly described?

a. If yes, please provide an explanation.

b. If no, please provide an alternative study(ies) and/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 responses in "a" or "b" above, please comment on whether the effects selected are appropriate for use in deriving the RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for their selection.

d. Given the lack of studies on inhalation exposure to PFHxA, no "subchronic" RfC is derived. Please comment on this decision.

E.3.1. External Peer Review Comments on Noncancer Toxicity Values Data Selection

For charge question 4, three reviewers concurred with the selection of the Loveless et al. (2009) study and the effect of decreased offspring body weight as scientifically justified for derivation of an RfD for PFHxA. Two reviewers recommended the NTP (2018) study with serum T4 as an endpoint be used as an alternative." These comments are described below. Two reviewers noted that the topic is "outside of their expertise" with one stating that "the reasoning presented for RfD derivation appeared sound" while the other declined to comment. "All reviewers who provided comments agreed with the decision to not derive a reference concentration."

For charge question 5, "reviewers' comments on the charge questions related to the derivation of the subchronic RfD were similar to those made for the chronic RfD." Four reviewers "concurred with the selection of the Loveless et al. (2009) study and the selected effect as scientifically justified for derivation of the subchronic RfD for PFHxA. As with the chronic RfD, one reviewer "suggested using the NTP (2018) study with the endpoint of T4 suppression, although they did not include this comment as a tiered recommendation." Two reviewers noted that the topic is "outside of their expertise" with one stating that "the reasoning presented for RfD derivation appeared sound" while the other declined to comment. "All reviewers who provided comments agreed with the decision to not derive a subchronic reference concentration."

Tier 1 Recommendations

Comment: Two reviewers commented that the EPA should calculate an osRfD using the T4 endpoint from the NTP (2018). Of these, one reviewer recommended EPA "…also calculate this value using the T4 endpoint from the NTP, 2018 study and to determine if this has significant impact on the calculation of the RfD." This reviewer recommended that, if it does "have a significant impact, then EPA should prioritize the use of the T4 endpoint" for the RfD. The second reviewer recommended EPA use serum T4 as an endpoint should be used as an alternative to support the derivation of an RfD.

EPA Response: Decreases in free and total T4 observed in male rats in the 28 day study by NTP (2018). Total T4 was advanced for derivation of a POD for endocrine effects over free T4 because of concerns about the measurement method variability of the assay used to measure free T4 (see Section 5.2.1). The POD for total T4 was higher than that selected for the developmental osRfD (see Table 5-5). This endpoint was not considered for derivation of a lifetime toxicity value due to the high level of uncertainty associated with use of a short-term study to protect against the effects of a chronic, lifetime exposure. Therefore, total T4 was prioritized for subchronic candidate value derivation.

Calculation of a candidate subchronic toxicity value for total T4 did not affect the overall subchronic RfD selection for the Toxicological Review. As described in Section 5.2.1 (Selection of Subchronic RfD and Confidence Statement), "a ***subchronic RfD of 5 × 10−4 mg/kg-day based on decreased postnatal body weight*** is selected for less-than-lifetime exposure. The confidence in the selected subchronic RfD is equivalent to that of the hepatic subchronic RfDs but lower than the hematopoietic subchronic RfD. The developmental subchronic RfD is expected to be protective of all life stages. The UF_C (see Table 5-13) is lower than or equivalent to the other subchronic osRfDs and the endpoint has the lowest POD_{HED} (0.048 mg/kg-day, see Table 5-11). The decision to select the developmental subchronic RfD was based on all of the available subchronic osRfDs in addition to overall confidence and composite uncertainty for those subchronic osRfDs."

Tier 2 Suggestions

Comment: EPA should consider adding text to the organ-specific narrative for hepatic effects and for developmental impacts, regarding adversity versus adaptation that may be relevant for the study selection justification and health impacts to the human population. The reviewer noted that these studies were either medium or high confidence studies with good annotation and discussion of observations, and the quantitative estimates resulting from these calculations indicate that these are sensitive hence protective endpoints for use in the RfD development. The reviewer also noted that these endpoint choices for the RfD are highly relevant for human populations.

EPA Response: Additional text was added to Sections 3.2.1, 3.2.2, 4.1 and 5.2.1 to clarify how the observations for hepatic and developmental effects are expected to be adverse, potentially

relevant to humans, and coherent across different layers of biology (i.e., from chemical -molecular interaction) to organ level effects (e.g., increased liver weight and necrosis for hepatic effects).

E.3.2. Public Comments on Noncancer Toxicity Values Data Selection

Comment: One commenter agreed with the endpoints selected for POD derivation and a second agreed with the decision to use high confidence animal studies.

EPA Response: The external peer review panel supported the selection of the endpoints for POD derivation in the draft, although a tier 1 recommendation was made to add decreased T4 for endocrine effects. The assessment has been updated to reflect the panel recommendations.

Comment: Two commenters disagreed with the selection of decreased postnatal body weight for the RfD on the basis that these effects could be driven by maternal toxicity rather than a direct developmental effect and the outcome is non-specific. One also suggested that the RfD should be based on renal effects (papillary necrosis and tubular degradation) from the Klaunig (2015) study.

EPA Response: EPA considered the available evidence base, key science questions, and extensive peer review to develop and justify conclusions that are based on the PFAS protocol and the IRIS Handbook that was favorably reviewed by the National Science Academy. Additional justification and documentation for the rationale underlying this decision is provided in Section 5.

E.4. CHARGE QUESTIONS 6, 7, AND 8: NONCANCER TOXICITY VALUE DERIVATION

6) EPA used benchmark dose modeling (U.S. EPA, 2012, 1239433) to identify points-of-departure (PODs) for oral exposure to PFHxA. Are the modeling approaches used, selection and justification of benchmark response levels, and the selected models used to identify each POD for toxicity value derivation scientifically justified and clearly described?

7) Appendix A identifies the potential for pharmacokinetic differences across species and sexes as a key science issue and lays out a hierarchy for using relevant pharmacokinetic data in extrapolating oral doses between laboratory animals and humans. Section 5.2.1 describes the various approaches considered and the rationale for the selected approach. Given what is known and not known about the potential interspecies differences in PFHxA pharmacokinetics, EPA used the ratio of human-to-animal serum clearance values assuming the volume of distribution (V_d) in humans is equivalent to that in monkeys to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs.

   a. Is applying the ratio of human-to-animal serum clearance values for PFHxA scientifically justified and clearly described? If not, please provide an explanation and detail the preferred alternative approach.

   b. Does the Toxicological Review clearly describe the uncertainties in evaluating the pharmacokinetic differences between the experimental animal data and humans?

8) EPA has evaluated and applied uncertainty factors to account for intraspecies variability (UF_H), interspecies differences (UF_A), database limitations (UF_D), exposure duration (UF_S), and LOAEL-to-NOAEL extrapolation (UF_L) for PFHxA.

   a. Is uncertainty in the derivation of the toxicity values scientifically justified and clearly described? Please describe and provide comments, if needed.

   b. For uncertainty in interspecies differences (UF_A), a value of 3 is applied to account for remaining uncertainty in characterizing the pharmacokinetic and pharmacodynamic differences between laboratory animals and humans after calculation of the HED. For developmental and hematopoietic outcomes, the evidence base lacked chemical-and species-specific information that would have been useful for informing the UFA; for hepatic outcomes, however, available mechanistic and supplemental information was useful for further evaluating the interspecies uncertainty factor. Some data indicate a PPARα-dependent pathway that might support a UF_A of 1. Evidence for non-PPARα modes of action, however, is available in the PFHxA (and larger PFAS) database. Thus, uncertainty remains regarding the potential differences in sensitivity across species due to the involvement of both PPARα-dependent and-independent pathways. Further, data are lacking to determine with confidence the relative contribution of each of these pathways. 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 hepatic effects and that a value of $UF_A = 3$ is warranted to account for the residual uncertainty in pharmacodynamic 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 scientifically justified and clearly described.

   c. To inform uncertainty in intraspecies variability (UF_H), the assessment evaluates and considers the available evidence on potential susceptibility to PFHxA within different populations or lifestages, including any potential human health impacts from early life exposure. Are the available information and data appropriately considered and the resultant UF_H values scientifically justified and clearly described?

   d. Are the provided rationales for the remaining uncertainty factors (UF_L, UF_D, UF_S) scientifically justified and clearly described? If not, please explain.

E.4.1. External Peer Review Comments on Noncancer Toxicity Value Derivation

For charge question 6, as summarized in the contractor report, "all reviewers who provided responses to this charge question concurred that the approaches used, and the identification of PODs were scientifically justified and clearly described. Faustman was impressed with the details provided to identify the PODs for exposure to PFHxA and found the tables very easy to use." Two reviewers "declined to comment, stating that this topic was outside of their area of expertise."

For charge question 7, "reviewers who provided responses to this charge question generally concurred that the approach used for potential interspecies differences in PFHxA pharmacokinetics was scientifically justified and clearly described. The same reviewers stated that the Toxicological Review clearly described the uncertainties. Several reviewers provided recommendations for

improving the clarity." These are described below. Two reviewers "declined to comment, stating that this topic was outside of their area of expertise."

For charge question 8, "reviewers had mixed responses when commenting on the UF_A of 3. All reviewers who responded to Charge Question 8c concurred with a UF_H of 10." Two reviewers "declined to comment, stating that this topic was outside of their area of expertise." Four reviewers "provided several comments related to the remaining uncertainty factors." These comments are described below.

Tier 1 Recommendations

Comment: If models that do not provide adequate fit are included in the tables summarizing benchmark dose modeling results for different endpoints (in Appendix B), they should be marked/identified as such in these tables (e.g., by placing the model names and associated estimates in parentheses)

EPA Response: Appendix B has been edited to provide additional clarity and transparency on the modeling results and decisions. All model results are provided in the summary tables and footnotes indicate whether data sets were determined to be inappropriate for modeling or when there was inadequate fit for all models. In instances where there was adequate fit for one or more models, bolded text indicates the selected model and associated values (explained in footnote).

Comment: In Table B-25, the selected model (indicated by bold type in the table and shown in the proceeding figure) has neither the lowest AIC nor lowest BMDL. While an explanation of this was provided by EPA during the peer review meeting, EPA should provide an explanation in the modeling appendix.

EPA Response: After additional review and discussion with BMD modelers, it was determined that this data set is not appropriate for BMD modeling because there is a single dose group showing a high incidence response (50%) in contrast to no response in all other groups. On this basis, a NOAEL approach was used for POD derivation for this endpoint. Tables 5-5 and 5-10 which show the PODs considered for derivation of the RfD and subchronic have been updated to reflect this change in the toxicity value derivation approach. Because this endpoint was not prioritized for derivation of the hepatic chronic or subchronic osRfD selections or the overall RfD or subchronic RfD there was no effect on the derived toxicity values for the Toxicological Review.

Comment: The pharmacokinetic assumptions and parameterizations used by EPA in the httk: High-Throughput Toxicokinetics package should be briefly mentioned/discussed in the Toxicological Review (since httk is a publicly available EPA "product") and the context for making comparisons with the assumptions and parameterizations of the pharmacokinetic modeling performed for this Review should be clarified.

EPA Response: httk is a tool for rapid risk ranking to identify chemicals for which more in-depth, chemical-specific analyses should be conducted. The httk project at EPA advises against using this approach for this IRIS assessment, noting that the in vitro data used as inputs do not

capture the large sex differences seen for many PFAS. On this basis, EPA determined that such an evaluation of httk would not be an appropriate addition to this assessment.

Comment: The reasoning behind using CL as opposed to t1/2 uses two conflicting lines of reasoning and clarification is needed.

EPA Response: Clarifying text was added to Section 5.2.1 (Approach for Animal-Human Extrapolation of PFHxA Dosimetry) that included explanation of EPA's guidelines on using allometric scaling for deriving oral reference doses and that while there was not PBPK data available for PFHxA, there was TK data.

Tier 2 Suggestions

Comment: Given the lack of sex differences observed in human studies, EPA should consider clarifying the text implying that female human and male human equivalent doses will be calculated on the basis of sex-specific PODs in animals.

EPA Response: Clarifying text was added to Section 5.2.1 (Approach for Animal-Human Extrapolation of PFHxA Dosimetry).

Comment: Discussion of the Pérez et al. study should note that some of the results were called into question for PBFA and some of these issues could also apply to PFHxA. EPA should also consider avoiding use of the Pérez study as supplemental information, or if used, to include a caveat per the additional studies referenced by the reviewer.

EPA Response: Clarifying text was added to Section 3.1.2 (Distribution in Humans).

Comment: The reference to slower elimination at higher concentrations (Dzierlenga et al.) was noted as opposite the expectation of saturable renal absorption (mediated by Oatp1a1). The reviewer noted that Han et al. mentions other transporters that have been tested for activity with PFAS and suggested EPA consider adding a clarification such as: "While saturation of reabsorption transporters would lead to decreased half-life, there are also transporters responsible for elimination of PFAS to urine, and saturation of these transporters, such as Oat 1 and Oat3, could lead to an increase in observed half-life and could thereby help explain the observations of Dzierlenga et al."

EPA Response: Clarifying text was added to Section 5.2.1 (Approach for Animal-Human Extrapolation of PFHxA Dosimetry).

Comment: If EPA decides to maintain a value of 3 for UF_A, then a value of 10 should be adopted for UF_D.

EPA Response: As described in EPA's A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002), the interspecies uncertainty factor (UF_A) is applied to account for extrapolation of animal data to humans; it accounts for uncertainty regarding the pharmacokinetic and pharmacodynamic differences across species. Although the pharmacokinetic uncertainty is mostly addressed through the application of dosimetric approaches for estimating human equivalent doses, there is residual uncertainty around the pharmacokinetics and the uncertainty surrounding pharmacodynamics. Typically, a threefold UF is applied for this

uncertainty in the absence of chemical-specific information. This is the case for the hematopoietic and developmental endpoints. For the hepatic endpoints, known species differences exist between rodent and human hepatic response to hepatotoxicant, particularly for effects mediated by PPARα (Hall et al., 2012). Although the available evidence from PFAS structurally similar PFHxA were available, experiments specifically challenging the role of PPARα in PFHxA mediated hepatotoxicity were not available. Thus, based on the residual uncertainty surrounding the interspecies differences in pharmacodynamics described above, a factor of 3 is applied to account for the pharmacodynamic uncertainty of the UFA for all potential health effect consequences of PFHxA exposure.

A UF_D of 3 is applied because the evidence base for hepatic, hematopoietic, and developmental endpoints included two subchronic studies and one chronic study in Sprague Dawley rats and developmental/reproductive studies in Sprague Dawley rats and Crl:CD1 mice. Limitations, as described in U.S. EPA (2002) were used as the basis for a $UF_D = 3$. These limitations included a lack of informative human studies for most outcomes, subchronic or chronic toxicity studies in more than one species, multigenerational study, a developmental neurotoxicity study. Additionally, the data to inform effects on thyroid hormones is limited to a single short term study.

Additional justification has been provided in the draft in Section 5 to clearly document the rationale for UF selection.

Comment: For the UF_S for hepatocellular hypertrophy, EPA should consider including a discussion of the specific study results justifying the specific UF_S value proposed (i.e., 3 instead of 10).

EPA Response: Additional text was added to Table 5-6 to clarify EPA's rationale for selection of a $UF_S = 3$ for hepatic effects. Briefly, hepatocellular hypertrophy observed in the subchronic study is expected to represent a less severe adverse hepatic response than would be expected to occur with chronic exposure. This is expected to reduce the uncertainty with use of a subchronic study.

Comment: For the UF_D, EPA should consider modifying Table 5-6 to delete "the dose received by the pups is unclear and might be significantly less than that administered to the dams" as a cited factor that in a meaningful way diminishes confidence in the database relevant to deriving the RfD. Otherwise, since developing organism (e.g., pup) doses are commonly unknown, by EPA's reasoning a UF_D of 3 might automatically be applied any time the basis for an RfD or candidate RfD is developmental effects. Moreover, it is not needed as the EPA cites other considerations that are sufficient to support a UF_D of 3.

EPA Response: This text was removed.

Comment: EPA should consider adding a more explicit description of the reasoning for choosing a UF_A of 3 instead of 1 or 10.

EPA Response: Clarifying text was added to Section 5.2.1 (Derivation of Candidate Toxicity Values for the RfD) to support the rationale for the $UF_A = 3$.

Comment: EPA should consider revising the UF_S of 1 to 10. The reviewer stated that the UF_S of 1 does not seem to consider the data showing that PFHxA exposure causes a reduction in serum thyroid hormone, but there is little information beyond that. Moreover, there is data suggesting that eye-opening is delayed by PFHxA exposure, which is a potential thyroid endpoint, but this relationship is not evaluated empirically. Considering this, the UF_S of 1 does not appear to cover this level of uncertainty for development.

EPA Response: The reviewers concerns seem to be more directly related to lack of additional data to inform the endocrine effects of PFHxA exposure. Limitations of the evidence base are accounted for with the database uncertainty factor (UF_D). Additional text was added to the UF_D justification in Table 5-6 to address the data gap described by the reviewer. Additionally, in the current version of the draft, decreased serum free T4 was brought forward for dose response analysis in support of the subchronic reference dose (RfD) and a $UF_S = 10$ was applied to this endpoint.

E.4.2. Public Comments on Noncancer Toxicity Value Derivation

Comment: Two commenters agreed with the data-driven HED approach used in the toxicological review. One commenter disagreed with the selected approach on the basis that there is insufficient evidence to support the data driven approach used in the assessment and suggest that the BW3/4 approach should be used for calculation of the POD_{HED}.

EPA Response: The HED approach in the draft Toxicological Review was supported by the external peer review committee. Some clarifying text was added to the draft in response to some tier 1 and tier 2 recommendations from the panel.

Comment: One commenter indicated that they supported selection of a $UF_S = 1$ for hematopoietic effects but suggested EPA reconsider a $UF_S = 3$ for hepatic effects. Specifically, they state that "the rationale for application of a UF_S should consider whether adverse effects occur at a lower dose with longer exposure, not only that adverse effects at a certain dose become more severe with longer exposure" They suggest that a $UF_S = 1$ may be appropriate for hepatic effects similar to that used by DWQI (2017) for PFOA. Another commenter suggested that it was inappropriate to EPA should not use a subchronic study and an uncertainty factor for derivation of a lifetime toxicity value when data are available from a chronic study.

EPA Response: As explained in the assessment, a UF_S of 3 is applied to hepatocellular hypertrophy for the purpose of deriving a lifetime RfD. Although the endpoint was derived from a 90-day subchronic study (Loveless et al., 2009), which would typically warrant application of a $UF_S = 10$, there are some other sparse data that mitigate this uncertainty, to an extent. Specifically, significant hepatocellular hypertrophy was not observed in the chronic study in male or female rats (Klaunig et al., 2015). However, a $UF_S = 1$ was not applied as the evidence supports a pathway where hepatocellular hypertrophy is an adverse event leading to more severe outcomes with longer exposure durations, such as the necrosis that was observed in female rats in the chronic study. Additionally, the highest dose levels used in the chronic study were at or below the LOAEL

for this effect in the available subchronic studies (see Section 3.2.1). Thus, some uncertainty remains and a UF_S of 3 is applied.

A UF_S of 3 is also applied to the hematopoietic endpoint (i.e., decreased RBCs) from the 90-day subchronic study (Chengelis et al., 2009b). Specifically, a UF_S lower than 10 was warranted as more significant effects on RBCs were not observed after chronic exposure at the same PFHxA doses (RBCs decreases of the same magnitude were observed at matched doses and sexes across exposure durations see Section 3.2.4); however, uncertainty remains when considering the doses tested in the chronic as compared to the subchronic study. Further, the subchronic study may poorly predict a chronic exposure setting across multiple RBC life cycles (one cycle is ~60 days), which could reflect cumulative effects as greater proportions of RBCs across stages are affected, or possibly even reduced effects (compensatory responses) warranting a UF_S higher than 1. Thus, a UF_S of 3 was applied.

Comment: One commenter suggested that the lack of informative human data should not be accounted for in the UF_D = 3 and indicates questionable human relevance of the animal findings.

EPA Response: EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) states that the "database UF is intended to account for the potential for deriving an underprotective RfD/RfC as a result of an incomplete characterization of the chemical's toxicity." The document recommends "…the assessor should consider both the data lacking and the data available for particular organ systems as well as life stages" when determining the value of the UF_D. Because reliable human data is the most relevant for assessing risk of an exposure to humans, the lack of informative human studies presents an important gap in the evidence base.

Comment: One commenter agreed with the BMR selections. Another suggested that additional support including references are needed to support the BMR justifications.

EPA Response: The modeling approach in the draft Toxicological Review was supported by the external peer review committee. In addition, the assessment cites and follows EPA guidance on BMR selection, which includes additional references and information.

Comment: One commenter disagrees with the decision to use a BMD approach for datasets where there is a response only at one dose (e.g., the highest dose group) regardless of whether the software states that models are viable suggesting that a NOAEL/LOAEL approach be used in these instances. They specifically cite the following endpoints but indicate this list may not be exhaustive: decreased RBC in female rats (Klaunig 2015); decreased hemoglobin in female rats (Klaunig 2015; Loveless 2009); and increased hepatocellular hypertrophy in female rats (Loveless 2009)

EPA Response: All datasets were reviewed for the appropriateness of a BMD approach for POD derivation. For decreased hemoglobin in male rats from Chengelis et al. (2009b) it was determined that the data set was not appropriate for BMD modeling on the basis that the response in the high dose group was much larger than the BMR and there was no response in all other dose groups thus the NOAEL/LOAEL approach was applied to this endpoint (Appendix B, Section B.5). For increased hepatocellular hypertrophy in female rats from Loveless et al. (2009) the dataset was

not appropriate for BMD modeling on the basis that the response in the high dose group (50%) is much larger than the BMR and there was no response in all other dose groups (Appendix B, Section B.16). Additional text has been added to modeling appendix has been updated to provide clarify situations in which a NOAEL/LOAEL approach would be preferred over a BMD approach (Appendix B, Section B.3).

Comment: One commenter expressed concern that the osRfD for hematopoietic effects is higher than the subchronic osRfD on the basis that, "It is not logical or supportable to conclude that a specific toxicological effect will occur at a much lower dose from subchronic exposure than from chronic exposure."

EPA Response: The EPA carefully evaluated the hematopoietic endpoints for toxicity value derivation considering several factors that formed the basis for the overall osRfD and subchronic osRfD. The specific toxicological endpoint, decreased red blood cells, was available from both the chronic and subchronic studies, however it was noted that while the subchronic osRfD is lower (~7-fold) than the chronic osRfD and both subchronic and chronic exposure designs and study durations include the life cycle of a red blood cell (~60 days in rats), the subchronic study duration may miss longer term (or even compensatory) effects on RBCs (i.e., regeneration) that would be observable in a chronic study. Further, confidence in the quantification of the POD for the subchronic osRfD is *low* given the POD was far below the NOAEL (50 mg/kg-d) and the osRfD is far below toxicity values derived for the same finding from other subchronic studies suggesting some underlying variability is driving the POD lower. These weaknesses and uncertainties in the ability to reliably estimate toxicity values for the hematopoietic effects observed in the 90-day study by (Chengelis et al., 2009b) reduce the confidence in those estimates, which is reflected in two ways. First, there is less confidence in the (Chengelis et al., 2009b) candidate value for lifetime exposure, as compared to the value based on the chronic study, and, although the candidate value from the subchronic study is lower, the higher confidence data from the longer-term study is selected for the lifetime osRfD. Second, for the subchronic osRfD, although data from the (Chengelis et al., 2009b) is ultimately selected because the chronic study is not applicable and the POD from (Chengelis et al., 2009b) was much lower and more protective than PODs from the other subchronic studies, this value was interpreted as medium-low confidence overall given the aforementioned uncertainties. Based on this lower confidence determination, this subchronic osRfD is not used to support the overall subchronic RfD. The EPA added this additional clarification to **Section 3.2.1**, and **Section 5.2.1.**

E.5. CHARGE QUESTION 9 AND 10: CARCINOGENICITY HAZARD IDENTIFICATION AND TOXICITY VALUE DERIVATION

9) The Toxicological Review concludes that there is inadequate information to assess carcinogenic potential for PFHxA and that this descriptor applies to oral and inhalation routes of human exposure. Please comment on whether the available animal and

mechanistic studies and the analysis presented in the Toxicological Review are scientifically justified and clearly described.

10) Given the conclusion there was inadequate information to assess carcinogenic potential for PFHxA (Charge Question 5), the Toxicological Review does not derive quantitative estimates for cancer effects for either oral or inhalation exposures. Is this decision scientifically justified and clearly described?

E.5.1. External Peer Review Comments on Carcinogenicity Hazard Identification and Toxicity Value Derivation

"All reviewers concurred that the analysis presented in the Toxicological Review was scientifically justified and clearly described."

Tier 1 Recommendations and Tier 2 Suggestions

Reviewers had no Tier 1 recommendations or Tier 2 suggestions.

E.5.2. Public Comments on Carcinogenicity Hazard Identification and Toxicity Value Derivation

Comment: One commenter agreed with the conclusion that there is inadequate information to assess carcinogenic potential of PFHxA, noting that carcinogenicity has only been evaluated in a single study of one species (rat).

EPA Response: NA

E.6. ADDITIONAL COMMENTS

E.6.1. Additional External Peer Review Comments

Two reviewers "provided additional comments separately from their responses to the charge questions. These included the following tiered comments not already covered in their responses to charge questions."

Tier 1 Recommendations

Reviewers had no Tier 1 recommendations.

Tier 2 Suggestions

Comment: EPA should consider how data from other PFAS either support or differ from PFHxA observations and how those could be explained by structure-activity relationships (e.g., chain length vs. half-live observations) as well as how data from other model systems (e.g., zebrafish) could help to fill data gaps.

EPA Response: Additional comparisons from other PFAS were added to the draft toxicological review, specifically drawing from other observations in PFAS. Examples include the discussion in the hepatic effects Section 3.2.1, subsection Considerations Related to Human relevance, and Table 4-1 in Section 4.1. Note that information from the supplemental evidence that

was determined to be potentially impactful to assessment conclusions (that may evaluate effects on model systems other than animal and human) were captured and incorporated into the assessment.

Comment: EPA should consider harmonizing the discussion of supporting evidence across the different endpoints considered. For example, if structure-activity relationship information is available for hepatic effects and the document includes text on what should be expected for PFHxA based on observations for other PFAS, then under developmental effects, the document should state whether similar structure-activity relationships could be considered or if such information is not available

EPA Response: Similar to the comment above, the EPA included additional information from other PFAS particularly in Section 4, and summarized in Table 4.1, into the assessment. The evidence from other PFAS in models evaluating effects similar to PFHxA were considered.

Comment: EPA should consider adding context on reliability for the information presented in Table 1-1 on the available physicochemical properties of PFHxA. The reviewer noted, for example, that water solubility of ammonium vs. sodium salts varies five orders of magnitude and stated that "clearly one of these values is wrong as once dissociated these should behave similarly." Similarly, the reviewer noted that the same is true for the bioconcentration factor.

EPA Response: Text was added to Section 1.1.1 to clarify that the data in the table represent both experimental and predicted values and that the predicted values may be less reliable. Footnotes are used in Table 1-1 to indicate which values are experimental and which are predicted.

Comment: In the pharmacokinetics background (Section 3.1) of the Toxicological Review, EPA should consider clarifying how "substantial binding" to serum proteins is defined (see page 3-5, lines 6-7). The reviewer noted that PFHxA has been shown in in vitro studies to bind less strongly than long-chain PFAS.

EPA Response: The text was edited to indicate the percent binding reported in the study (>99% bound to serum albumin).

E.6.2. Additional Public Comments

Comment: One commenter suggested that EPA expand the background information section on sources and relative contributions of PFHxA sources to better support the case for human exposures. They also recommend that EPA present exposure information for PFHxA relative to other PFAS compounds.

EPA Response: 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 PFHxA and related salts, and information on human exposure is tagged as supplemental information during the screening process.

Comment: One commenter suggested changes to the integration judgement language (i.e., "the available evidence indicates that PFHxA exposure is likely to cause X effects in humans, given relevant exposure circumstances") on the basis that it implies causation. Alternative language

was suggested. Specifically, "the available evidence indicates that PFHxA could potentially result in an increased risk of X in humans if exposure to exceeds XXX on a mg/kg-day basis."

EPA Response: The integration judgement language in the Toxicological Review is consistent with the peer-reviewed IRIS Handbook.

Comment: One commenter suggested that EPA apply an additional uncertainty factor to account for potential additive effects of exposure to multiple PFAS chemicals.

EPA Response: EPA applied uncertainty factors to account for five possible areas of uncertainty as described in "Derivation of Candidate Values for the RfD," and in U.S. EPA (2002). This assessment is specific to PFHxA 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.

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