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Toxicological Review of Perfluorohexanoic Acid (PFHxA) and Related Compounds Ammonium and Sodium Perfluorohexanoate (PFHxA-NH_4 and PFHxA-Na)

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Supplemental Information

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Integrated Risk Information System
Center for Public Health and Environmental Assessment
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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 revisions 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 (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 study were used in the BMD modeling and then converted to human equivalent doses (HEDs) using the ratio of animal-to-human serum half-lives. For endpoints with successful BMD model fit, the modeling results are presented in this Appendix.

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 is deemed appropriate on the basis of the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated from a homogeneous variance model. If the test for homogeneity of variance is rejected ($p < 0.05$), the model is run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model does not adequately fit the data (i.e., Test 3; $p < 0.05$), the data set is considered unsuitable for BMD modeling. 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 relative liver weight, a BMR equal to 10% increase 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.

B.2. 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-day)	*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	p-Values	Test 2 p-values	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**

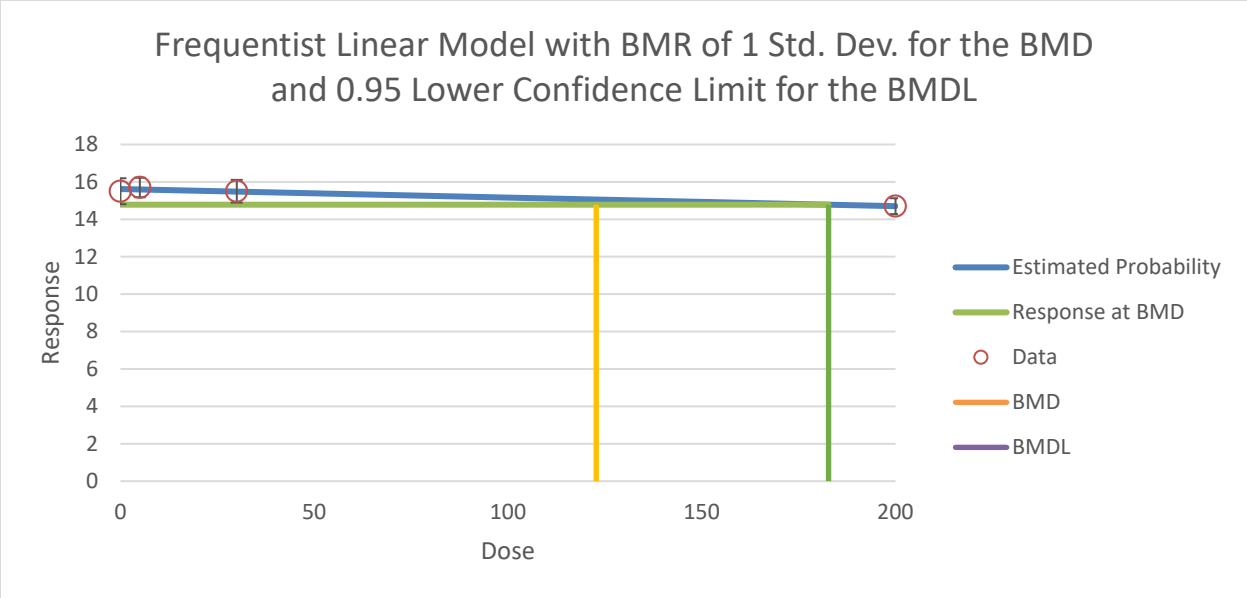

Figure B-1. Dose response curve for the Linear model fit to hemoglobin in female rats (Klaunig et al., 2015).

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B.3. 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-day)	*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

Table B-4. Benchmark dose results for hemoglobin in male rats—constant variance, BMR = 1 standard deviation ((Chengelis et al., 2009b)

Models	*p*-Values	Test 2 *p*-values	AIC	1 SD		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential 2	0.704934	0.064112	91.82015	110.5213	77.18423	0.615129749
Exponential 3	0.539535	0.064112	93.49724	142.4976	78.66252	-0.004155158
Exponential 4	0.704934	0.064112	91.82015	110.5301	77.18593	0.615097154
Exponential 5	0.539535	0.064112	93.49724	142.4988	78.6632	-0.004163015
Hill	NA	0.064112	95.52553	58.25582	51.28692	1.3298×10^{-6}
Polynomial (Poly 3)	**0.853527**	**0.064112**	**91.4376**	**151.5179**	**81.3468**	**-0.001390585**
Polynomial (Poly 2)	0.558344	0.064112	93.46342	143.161	81.22378	-0.006077488
Power	0.539814	0.064112	93.49673	141.0967	81.06874	-0.007648406
Linear	0.719614	0.064112	91.77892	112.5099	79.80677	0.5847594

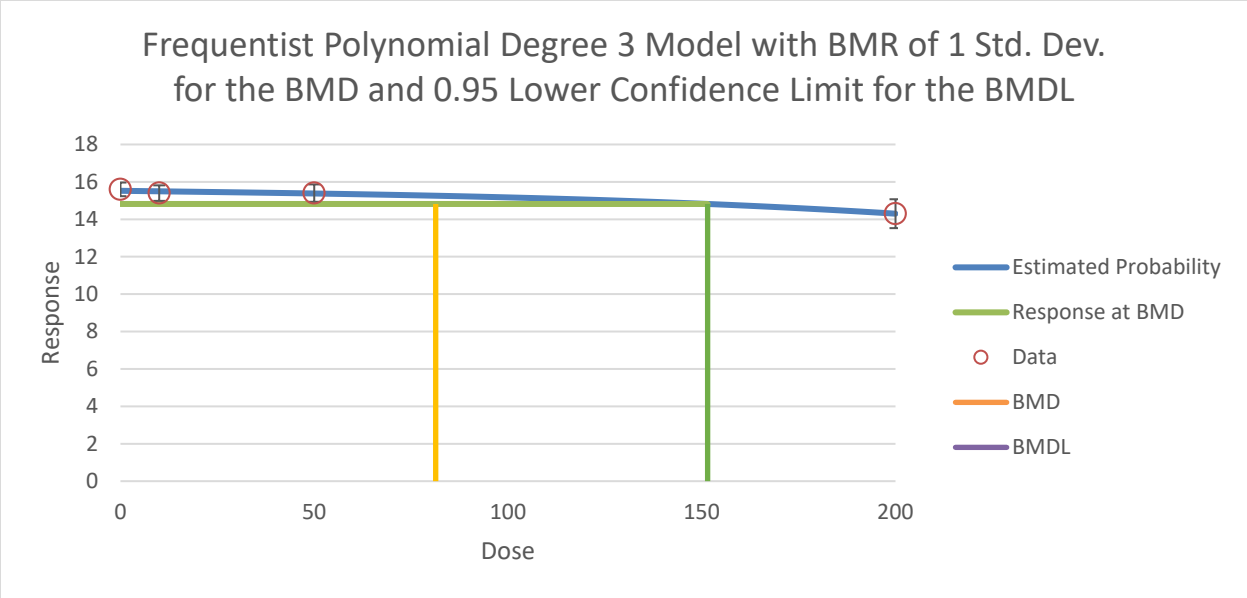

Figure B-2. Dose response curve for the Polynomial Degree 3 model fit to hemoglobin in male rats (Chengelis et al., 2009b).

B.4. HEMOGLOBIN—FEMALE RATS (CHENGELIS ET AL., 2009B)

Table B-5. Dose response data for hemoglobin in female rats (Chengelis et al., 2009b)

Dose (mg/kg-day)	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-6. Benchmark dose results for hemoglobin in female rats—nonconstant variance, BMR = 1 standard deviation (Chengelis et al., 2009b)

Models	*p*-Values	Test 3 *p*-values	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
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. Therefore, this data set is not amendable for BMD modeling.

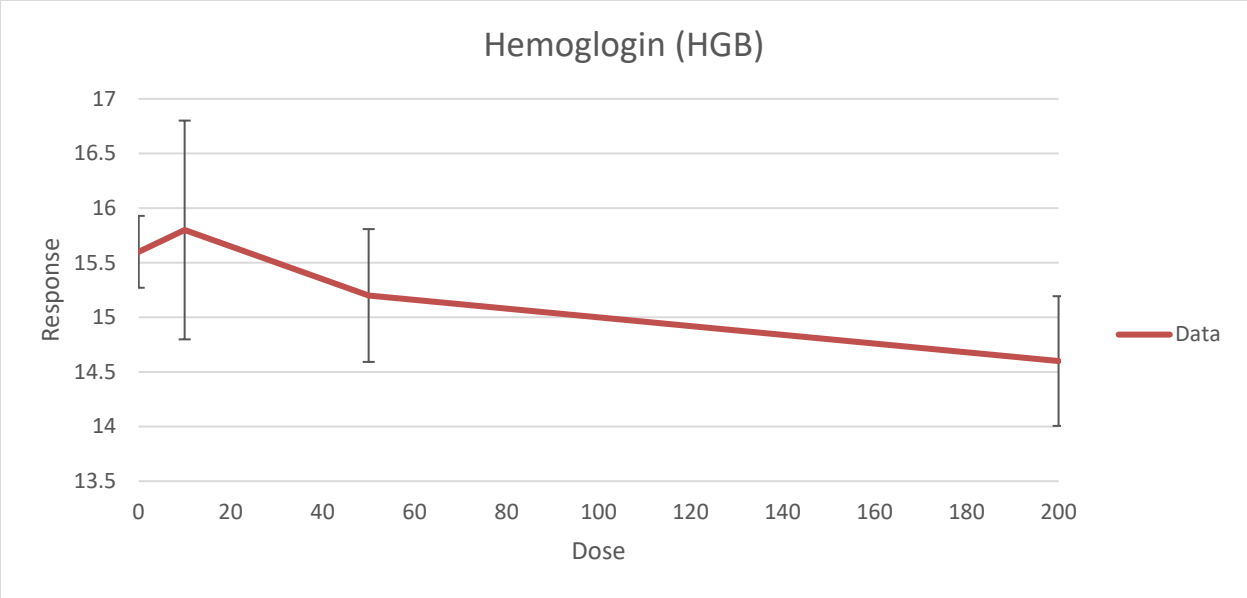

Figure B-3. Dose response data for hemoglobin in female rats (Chengelis et al., 2009b).

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

Table B-7. Dose response data for hemoglobin in male rats (Loveless et al., 2009)

Dose (mg/kg-day)	*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-8. Benchmark dose results for hemoglobin in male rats nonconstant variance, BMR = 1 standard deviation (Loveless et al., 2009)

Models	*p*-Values	Test 3 *p*-values	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. Therefore, this data set is not amendable for BMD modeling.

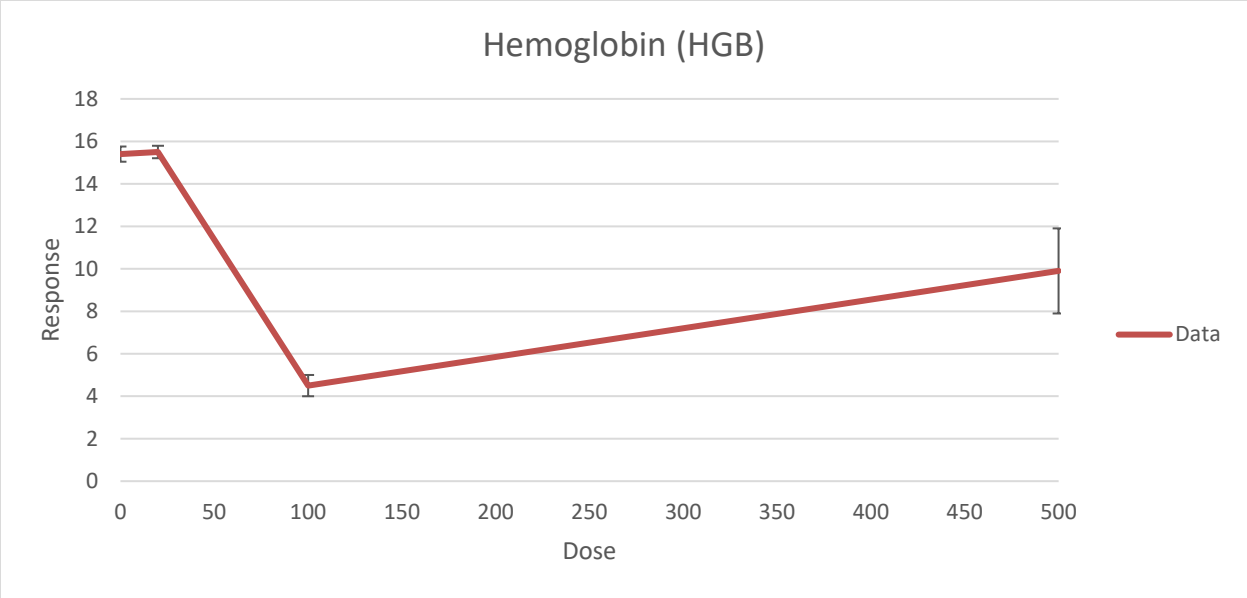

Figure B-4. Dose response data for hemoglobin in male rats (Loveless et al., 2009).

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

Table B-9. Dose response data for hemoglobin in female rats (Loveless et al., 2009)

Dose (mg/kg-day)	*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-10. Benchmark dose results for hemoglobin in female rats constant variance, BMR = 1 standard deviation (Benchmark dose results for hemoglobin in female rats constant variance, BMR = 1 standard deviation (Loveless et al., 2009)

Models	*p*-Values	Test 2 *p*-values	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	-1.06893E-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

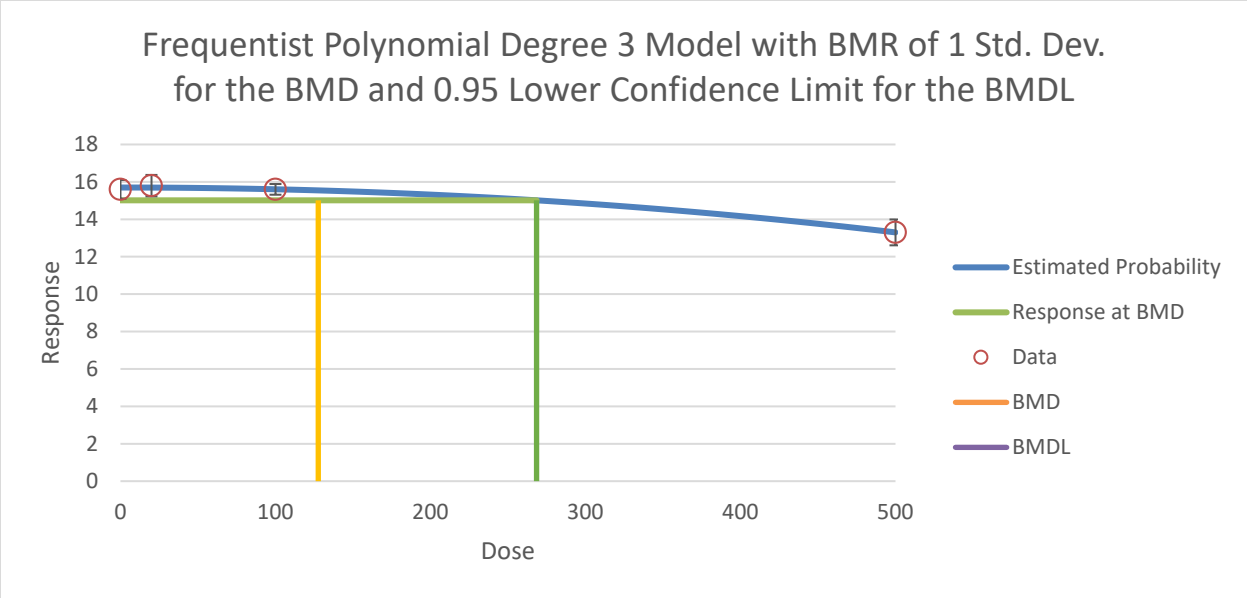

Figure B-5. Dose response curve for the Polynomial Degree 3 model fit to hemoglobin in female rats (Loveless et al., 2009).

B.7. RED BLOOD CELLS—MALE RATS (KLAUNIG ET AL., 2015)

Table B-11. Dose response data for red blood cells in male rats (Klaunig et al., 2015)

Dose (mg/kg-day)	*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-12. Benchmark dose results for red blood cells in male rats—nonconstant variance, BMR = 1 standard deviation (Klaunig et al., 2015)

Models	*p*-Values	Test 3 *p*-values	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
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

The only model provides adequate fit is the Hill model, but the Hill model failed to estimate BMDL.
Both constant and nonconstant variance models failed to model the variance data.

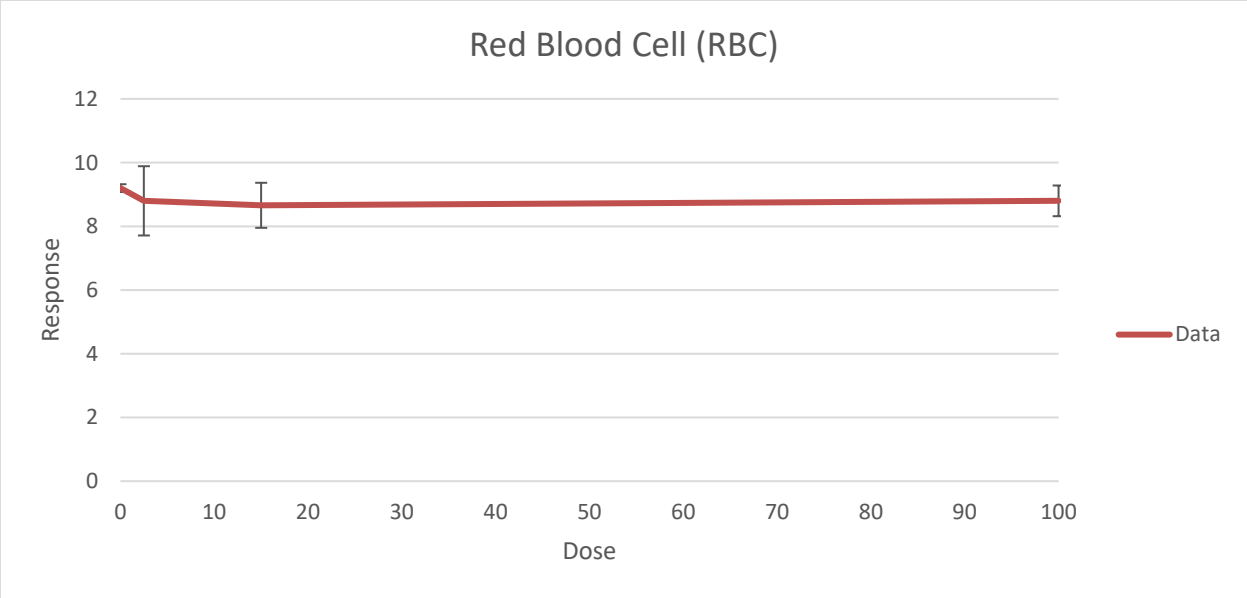

Figure B-6. Dose response data hemoglobin in male rats (Klaunig et al., 2015).

B.8. RED BLOOD CELLS—FEMALE RATS (KLAUNIG ET AL., 2015)

Table B-13. Dose response data for red blood cells in female rats (Klaunig et al., 2015)

Dose (mg/kg-day)	*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-14. Benchmark dose results for red blood cells in female rats—constant variance, BMR = 1 standard deviation (Klaunig et al., 2015)

Models	*p*-Values	Test 2 *p*-values	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**

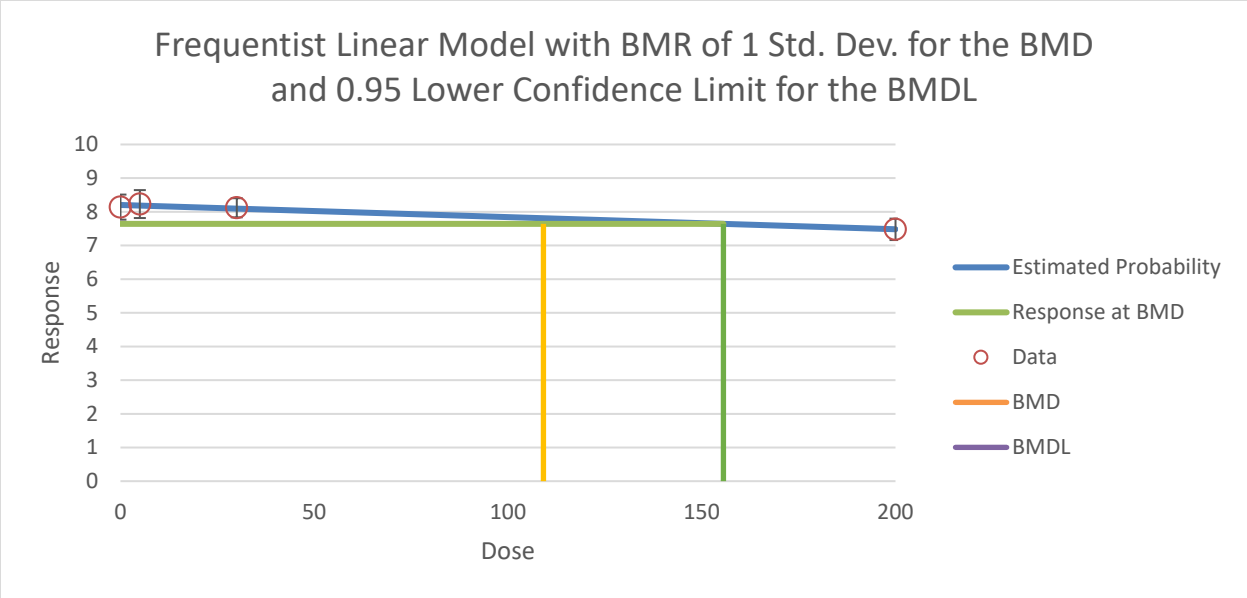

Figure B-7. Dose response curve for the Linear model fit to hemoglobin in female rats (Klaunig et al., 2015).

B.9. RED BLOOD CELLS—MALE RATS (CHENGELIS ET AL., 2009B)

Table B-15. Dose response data for red blood cells in male rats (Chengelis et al., 2009b)

Dose (mg/kg-day)	*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-16. Benchmark dose results for red blood cells in male rats—nonconstant variance, BMR = 1 standard deviation (Chengelis et al., 2009b)

Models	*p*-Values	Test 3 *p*-values	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.

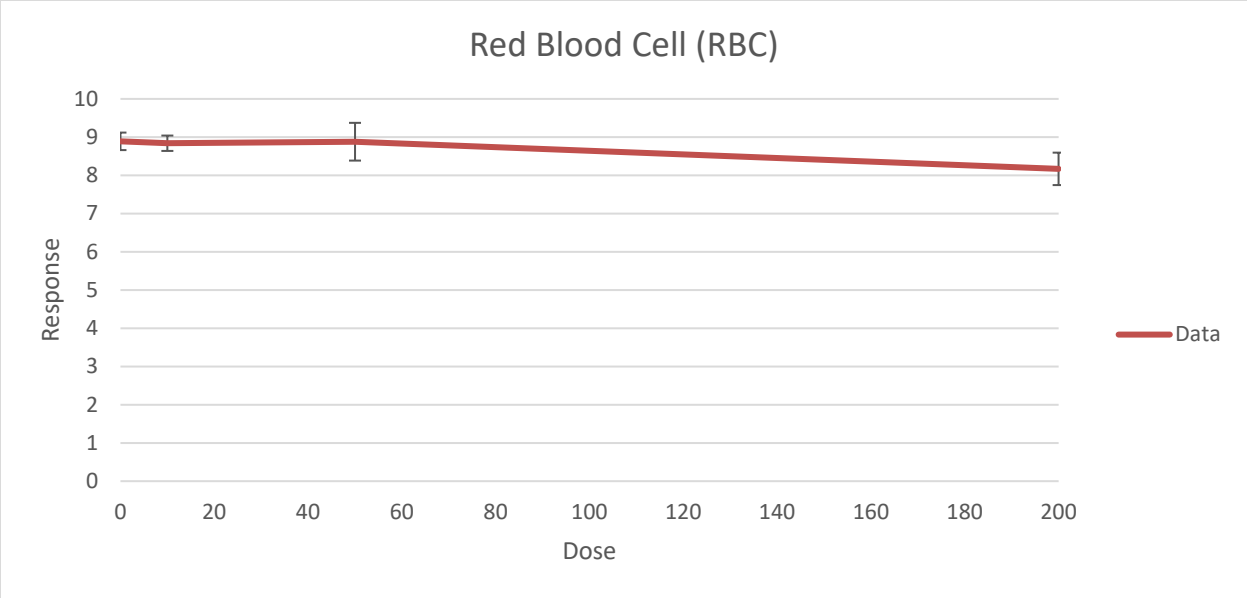

Figure B-8. Dose response data for red blood cells in male rats (Chengelis et al., 2009b).

B.10. RED BLOOD CELLS—FEMALE RATS (CHENGELIS ET AL., 2009B)

Table B-17. Dose response data for red blood cells in female rats (Chengelis et al., 2009b)

Dose (mg/kg-day)	*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-18. Benchmark dose results for red blood cells in female rats—constant variance, BMR = 1 standard deviation (Chengelis et al., 2009b)

Models	*p*-Values	Test 2 *p*-values	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
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

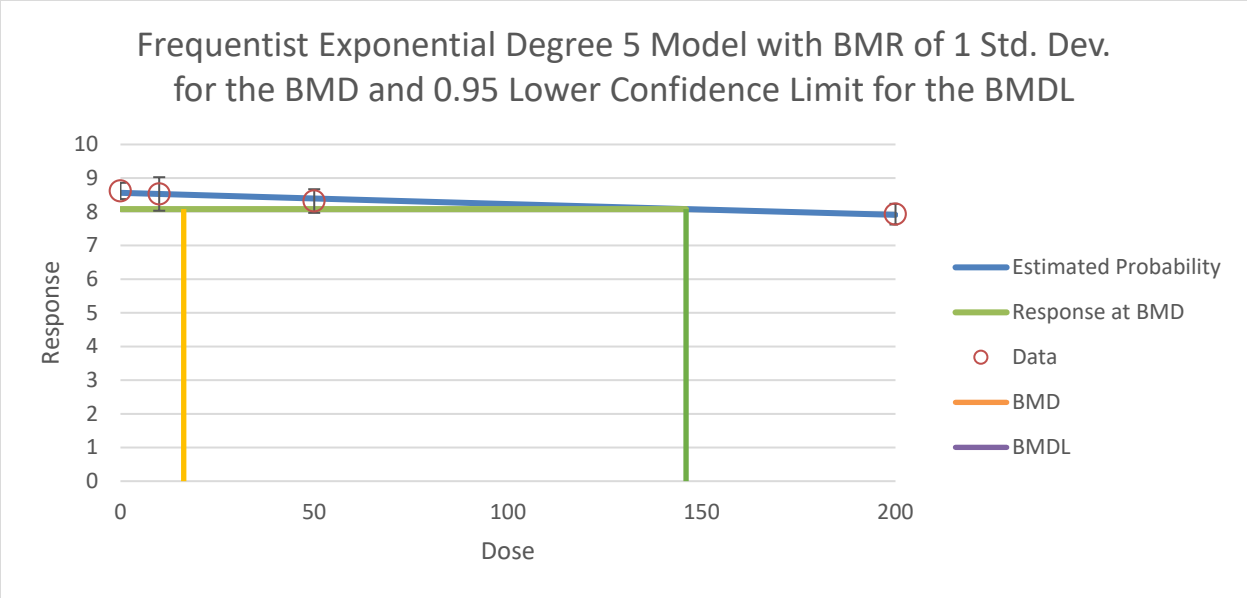

Figure B-9. Dose response curve for the Exponential 5 model fit to red blood cells in female rats (Chengelis et al., 2009b).

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

Table B-19. Dose response data for red blood cells in male rats (Loveless et al., 2009)

Dose (mg/kg-day)	*Number of animals*	Mean (million/µL)	Standard deviation
0	10	8.89	0.36
20	10	8.95	0.34
100	10	8.46	0.41
500	10	6.09	1.27

Table B-20. Benchmark dose results for red blood cells in male rats—nonconstant variance, BMR = 1 standard deviation (Loveless et al., 2009)

Models	*p*-Values	Test 3 *p*-values	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**

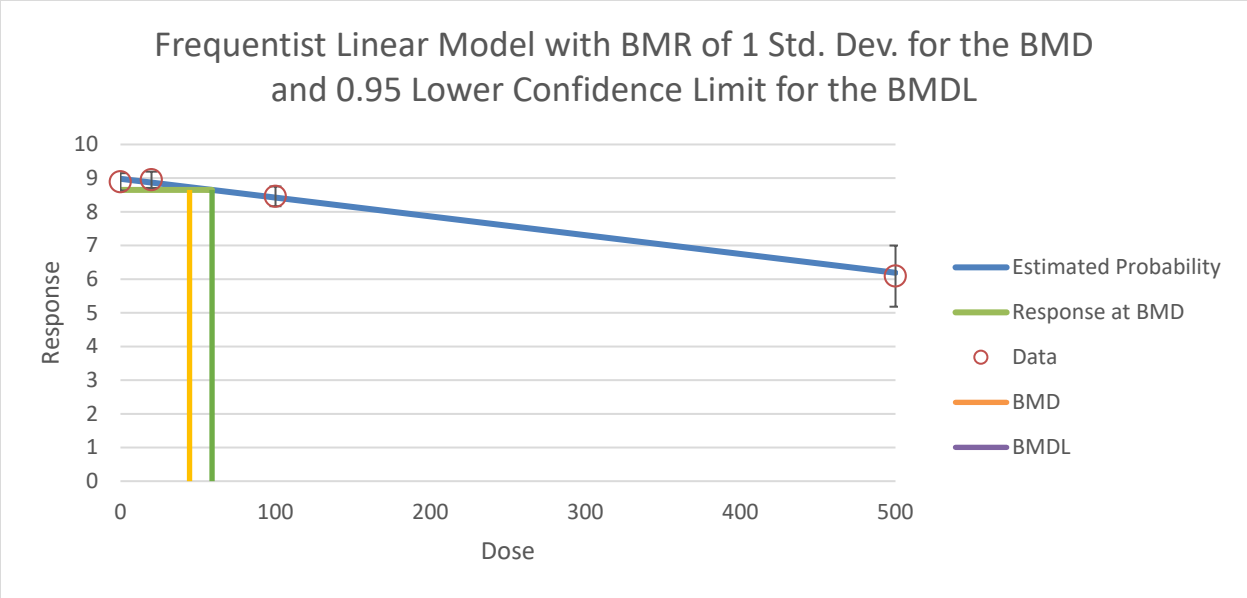

Figure B-10. Dose response curve for the Linear model fit to red blood cells in male rats (Loveless et al., 2009).

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

Table B-21. Dose response data for red blood cells in female rats (Loveless et al., 2009)

Dose (mg/kg-day)	*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-22. Benchmark dose results for red blood cells in female rats—constant variance, BMR = 1 standard deviation (Loveless et al., 2009)

Models	*p*-Values	Test 2 *p*-values	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
Exponential 5	0.331861	0.087567	57.49047	238.0095	116.9523	-0.08345
Hill	NA	0.087567	59.42671	113.2878	101.18	-2.4E-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**

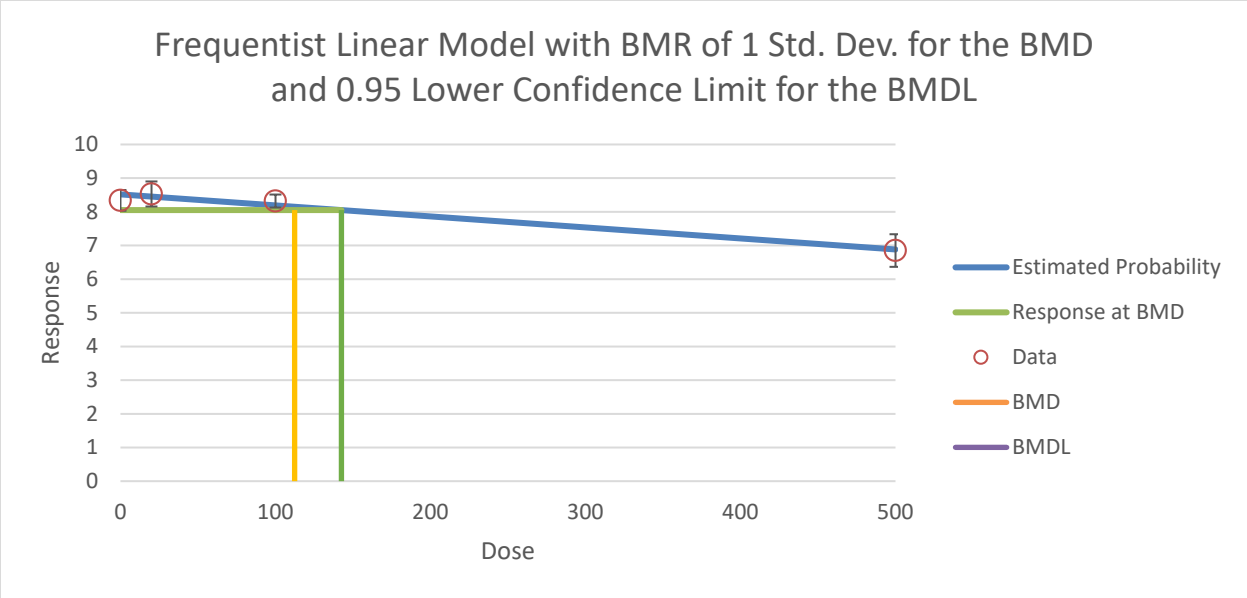

Figure B-11. Dose response curve for the Linear model fit to red blood cells in female rats (Loveless et al., 2009).

B.13. HEPATOCELLULAR HYPERTROPHY—MALE RATS (CHENGELIS ET AL., 2009B)

Table B-23. Dose response data for hepatocellular hypertrophy in male rats (Chengelis et al., 2009b)

Dose (mg/kg-day)	*Number of animals*	Incidence	Percentage of incidence
0	10	0	0
10	10	0	0
50	10	0	0
200	10	7	70

This data set is not considered appropriate for BMD modeling because there is a single dose group showing a high incidence response (70%) in contrast to no response in all other groups.

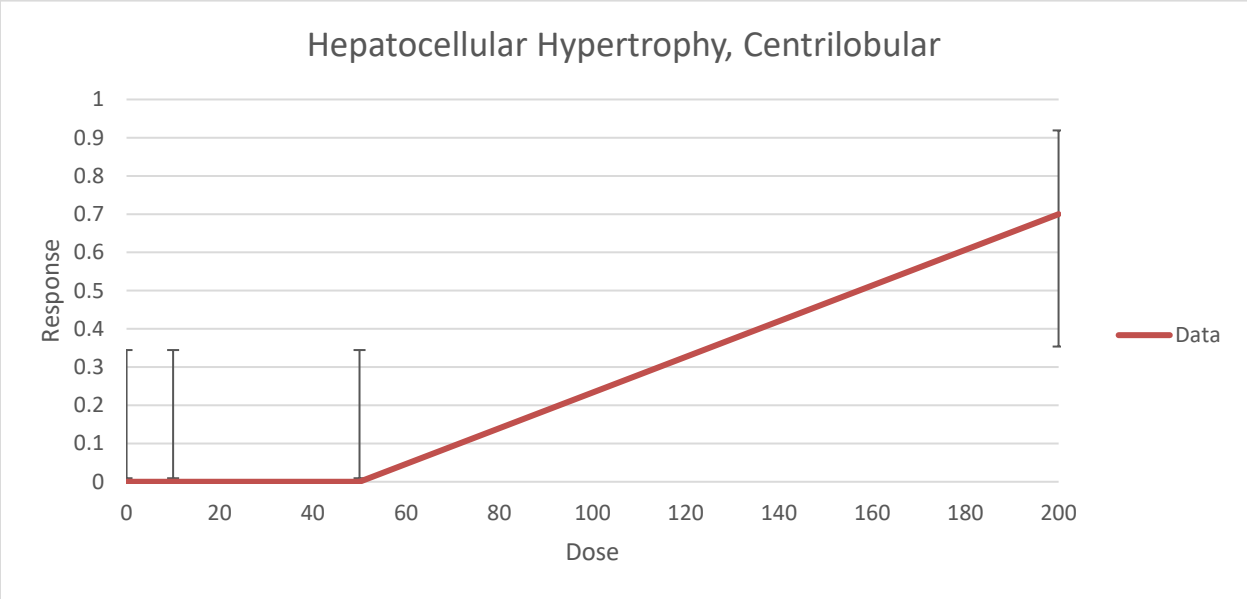

Figure B-12. Dose response data for hepatocellular hypertrophy in male rats (Chengelis et al., 2009b).

B.14. HEPATOCELLULAR HYPERTROPHY—FEMALE RATS (LOVELESS ET AL., 2009)

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

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

Table B-25. Benchmark dose results for hepatocellular hypertrophy in female rats—nonconstant variance, BMR = 10% Extra Risk (Loveless et al., 2009)

Models	*p*-Values	AIC	10% Extra Risk		Scaled residual for dose group near BMD
			BMD	BMDL	
Dichotomous Hill	1	17.86294	405.8782	97.0233	1.22E-07
Gamma	0.98176	19.86399	319.4271	97.38679	0.000597
Log-Logistic	1	17.86294	442.544	96.1137	-1.2E-09
Multistage Degree 3	**0.996008**	**15.98505**	**267.9741**	**96.31573**	**-0.24575**
Multistage Degree 2	0.956334	16.4752	201.2327	82.64841	-0.53848
Multistage Degree 1	0.610724	18.92693	104.2041	53.56868	-1.08184
Weibull	1	17.86294	449.1777	97.95169	-1.1E-08
Logistic	1	15.86296	438.966	209.1724	4.68E-07
Log-Probit	1	17.86294	402.6258	93.01619	7.1E-07
Probit	1	17.86294	411.492	185.6167	1.31E-09

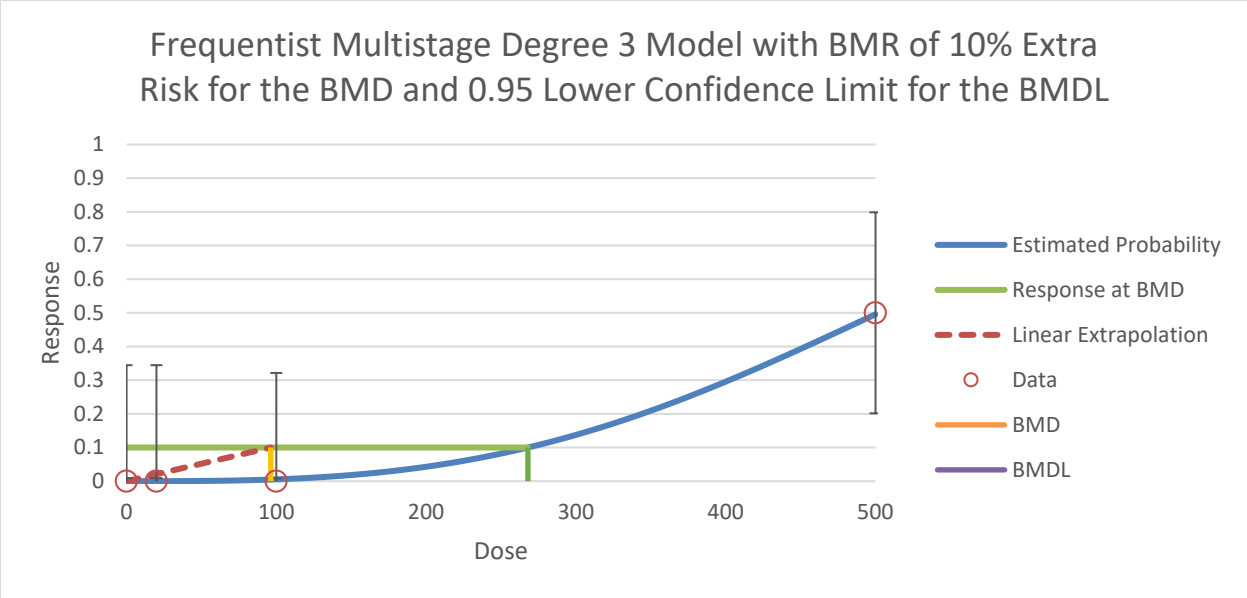

Figure B-13. Dose response curve for the Multistage Degree 3 model fit to hepatocellular hypertrophy in female rats (Loveless et al., 2009).

B.15. HEPATOCELLULAR HYPERTROPHY—MALE RATS (LOVELESS ET AL., 2009)

Table B-26. Dose response data for hepatocellular hypertrophy in male rats (Loveless et al., 2009)

Dose (mg/kg-day)	*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-27. Benchmark dose results for hepatocellular hypertrophy in male rats—nonconstant variance, BMR = 10% Extra Risk (Loveless et al., 2009)

Models	*p*-Values	AIC	10% Extra Risk		Scaled residual for dose group near BMD
			BMD	BMDL	
Dichotomous Hill	1	17.46023	85.47371	28.3855	-1.1E-06
Gamma	0.999944	17.46046	70.57884	20.71965	0.00025
Log-Logistic	1	15.46023	85.49796	28.38513	2.91E-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	4.78E-05
Log-Probit	1	17.46023	78.71963	26.71976	-7.5E-12
Probit	0.999765	15.47853	71.58692	37.71366	0.012573

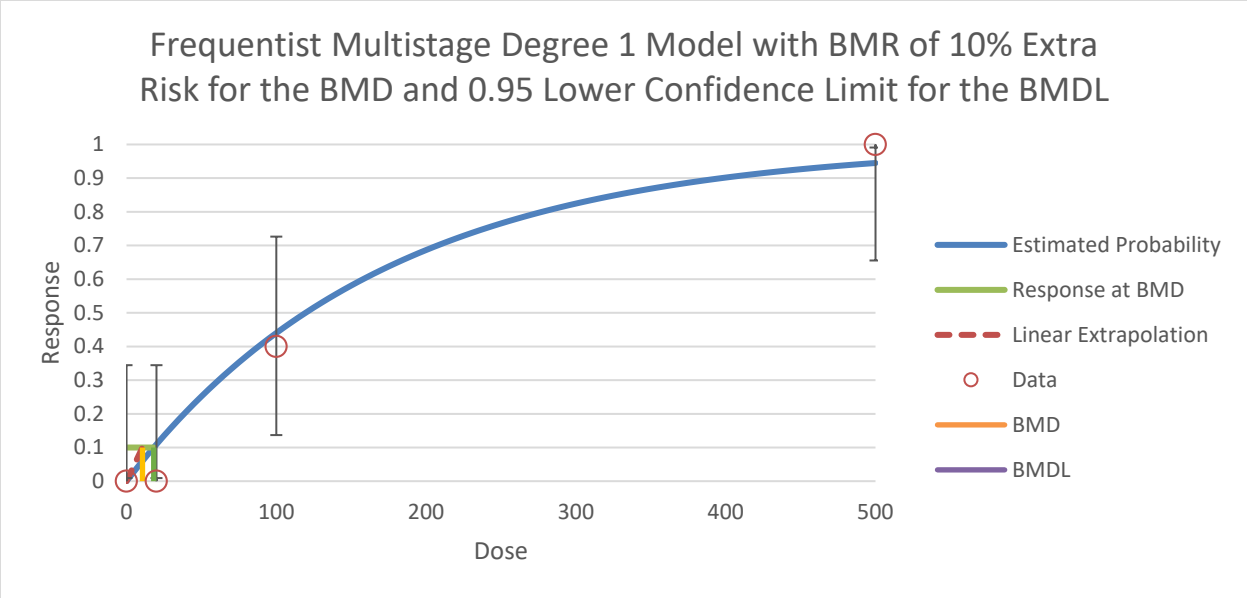

Figure B-14. Dose response curve for the Multistage Degree 1 model fit to hepatocellular hypertrophy in male rats (Loveless et al., 2009).

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B.16. POSTNATAL (F_1) COMBINED RAT BODY WEIGHT ON PND 0 (LOVELESS ET AL., 2009)

Table B-28. Dose response data for postnatal (F1) combined rat body weight on PND 0 (Loveless et al., 2009)

Dose (mg/kg-day)	*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-29. Benchmark dose results for postnatal (F1) combined rat body weight on PND 0—nonconstant variance, BMR = 5% relative deviation (Loveless et al., 2009)

Models	*p*-Values	Test 3 *p*-values	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

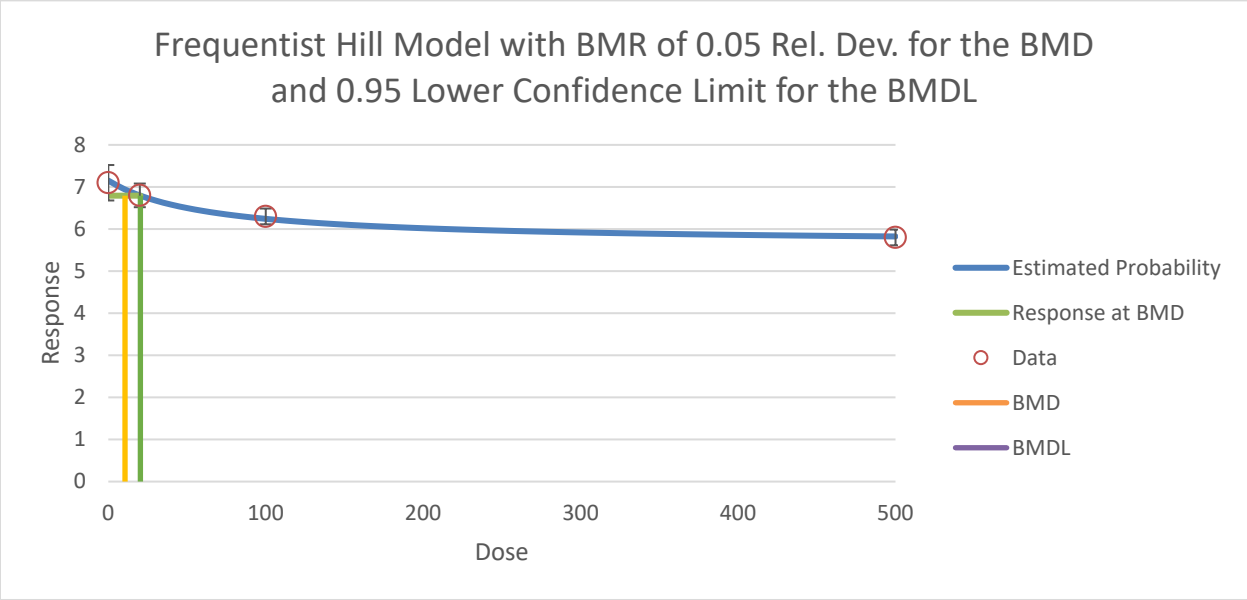

Figure B-15. Dose response curve for the Hill model fit to postnatal (F1) combined rat body weight on PND 0 (Loveless et al., 2009).

B.17. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASE 2) ON PND 0 (IWAI AND HOBERMAN, 2014)

Table B-30. Dose response data for postnatal (F1) combined mouse body weight (Phase 2) on PND 0 (Iwai and Hoberman, 2014)

Dose (mg/kg-day)	*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-31. Benchmark dose results for postnatal (F1) combined mouse body weight (Phase 2) on PND 0—constant variance, BMR = 5% relative deviation (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)

Models	*p*-Values	Test 2 *p*-values	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential2	0.5476652	0.11356	-83.22986065	110.1988	72.6152	-0.18098
Exponential3	0.6427413	0.11356	-82.21886799	162.9802	78.154	-0.00108
Exponential4	0.5476662	0.11356	-83.22986423	110.2315	72.6152	-0.18210
Exponential5	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	2.26045E-05
Linear	0.5601161	0.11356	-83.27482038	111.6004	75.16344	-0.16700

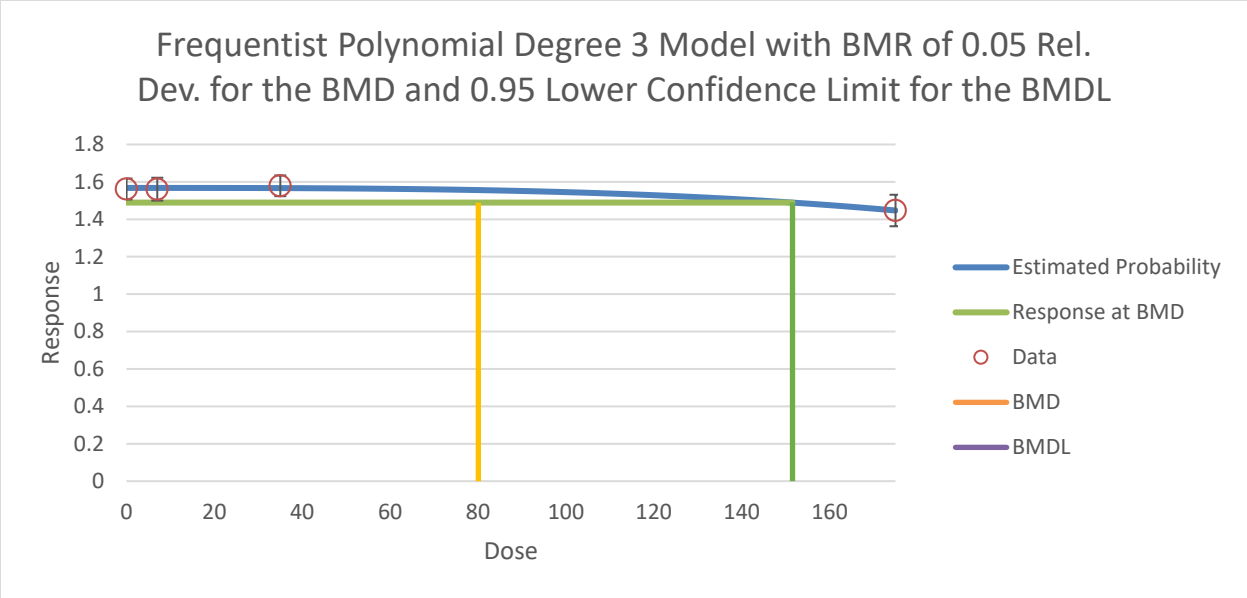

Figure B-16. Dose response curve for the Polynomial Degree 3 model fit to postnatal (F1) combined rat body weight (Phase 2) on PND 0 (Iwai and Hoberman, 2014). X-axis is dose (mg/kg-day) and y-axis is mean body weight (g).

B.18. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASE 1) ON PND 0 (IWAI AND HOBERMAN, 2014)

Table B-32. Dose response data for postnatal (F1) combined mouse body weight (Phase 1) on PND 0 (Iwai and Hoberman, 2014)

Dose (mg/kg-day)	*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-33. Benchmark dose results for postnatal (F1) combined mouse body weight (Phase 1) on PND 0—nonconstant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Models	*p*-Values	Test 3 *p*-values	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.

B.19. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASES 1 AND 2) ON PND 0 (IWAI AND HOBERMAN, 2014)

Table B-34. Dose response data for postnatal (F1) combined mouse body weight (Phases 1 and 2) on PND 0 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-day)	*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
175	20	1.447	0.180
100	19	1.484	0.100
350	19	1.365	0.237
500	13	1.396	0.187

Table B-35. Benchmark dose results for postnatal (F1) combined mouse body weight (Phase 1 and 2) on PND 0—nonconstant variance, BMR = 5% relative deviation Benchmark dose results for postnatal (F_1) combined mouse body weight (Phase 1 and 2) on PND 0—nonconstant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Models	*p*-Values	Test 3 *p*-values	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential2	<0.0001	<0.0001	-113.7083011	143.1894	105.9785	-0.7569
Exponential3	<0.0001	<0.0001	-113.7082872	143.218	105.9774	-0.7574
Exponential4	<0.0001	<0.0001	-111.765811	142.2114	45.41936	-0.7464
Exponential5	<0.0001	<0.0001	-114.9537952	124.9707	78.92966	-1.0491
Hill	<0.0001	<0.0001	-114.8291753	120.1336	86.39634	-0.9939
Polynomial (Poly 6)	<0.0001	<0.0001	-113.1738765	151.9313	116.5059	-0.8482
Polynomial (Poly 5)	<0.0001	<0.0001	-113.1738878	151.8383	114.2464	-0.8469
Polynomial (Poly 4)	<0.0001	<0.0001	-113.1738762	151.9337	114.3852	-0.8482
Polynomial (Poly 3)	<0.0001	<0.0001	-113.1738765	151.9313	114.3477	-0.8482
Polynomial (Poly 2)	<0.0001	<0.0001	-113.1738765	151.9313	114.3472	-0.8482
Power	<0.0001	<0.0001	-113.1738821	151.8842	114.494	-0.8473
Linear	<0.0001	<0.0001	-113.1738823	151.8741	114.3477	-0.8474

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

B.20. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASE 2) ON PND 4 (IWAI AND HOBERMAN, 2014)

Table B-36. Dose response data for postnatal (F1) combined mouse body weight (Phase 2) on PND 4 Dose-response data for postnatal (F_1) combined mouse body weight (Phase 2) on PND 4 (Iwai and Hoberman, 2014)

Dose (mg/kg-day)	*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-37. Benchmark dose results for postnatal (F1) combined mouse body weight (Phase 2) on PND 4—constant variance, BMR = 5% relative deviation 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)

Models	*p*-Values	Test 2 *p*-values	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	4.3382E-06
Linear	0.2746896	0.3216	62.89279543	168.0092	81.96061	-0.247433913

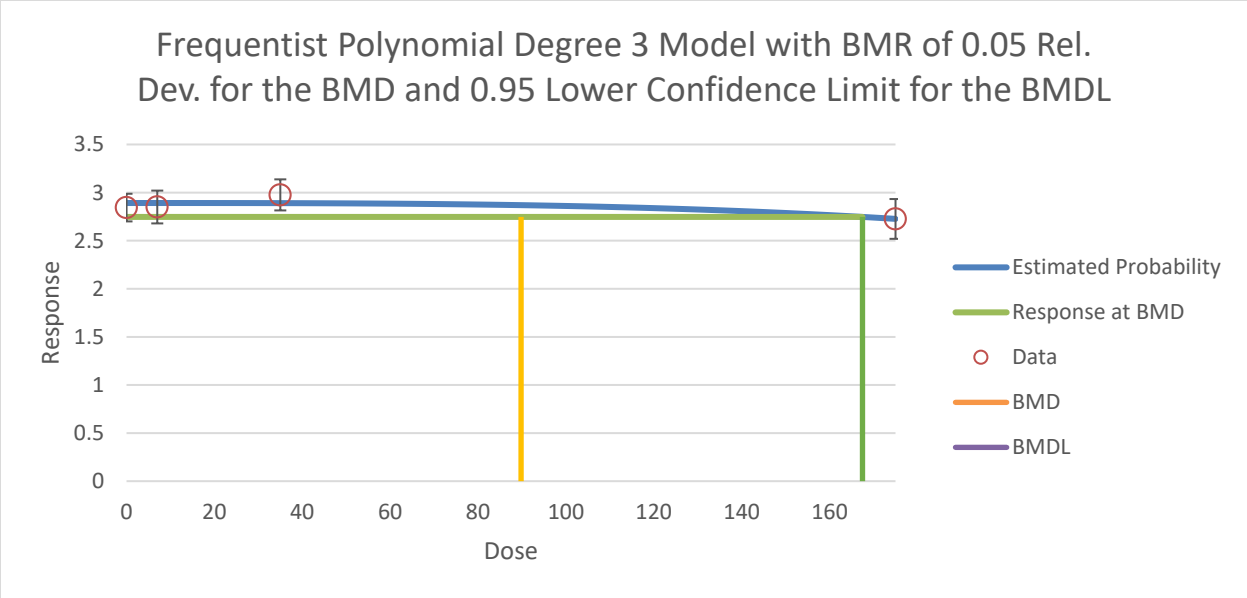

Figure B-17. Dose response curve for the Polynomial model (poly 3) fit to postnatal (F1) combined rat body weight (Phase 2) on PND 4 Dose-response curve for the Polynomial model (poly 3) fit to postnatal (F_1) combined rat body weight (Phase 2) on PND 4 (Iwai and Hoberman, 2014). X-axis is dose (mg/kg-day) and y-axis is mean body weight (g).

B.21. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASE 1) ON PND 4 (IWAI AND HOBERMAN, 2014)

Table B-38. Dose response data for postnatal (F1) combined mouse body weight (Phase 1) on PND 4 Dose-response data for postnatal (F_1) combined mouse body weight (Phase 1) on PND 4 (Iwai and Hoberman, 2014)

Dose (mg/kg-day)	*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-39. Benchmark dose results for postnatal (F1) combined mouse body weight (Phase 1) on PND 4—nonconstant variance, BMR = 5% relative deviation Benchmark dose results for postnatal (F_1) combined mouse body weight (Phase 1) on PND 4—nonconstant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Models	*p*-Values	Test 3 *p*-values	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
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

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

B.22. POSTNATAL (F_1) COMBINED MOUSE BODY WEIGHT (PHASES 1 AND 2) ON PND 4 (IWAI AND HOBERMAN, 2014)

Table B-40. Dose response data for postnatal (F1) combined mouse body weight (Phase 1) on PND 4 Dose-response data for postnatal (F_1) combined mouse body weight (Phase 1) on PND 4 (Iwai and Hoberman, 2014)

Dose (mg/kg-day)	*Number of litters*	Mean (g)	Standard deviation
0	38	2.902	0.387
7	16	2.85	0.320
35	19	2.976	0.335
175	20	2.726	0.442
100	19	2.771	0.248
350	17	2.256	0.650
500	11	2.382	0.482

Table B-41. Benchmark dose results for postnatal (F1) combined mouse body weight (Phase 1 and 2) on PND 4—nonconstant variance, BMR = 5% relative deviation Benchmark dose results for postnatal (F_1) combined mouse body weight (Phase 1 and 2) on PND 4—nonconstant variance, BMR = 5% relative deviation (Iwai and Hoberman, 2014)

Models	*p*-Values	Test 3 *p*-values	AIC	5% relative deviation		Scaled residual for dose group near BMD
				BMD	BMDL	
Exponential2	0.1128908	0.2000	147.7837189	96.33303	71.43861	-0.0192
Exponential3	0.0802807	0.2000	149.2060003	120.9626	73.66277	-0.3003
Exponential4	0.1128924	0.2000	147.78368	96.31896	71.43858	-0.0189
Exponential5	**0.4079141**	**0.2000**	**145.7743154**	**155.2243**	**103.1196**	**0.4065**
Hill	0.3365496	0.2000	146.2589902	165.6672	139.2494	0.3173
Polynomial (poly 6)	0.110707	0.2000	147.8372495	103.2643	78.89672	-0.0983
Polynomial (poly 5)	0.110707	0.2000	147.8372495	103.2643	79.3315	-0.0983
Polynomial (poly 4)	0.110707	0.2000	147.8372495	103.2643	78.87018	-0.0983
Polynomial (poly 3)	0.110707	0.2000	147.8372495	103.2643	78.84946	-0.0983
Polynomial (poly 2)	0.110707	0.2000	147.8372495	103.2643	78.84795	-0.0983
Power	0.0672452	0.2000	149.6433094	118.3717	79.54891	-0.2558
Linear	0.110707	0.2000	147.8372495	103.2643	78.84954	-0.0983

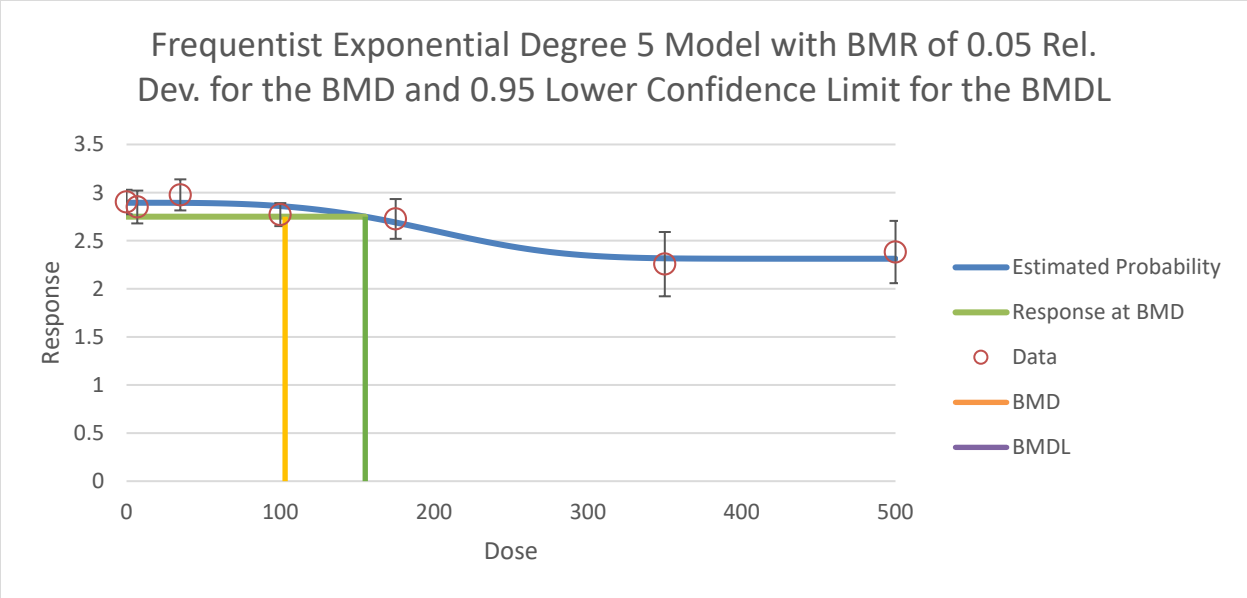

Figure B-18. Dose response curve for the Exponential 5 model fit to postnatal (F1) combined mouse body weight (Phases 1 and 2) on PND 4 Dose-response curve for the Exponential 5 model fit to postnatal (F_1) combined mouse body weight (Phases 1 and 2) on PND 4 (Iwai and Hoberman, 2014). X-axis is dose (mg/kg-day) and y-axis is mean body weight (g).

B.23. PERINATAL MORTALITY (PHASE 2) ON PNDS 0–21 (IWAI AND HOBERMAN, 2014)

Table B-42. Nested Model summary for perinatal mortality (Phase 2) on PNDs 0–21, BMR = 1% extra risk Nested Model summary for perinatal mortality (Phase 2) on PNDs 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
Nlogistic	Yes	Yes	0.2147	145.1	201.8	169.6
	Yes	No	0.006	158.29	157.6	39.26
	No	Yes	0.2107	141.2	151.4	24.77
	No	No	0.003	154.5	158.0	39.03
NCTR	Yes	Yes	0.2117	142.99	151.1	76.33
	Yes	No	0.0037	155.82	157.7	80.50
	No	**Yes**	**0.2213**	**139.20**	**151.5**	**78.90**
	No	No	0.0047	152.50	158.1	131.8
Rai Van Ryzin	Yes	Yes	0.1933	143.20	151.7	0.161
	Yes	No	0.0037	156.50	158.3	0.200
	No	**Yes**	**0.2053**	**139.20**	**151.7**	**126.4**
	No	No	0.0033	152.50	158.3	131.9

NCTR = National Center for Toxicological Research.

An average of BMDLs from NCTR (BMDL of 78.90 mg/kg-day) and Rai Van Ryzin (126.4 mg/kg-day) models with an identical AIC value is selected as the final BMDL (102.65 mg/kg/day).

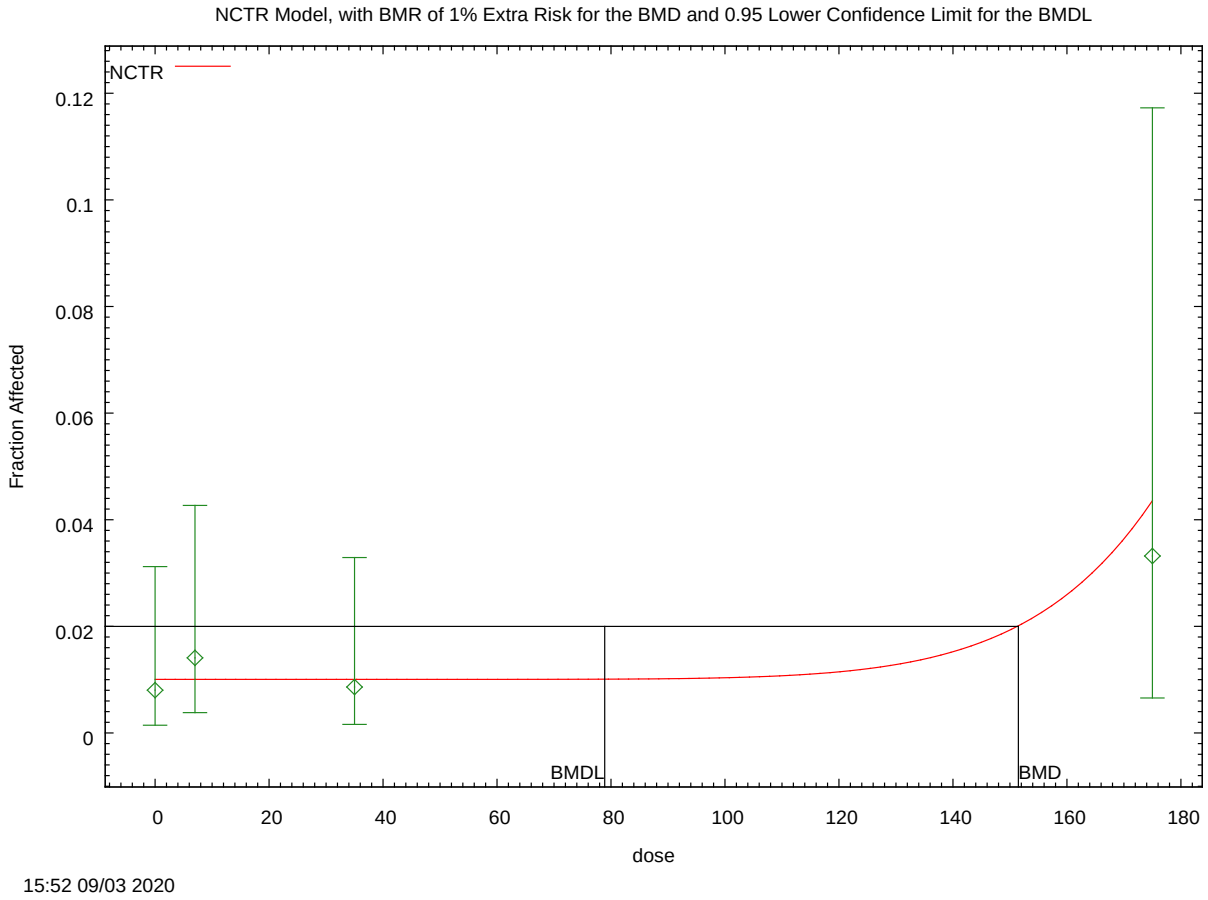

Figure B-19. Dose response curve for the Nested NCTR model fit to perinatal mortality (Phase 2) on PND 4 (0–21) Dose-response curve for the Nested NCTR model fit to perinatal mortality (Phase 2) on PND 4 (0–21) (Iwai and Hoberman, 2014). X-axis is dose (mg/kg-day) and y-axis is mean body weight (g).

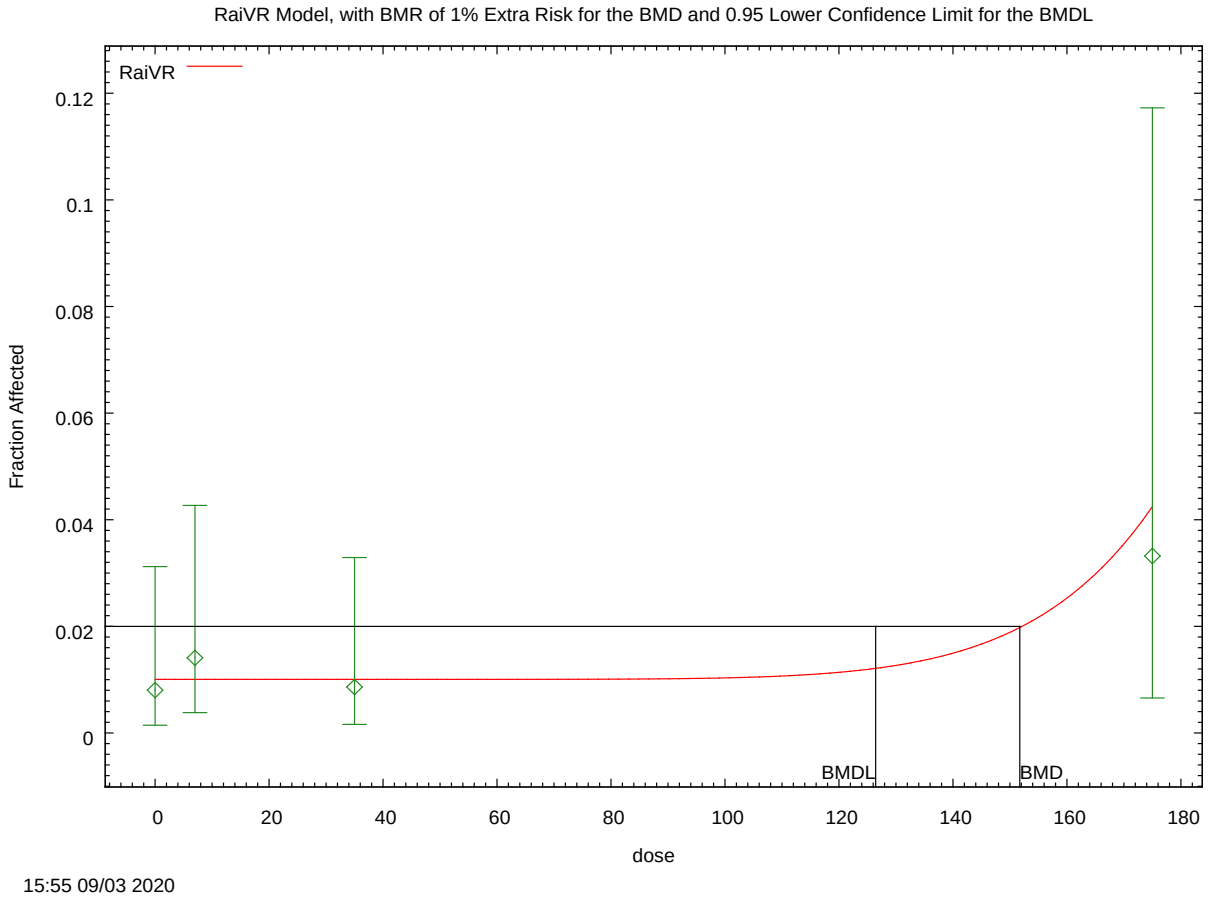

Figure B-20. Dose response curve for the Rai Van Ryzin model fit to perinatal mortality (Phase 2) on PND 4 (0–21) Dose-response curve for the Rai Van Ryzin model fit to perinatal mortality (Phase 2) on PND 4 (0–21) (Iwai and Hoberman, 2014). X-axis is dose (mg/kg-day) and y-axis is mean body weight (g).

B.24. PERINATAL MORTALITY (PHASE 1) ON PNDS 0–21 (IWAI AND HOBERMAN, 2014)

Table B-43. Nested model summary for perinatal mortality (Phase 1) on PNDs 0–21, BMR = 1% extra risk Nested model summary for perinatal mortality (Phase 1) on PNDs 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
Nlogistic	Yes	Yes	0.0553	356.23	206.1	105.8
	Yes	No	0	478.37	238.9	177.4
	No	**Yes**	**0.0613**	**353.33**	**201.7**	**98.61**
	No	No	0	477.04	233.1	162.70
NCTR	Yes	Yes	0.0557	356.27	199.3	99.65
	Yes	No	0	476.66	241.1	120.6
	No	Yes	0.0557	353.38	193.5	96.76
	No	No	0	477.06	216.1	108.0
Rai Van Ryzin	Yes	Yes	0.0627	357.38	193.5	1.422
	Yes	No	0	481.06	216.1	108.0
	No	Yes	0.0480	353.38	193.5	96.76
	No	No	0	477.06	216.1	108.0

NCTR = National Center for Toxicological Research.
NLogistic model with litter-specific covariate and intralitter correlation is selected as the best model based the lowest AIC value.

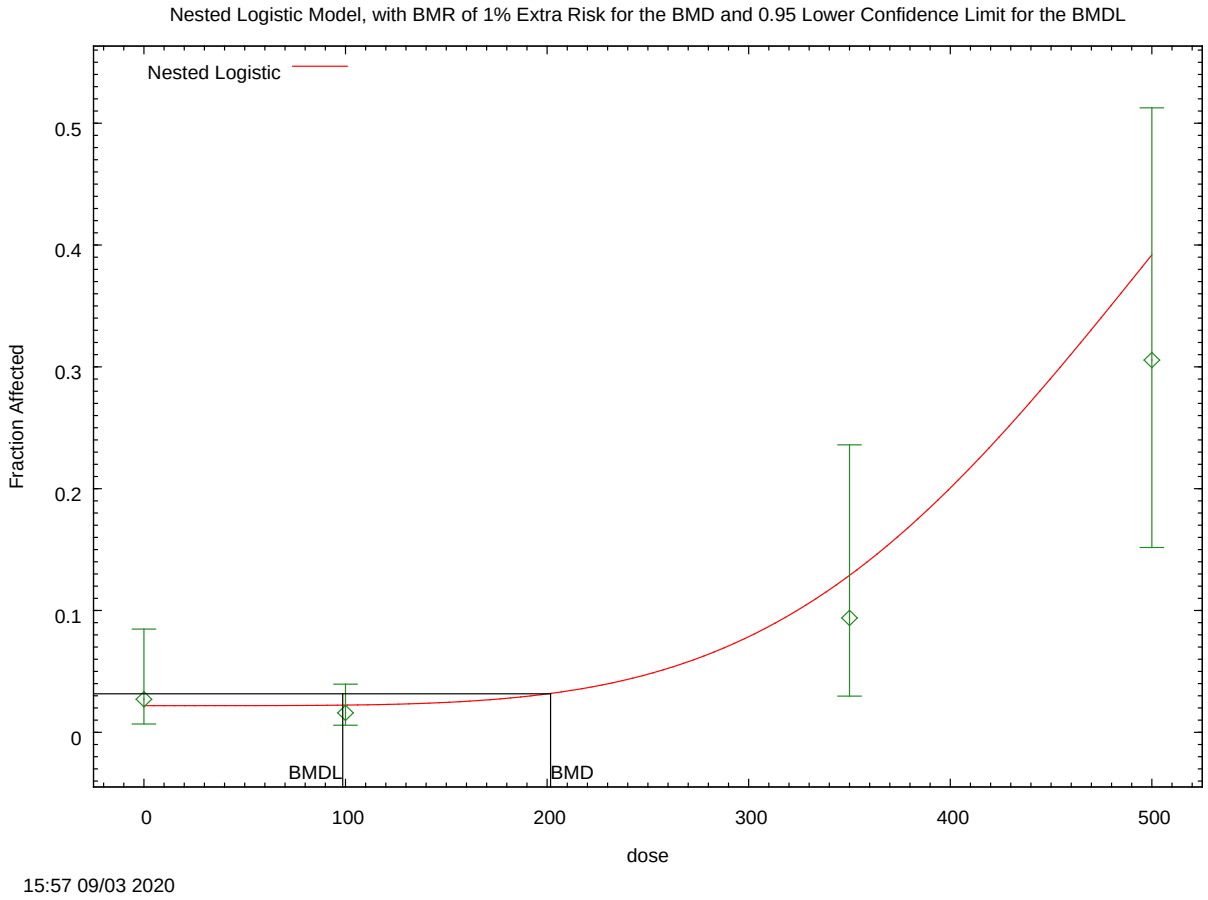

Figure B-21. Dose response curve for the Nested NLogistic model fit to perinatal mortality (Phase 1) on PND 4 (0–21) Dose-response curve for the Nested NLogistic model fit to perinatal mortality (Phase 1) on PND 4 (0–21) (Iwai and Hoberman, 2014). X-axis is dose (mg/kg-day) and y-axis is mean body weight (g).

B.25. PERINATAL MORTALITY (PHASES 1 AND 2) ON PNDS 0–21 (IWAI AND HOBERMAN, 2014)

Table B-44. Nested model summary for perinatal mortality (Phase 1 and 2) on PNDs 0–21, BMR = 1% extra risk Nested model summary for perinatal mortality (Phase 1 and 2) on PNDs 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
Nlogistic	Yes	Yes	0.0373	495.44	150.8	85.15
	Yes	No	0	632.14	199.7	138.2
	No	Yes	0.0357	491.8	147.7	83.59
	No	No	0	629.52	195.2	134.0
NCTR	Yes	Yes	0.0283	495.34	148.6	74.3
	Yes	No	0	630.65	194.4	97.2
	No	Yes	0.0327	491.70	145.0	72.5
	No	No	0	629.20	186.6	93.3
Rai Van Ryzin	Failed					

NCTR = National Center for Toxicological Research.

Because none of the models provided adequate fit (goodness of fit *p*-value > 0.1) to combined Phases 1 and 2 data, no BMDL could be estimated.

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, 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 in 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 Shawn Gannon, The Chemours Company, Wilmington, Delaware (personnel communication). 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 h. 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-h 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 h so the substitution was made prior to calculating the mean. Because all animals in this group were below LLOQ at 8 h, the value for that time point was treated as equal to LLOQ/$\sqrt{2}$, and the results for the 24-h time-point were censored. Finally, in the 175 mg/kg group, one animal in the 8-h group had a reported concentration 15 times higher than the other two animals in that group, 80% higher than the average concentration for that dose at 6 h and higher than any animal in the 350 mg/kg group at the same time point (8 h). 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 (Section 5.2.1, Approach for Animal-Human Extrapolation of PFHxA Dosimetry).

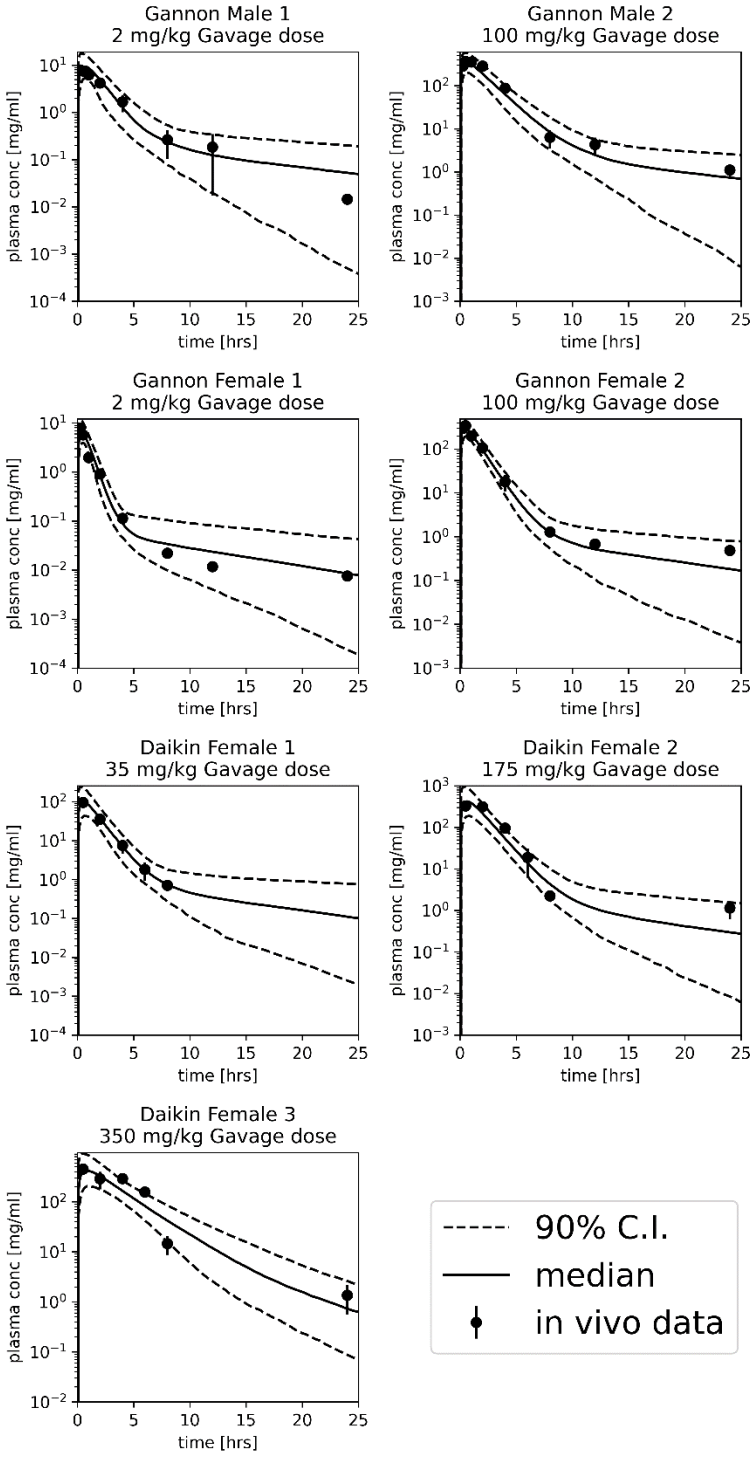

Figure C-1. Fits of population pharmacokinetic model to data for male (top row) and female (remaining rows) mice following 2–350 mg/kg oral exposure PFHxA.

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 (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 PHFxA 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, we 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 13.78 (5.51–32.86) days. Technicians 0–7 represent data from the 2007 ski season while technicians 8 and 9 are technician 0 and 1 measured again for the 2008 ski season.

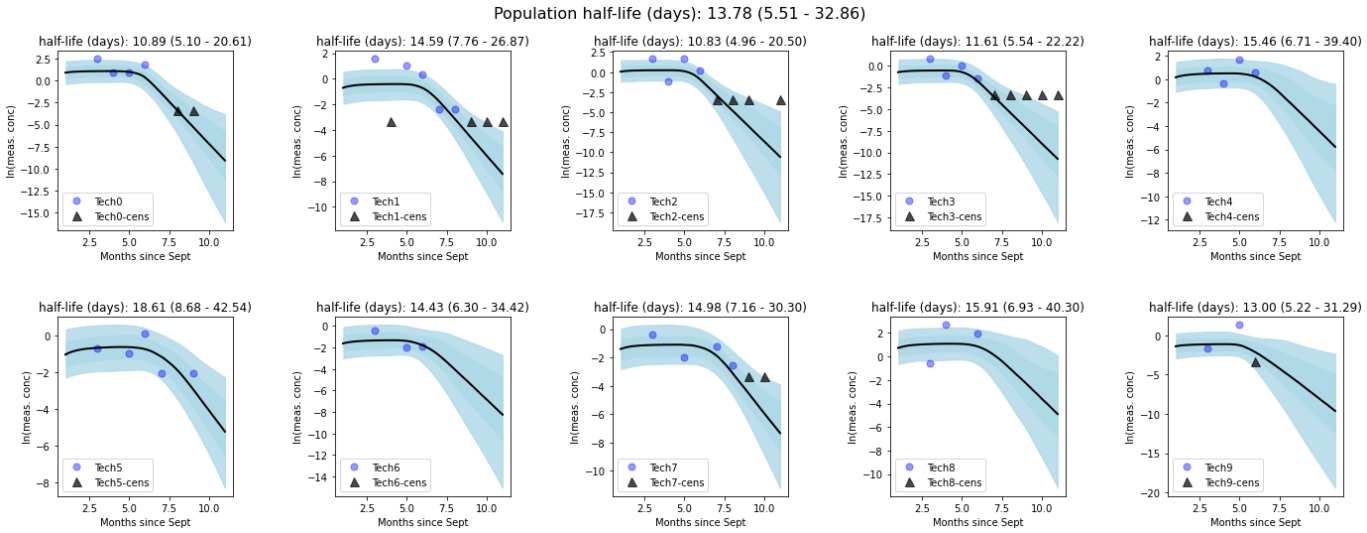

Figure C-2. Fits of human PFHxA data from ski-wax technician blood samples. Blue circles represent data above LOD while black triangles are data samples reported at the LOD (<0.05 ng/mL). 60% and 90% credible intervals are illustrated with the darker inside band and lighter outside band, respectively.

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 2020. 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-1-3	October 2020
An Umbrella Quality Assurance Project Plan (QAPP) for Dosimetry and Mechanism-Based Models (PBPK)	L-CPAD-0032188-QP-1-2	December 2020

Title	Document number	Date
Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS)	L-HEEAD-0032189-QP-1-2	September 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 2020	QA audit	None	None
July 2021	QA 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 *[insert chemical docket number—make sure the comments are in the docket]* on http://www.regulations.gov.

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

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