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Toxicological Review of Perfluorobutanoic Acid (PFBA) and Related Compound Ammonium Perfluorobutanoic Acid

(CASRN 375-22-4
CASRN 10495-86-0)

Supplemental Information—Appendices A though F

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Integrated Risk Information System
Center for Public Health and Environmental Assessment
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CONTENTS

TABLES

FIGURES

ABBREVIATIONS AND ACRONYMS

ACO	acyl-CoA oxidase
ADME	absorption, distribution, metabolism, and excretion
AFFF	aqueous film-forming foam
AIC	Akaike's information criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area-under-the-concentration curve
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BW	body weight
C_{AVG}	average concentration
C_{MAX}	maximum concentration
CA	Cochran-Armitage
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service registry number
CDR	Chemical Data Reporting
CI	confidence interval
CL	clearance
CL_A	clearance in animals
CL_H	clearance in humans
CPAD	Chemical and Pollutant Assessment Division
CPHEA	Center for Public Health and Environmental Assessment
CV	constant variance
CYP450	cytochrome P450 superfamily
DAF	dosimetric adjustment factor
DNA	deoxyribonucleic acid
DNT	developmental neurotoxicity
DOD	Department of Defense
EPA	Environmental Protection Agency
EOP	Executive Office of the President
ER	extra risk
FLR	full-litter resorption
FTOH	fluorotelomer alcohol
GD	gestation day
GFR	glomerular filtration rate
GGT	γ-glutamyl transferase
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
GSH	glutathione
HAWC	Health Assessment Workspace Collaborative
HED	human equivalent dose
HERO	Health and Environmental Research Online
HISA	highly influential scientific information
HPT	hypothalamic-pituitary-thyroid
IRIS	Integrated Risk Information System
i.v.	intravenous
IQ	intelligence quotient
IQR	interquartile range
ISI	influential scientific information
IUR	inhalation unit risk
LLOQ	lower limit of quantitation
LN	log-normal
LOAEL	lowest-observed-adverse-effect level
MBq	megabecquerel
MOA	mode of action
NCEA	National Center for Environmental Assessment
NCV	nonconstant variance
NIOSH	National Institute for Occupational Safety and Health
NIS	sodium-iodide symporter
NOAEL	no-observed-adverse-effect level
NPL	National Priority List
NTP	National Toxicology Program
OAT	organic anion transporter
OECD	Organisation for Economic Co-operation and Development
OMB	Office of Management and Budget
ORD	Office of Research and Development
OSF	oral slope factor
PC	partition coefficient
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PECO	Populations, Exposures, Comparators, Outcomes
PFAA	perfluoroalkyl acid
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFCA	perfluoroalkyl 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 proliferator-activated receptor
PQAPP	Programmatic Quality Assurance Project Plan
PT	prothrombin time
PXR	pregnane X receptor
QA	quality assurance
QAPP	Quality Assurance Project Plan
QMP	Quality Management Plan
RBC	red blood cell
RD	relative deviation
RfC	inhalation reference concentration
RfD	oral reference dose
RS	Rao-Scott
SD	standard deviation
S-D	Sprague-Dawley
SE	standard error
TD	toxicodynamic
TH	thyroid hormone
TK	toxicokinetic
TPO	thyroid peroxidase
TRI	Toxic Release Inventory
TSCA	Toxic Substances Control Act
TSCATS	Toxic Substances Control Act Test Submissions
TSH	thyroid-stimulating hormone
TSHR	thyroid-stimulating hormone receptor
UCMR	Unregulated Contaminant Monitoring Rule
UDP-GT	uridine 5′-diphospho-glucuronosyltransferase
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
V_d	volume of distribution
VOC	volatile organic compound
WOS	Web of Science

APPENDIX A. SYSTEMATIC REVIEW PROTOCOL FOR THE PFAS IRIS ASSESSMENTS

A single systematic review protocol was used to guide the development of five, separate IRIS PFAS assessments (i.e., PFBA, PFHxA, PFHxS, PFNA, and 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. ADDITIONAL DETAILS OF SYSTEMATIC REVIEW METHODS AND RESULTS

Table B-1. Perfluorobutanoic acid (PFBA) database search strategy

Search	Search strategy	Dates of search
PubMed		
Search terms	375-22-4[rn] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "Kyselina heptafluormaselna"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "2,2,3,3,4,4,4-heptafluoro-Butanoic acid"[tw] OR "Butanoic acid, 2,2,3,3,4,4,4-heptafluoro-"[tw] OR "Butanoic acid, heptafluoro-"[tw] OR "Perfluoro-n-butanoic acid"[tw] OR "Perfluorobutanoate"[tw] OR "2,2,3,3,4,4,4-Heptafluorobutanoic acid"[tw] OR "Butyric acid, heptafluoro-"[tw] OR "Fluorad FC 23"[tw] OR "H 0024"[tw] OR "NSC 820"[tw] OR ((PFBA[tw] OR "FC 23"[tw] OR HFBA[tw]) AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw] OR PFOS[tw] OR PFOA[tw]))	No date limit–7/19/2017
Literature update search terms	(((375-22-4[rn] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "Kyselina heptafluormaselna"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "2,2,3,3,4,4,4-heptafluoro-Butanoic acid"[tw] OR "Butanoic acid, 2,2,3,3,4,4,4-heptafluoro-"[tw] OR "Butanoic acid, heptafluoro-"[tw] OR "Perfluoro-n-butanoic acid"[tw] OR "Perfluorobutanoate"[tw] OR "2,2,3,3,4,4,4-Heptafluorobutanoic acid"[tw] OR "Butyric acid, heptafluoro-"[tw] OR "Fluorad FC 23"[tw] OR "H 0024"[tw] OR "NSC 820"[tw] OR ((PFBA[tw] OR "FC 23"[tw] OR HFBA[tw]) AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw] OR PFOS[tw] OR PFOA[tw])) AND ("2017/08/01"[PDAT] : "2018/02/14"[PDAT])	8/1/2017–2/14/2018

Search	Search strategy	Dates of search
Web of Science		
Search terms	TS="Heptafluoro-1-butanoic acid" OR TS="Heptafluorobutanoic acid" OR TS="Heptafluorobutyric acid" OR TS="Kyselina heptafluormaselna" OR TS="Perfluorobutanoic acid" OR TS="Perfluorobutyric acid" OR TS="Perfluoropropanecarboxylic acid" OR TS="2,2,3,3,4,4,4-heptafluoro-Butanoic acid" OR TS="Butanoic acid, 2,2,3,3,4,4,4-heptafluoro-" OR TS="Butanoic acid, heptafluoro-" OR TS="Perfluoro-n-butanoic acid" OR TS="Perfluorobutanoate" OR TS="2,2,3,3,4,4,4-Heptafluorobutanoic acid" OR TS="Butyric acid, heptafluoro-" OR TS="Fluorad FC 23" OR TS="H 0024" OR TS="NSC 820" OR (TS=(PFBA OR "FC 23" OR HFBA) AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR perfluoroo* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA))	No date limit−7/20/2017
Literature update search terms	((TS="Heptafluoro-1-butanoic acid" OR TS="Heptafluorobutanoic acid" OR TS="Heptafluorobutyric acid" OR TS="Kyselina heptafluormaselna" OR TS="Perfluorobutanoic acid" OR TS="Perfluorobutyric acid" OR TS="Perfluoropropanecarboxylic acid" OR TS="2,2,3,3,4,4,4-heptafluoro-Butanoic acid" OR TS="Butanoic acid, 2,2,3,3,4,4,4-heptafluoro-" OR TS="Butanoic acid, heptafluoro-" OR TS="Perfluoro-n-butanoic acid" OR TS="Perfluorobutanoate" OR TS="2,2,3,3,4,4,4-Heptafluorobutanoic acid" OR TS="Butyric acid, heptafluoro-" OR TS="Fluorad FC 23" OR TS="H 0024" OR TS="NSC 820") OR TS=(PFBA OR "FC 23" OR HFBA) AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR perfluoroo* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA)) AND PY=2017-2018	2017−2018
Toxline		
Search terms	(375-22-4 [rn] OR "heptafluoro-1-butanoic acid" OR "heptafluorobutanoic acid" OR "heptafluorobutyric acid" OR "kyselina heptafluormaselna" OR "perfluorobutanoic acid" OR "perfluorobutyric acid" OR "perfluoropropanecarboxylic acid" OR "2,2,3,3,4,4,4-heptafluoro-butanoic acid" OR "butanoic acid 2 2 3 3 4 4 4-heptafluoro-" OR "butanoic acid heptafluoro-" OR "perfluoro-n-butanoic acid" OR "perfluorobutanoate" OR "2,2,3,3,4,4,4-heptafluorobutanoic acid" OR "butyric acid heptafluoro-" OR "fluorad fc 23" OR "h 0024" OR "nsc 820" OR ((pfba OR "fc 23" OR hfba) AND (fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro* OR perfluorinated OR fluorinated OR pfas OR pfos OR pfoa))) AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]	No date limit−7/20/2017

Search	Search strategy	Dates of search
Literature update search terms	@AND+@OR+("heptafluoro-1-butanoic acid"+"heptafluorobutanoic+acid"+"heptafluorobutyric+acid"+"kyselina+heptafluormaselna"+"perfluorobutanoic+acid"+"perfluorobutyric+acid"+"perfluoropropanecarboxylic +acid"+"2 2 3 3 4 4 4-heptafluoro-butanoic+acid"+"butanoic+acid+2 2 3 3 4 4 4-heptafluoro-"+"butanoic+acid+heptafluoro-"+"perfluoro-n-butanoic acid"+"perfluorobutanoate"+"2 2 3 3 4 4 4-heptafluorobutanoic+acid"+"butyric+acid+heptafluoro-"+"fluorad+fc+23"+"h0024"+"nsc+820"+@TERM+@rn+375-22-4("pfba"+"fc+23"+"hfba"))+(fluorocarbon*+ fluorotelomer*+polyfluoro*+perfluoro*+perfluorinated+fluorinated+pfas+pfos+pfoa)+@RANGE+yr+2017+2018	2017−2018
TSCATS		
Search terms	375-22-4[rn] AND tscats[org]	No date limit−7/20/2017

Table B-2. Title/abstract-level screening criteria for the initial literature searches

	Inclusion criteria	Exclusion criteria
Populations	Humans Standard mammalian animal models, including rat, mouse, rabbit, guinea pig, hamster, monkey, dog Alternative animal models in standard laboratory conditions (e.g., *Xenopus*, zebrafish, minipig) Human or animal cells, tissues, or organs (not whole animals); bacteria, nonmammalian eukaryotes; other nonmammalian laboratory species	• Ecological species
Exposures	Exposure is to PFBA Exposure via oral, inhalation, dermal, intraperitoneal, or intravenous injection routes Exposure is measured in air, dust, drinking water, diet, gavage, injection or via a biomarker of exposure (PFBA levels in whole blood, serum, plasma, or breastmilk)	• Study population is not exposed to PFBA • Exposure is to a mixture only
Outcomes	Studies that include a measure of one or more health effect endpoints, including but not limited to, effects on reproduction, development, developmental neurotoxicity, liver, thyroid, immune system, nervous system, genotoxicity, and cancer In vivo or in vitro studies related to toxicity mechanisms, physiological effects/adverse outcomes, and studies useful for elucidating toxic modes of action (MOAs) Qualitative or quantitative description of absorption, distribution, metabolism, excretion, toxicokinetic or toxicodynamic models (e.g., PBPK, PBTK, PBTK/TD) Studies addressing risks to infants, children, pregnant women, occupational workers, the elderly, and any other susceptible or differentially exposed populations	

	Inclusion criteria	Exclusion criteria
Other	Structure and physiochemical properties Reviews and regulatory documents	Not on topic, including: • Abstract only, inadequately reported abstract, or no abstract and not considered further because study was not potentially relevant • Bioremediation, biodegradation, or chemical or physical treatment of PFBA, including evaluation of wastewater treatment technologies and methods for remediation of contaminated water and soil • Ecosystem effects • Studies of environmental fate and transport of PFBA in environmental media • Analytical methods for detecting/measuring PFAS compounds in environmental media and use in sample preparations and assays • Studies describing the manufacture and use of PFBA • Not chemical specific (studies that do not involve testing of PFBA) • Studies that describe measures of exposure to PFBA without data on associated health effects

MOA = mode of action; PBPK = physiologically based pharmacokinetic; PBTK = physiologically based toxicokinetic; TD = toxicodynamic.

Table B-3. Example DistillerSR form questions to be used for title/abstract-level and full text-level screening for literature search updates from 2019

	Used in title/abstract and full-text screening					Used in full text screening only	
Question	**Source of study if not identified from database search?**	**Does the article meet PECO criteria?**	**If meets PECO, what type of evidence?**	**If supplemental, what type of information?**	**Which PFAS did the study report?**	**If meets PECO, which health outcome(s) apply?**	**If meets PECO and endocrine outcome, which endocrine tags apply?**
Answer options (can select multiple options)	• Source other than HERO database search	• Yes • No • Unclear • Tag as potentially relevant supplemental information	• Human • Animal (mammalian models) • In vitro or in silico genotoxicity • PBPK or PK model	• In vivo mechanistic or MOA studies, including non-PECO routes of exposure (e.g., injection) and populations (e.g., nonmammalian) • In vitro or in silico studies (nongenotoxicity) • ADME/toxicokinetic (excluding models) • Exposure assessment or characterization (no health outcome) • PFAS mixture study (no individual PFAS comparisons) • Human case reports or case series • Ecotoxicity studies	• PFBA • PFHxA • PFHxS • PFNA • PFDA	• General toxicity, including body weight, mortality, and survival • Cancer • Cardiovascular, including serum lipids • Endocrine (hormone) • Gastrointestinal • Genotoxicity • Growth (early life) and development • Hematological, including nonimmune/hepatic/ renal clinical chemistry measures • Hepatic, including liver measures and serum markers (e.g., ALT; AST) • Immune/ inflammation	• Adrenal • Sex hormones (e.g., androgen; estrogen; progesterone) • Neuroendocrine • Pituitary • Steroidogenesis • Thyroid

	Used in title/abstract and full-text screening					Used in full text screening only	
Question	**Source of study if not identified from database search?**	**Does the article meet PECO criteria?**	**If meets PECO, what type of evidence?**	**If supplemental, what type of information?**	**Which PFAS did the study report?**	**If meets PECO, which health outcome(s) apply?**	**If meets PECO and endocrine outcome, which endocrine tags apply?**
				• Environmental fate or occurrence (including food) • Manufacture, engineering, use, treatment, remediation, or laboratory methods • Other assessments or records with no original data (e.g., reviews, editorials, commentaries)		• Musculoskeletal • Nervous system, including behavior and sensory function • Nutrition and metabolic • Ocular • PBPK or PK model • Renal, including urinary measures (e.g., protein) • Reproductive • Respiratory • Skin and connective tissue effects	

ADME = absorption, distribution, metabolism, and excretion; ALT = alanine aminotransferase; AST = aspartate aminotransferase; HERO = Health and Environmental Research Online; MOA = mode of action; PBPK = physiologically based pharmacokinetic; PECO = Populations, Exposures, Comparators, and Outcomes; PFAS = per- and polyfluoroalkyl substance; PFBA = perfluorobutanoic acid; PFDA = perfluorodecanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexanesulfonate; PFNA = perfluorononanoic acid; PK = pharmacokinetic.

APPENDIX C. ADDITIONAL TOXICOKINETIC INFORMATION IN SUPPORT OF DOSE-RESPONSE ANALYSIS

C.1. USE OF HALF-LIVES OF EXCRETION FOR DOSIMETRIC ADJUSTMENTS

The pharmacokinetics of PFBA have only been measured after direct administration of PFBA in single-exposure/single-day studies in animals (Chang et al., 2008). For the mouse, Chang et al. (2008) performed 24-hour toxicokinetic studies after 10, 30, and 100 mg/kg oral doses. Based on the area-under-the-concentration-curve (AUC) and maximum concentration (C_{max}), the data also appear approximately linear below 30 mg/kg but show some saturation above that dose rate (see Figure C-1, Figure C-2).

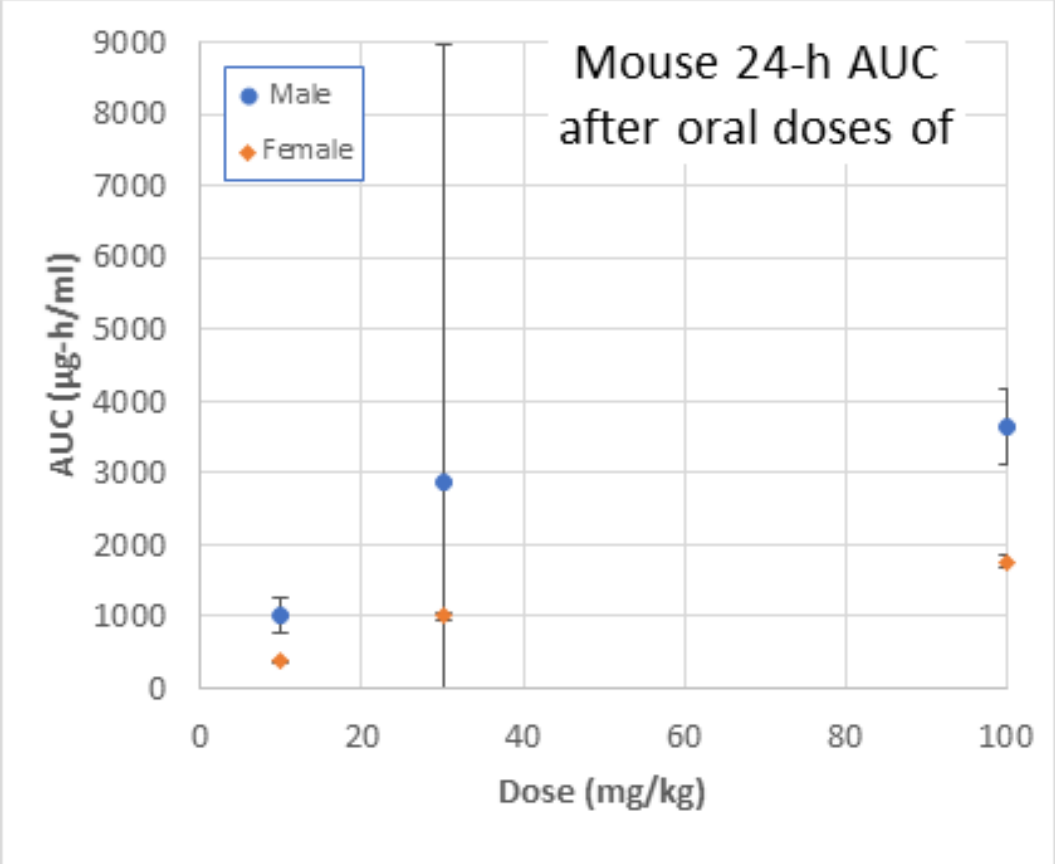

Figure C-1. Mouse AUC after oral doses of PFBA.

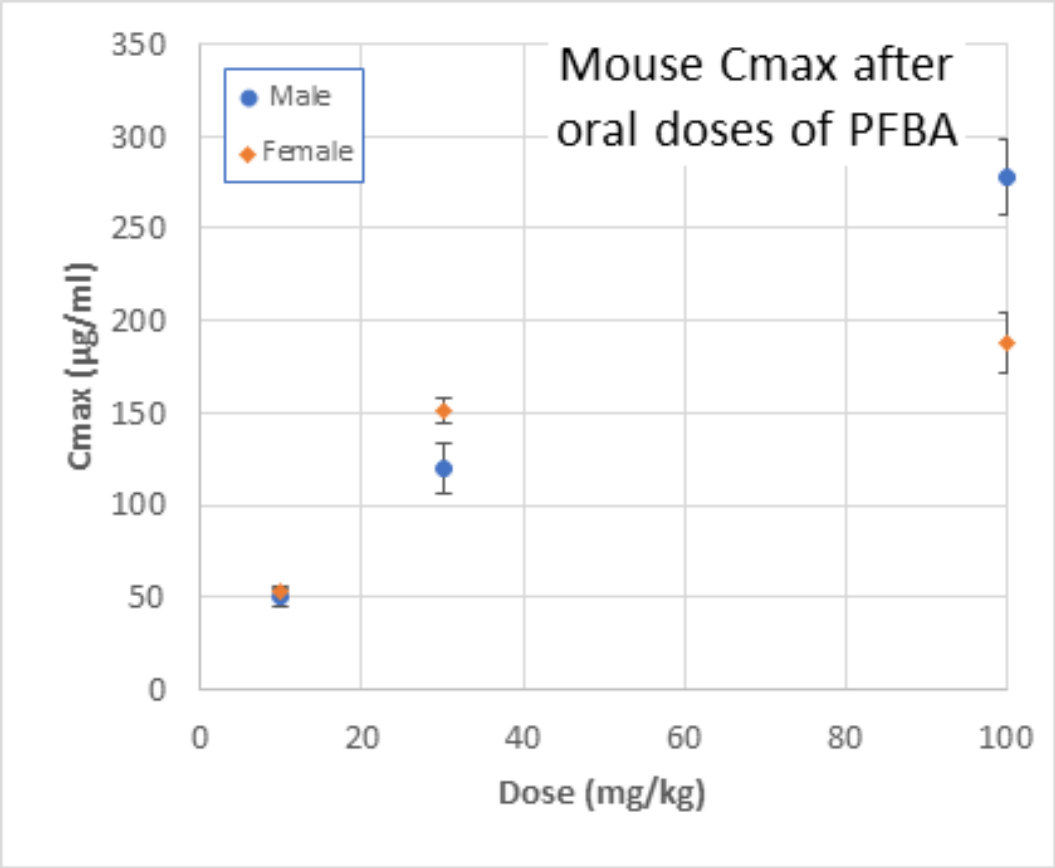

Figure C-2. Mouse C_{max} after oral doses of PFBA.

Chang et al. (2008) reported serum and liver concentrations in male rats and serum concentrations in female rats given a 3–300 mg/kg oral dose of PFBA at 24 hours after dosing. Although the time point for these measurements is not ideal given the short half-life of PFBA, the data indicate that the dosimetry is approximately linear up to 100 mg/kg in male rats and up to 30 mg/kg in female rats (see Figure C-3, Figure C-4). Tissue levels then appear to saturate or decline; this might be due to incomplete absorption at higher doses, saturable renal resorption, or both, whereby excretion is more rapid for concentrations above the level of saturable resorption in the kidney. With the half-life in female rats being ~3 hours, the female serum 24-hour data are particularly subject to experimental noise, but at least provide an indication that use of the half-life measured using a 30 mg/kg dose is applicable to BMD levels from bioassays at or below this dose rate.

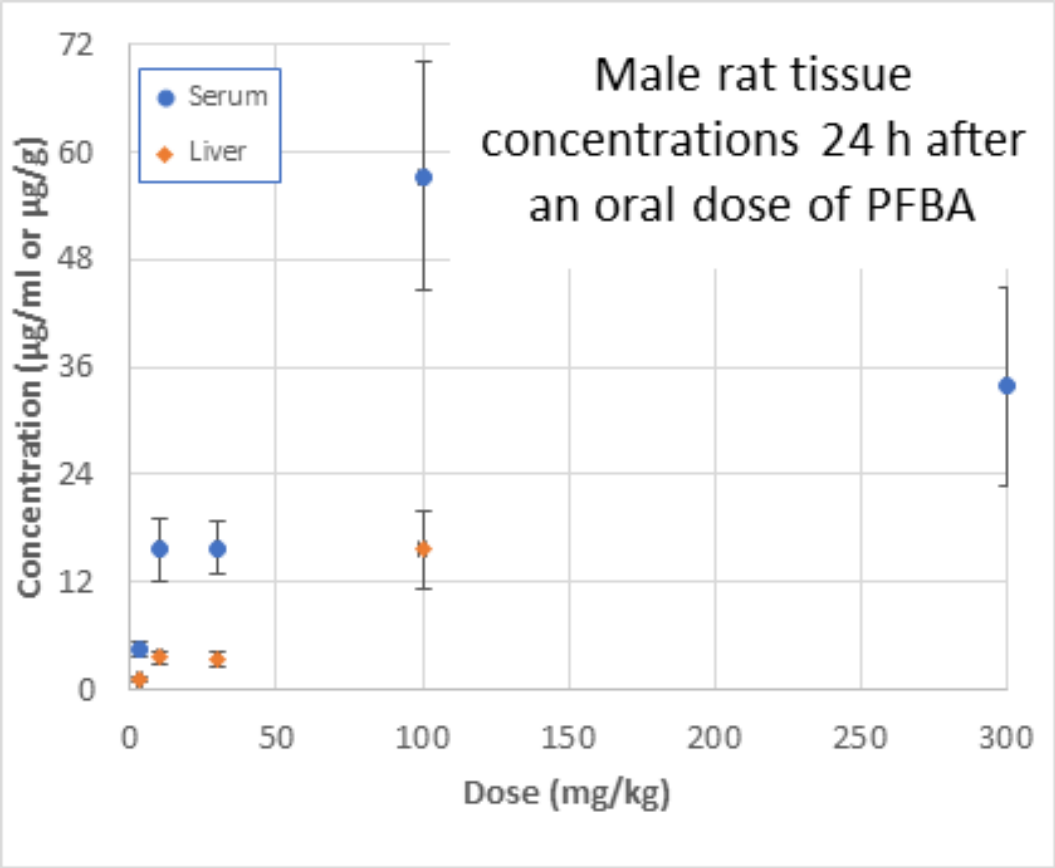

Figure C-3. Rat AUC after oral doses of PFBA.

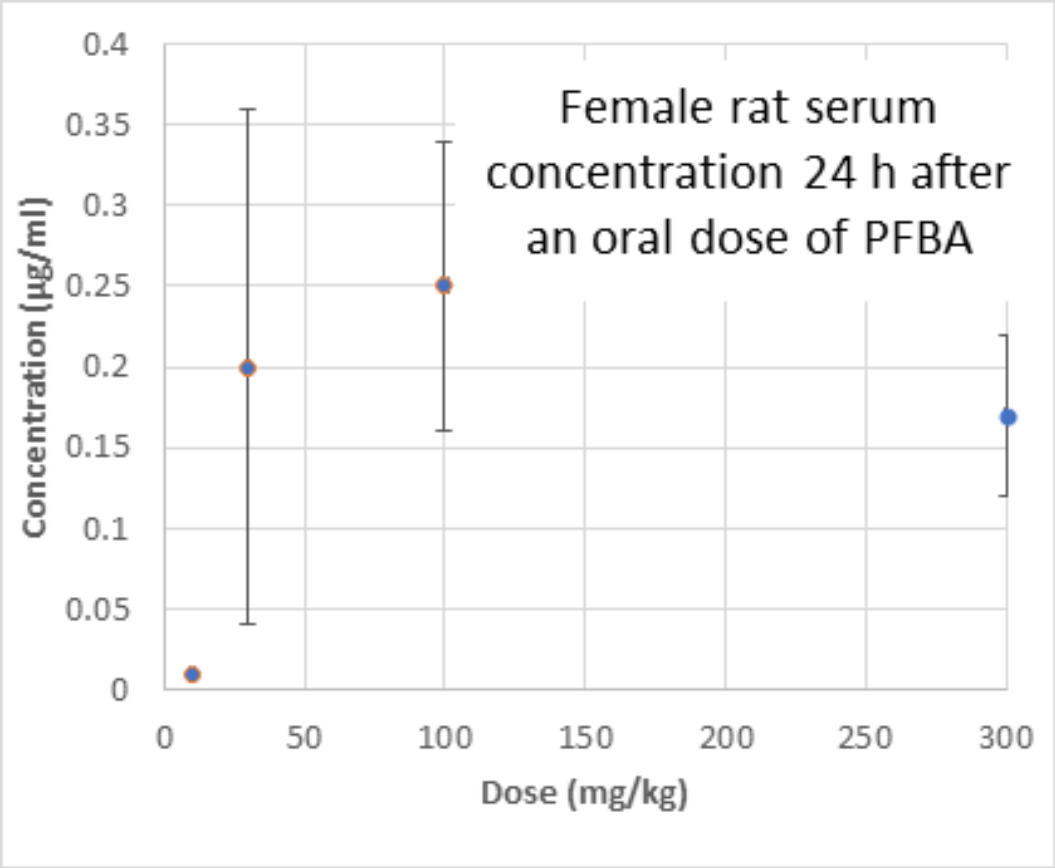

Figure C-4. Rat C_{max} after oral doses of PFBA.

For the human data analyzed by Chang et al. (2008), detailed toxicokinetic parameters are not available, but one can evaluate the relationship between the initial concentration and $t_{1/2}$. Here we only consider data for subjects in which the final concentration is greater than the limit of quantification to avoid statistical artifacts due to limited observational data. Although the lower half-life of the subject with the highest initial concentration indicates a possible negative trend, the half-life is in the range of subjects with lower initial concentrations. Thus, these data do not show a clear dose dependence for half-life and are interpreted as only showing interindividual variation (see Figure C-5). The human data appear consistent with first-order clearance across the range of concentrations observed.

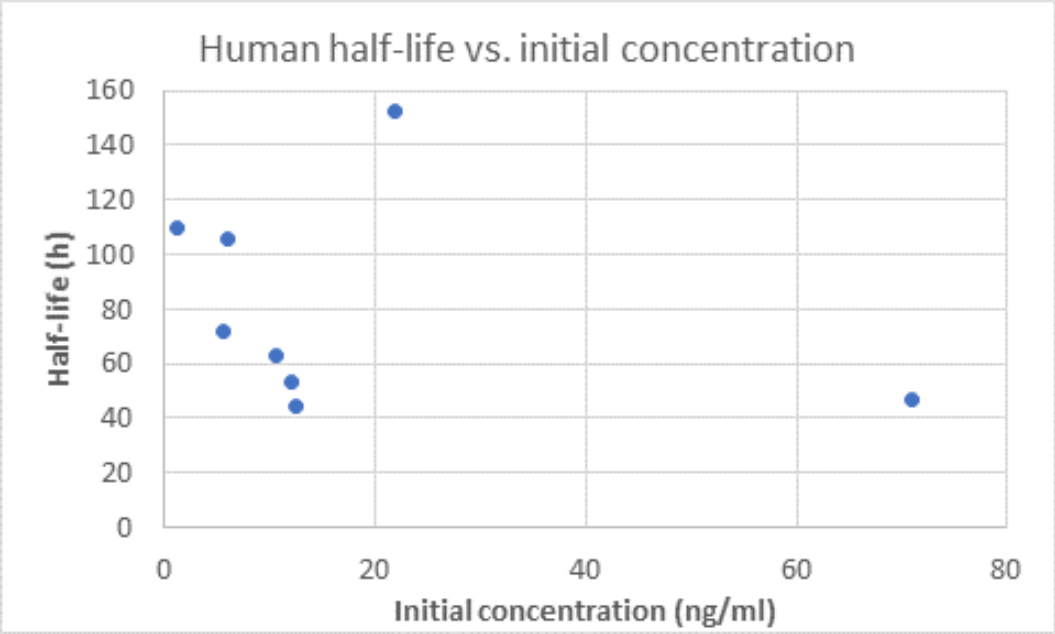

Figure C-5. Estimated human half-lives versus initial serum concentrations.

Chang et al. (2008) only evaluated one PFBA dose in monkeys, so determining whether the biphasic clearance pattern is due to the classical distinction between distribution and excretion phases or a nonlinearity in clearance is not possible. The data show linear clearance from 1–7 or 10 days after the i.v. dose was given, however, when serum concentrations were below 100 ng/mL. Thus, interpreting these data as showing linear kinetics for serum concentrations below 100 ng/mL under long-term exposure conditions seems reasonable. Because the highest initial condition of the human subjects in Chang et al. (2008) was 72 ng/mL, to the extent that kinetics in monkeys can be extrapolated to humans, the results for monkeys confirm the conclusion that human kinetics are also reasonably assumed linear below ~100 ng/mL. This is approximately 1,000-fold below the range of linearity in mice and rats, however, so uncertainty exists as to whether the range of linear kinetics in humans and monkeys extends into the range of rodent-based points of departure.

Russell et al. (2015) attempted to evaluate the kinetics of PFBA as a metabolite of 6:2 fluorotelomer alcohol (FTOH) during a 1-day inhalation study (6-hour exposure, 24-hour observation) and at the end of 23 days of exposure. The half-life of PFBA, however, could not be estimated from the single-day data for male rats and could be estimated only for the high-level exposure in female rats, with yields of PFBA 0.2% in males and not detectable or 0.02% in females. Also, three metabolic intermediates occur between 6:2 FTOH and PFBA, but the model appears to have assumed direct, instantaneous transformation through the first two steps. Assumptions about the volume of distribution were made by Russell et al. (2015). These simplifications in the model likely explain the large discrepancy between the PFBA half-life determined from the single-day exposure 6:2 FTOH for female rats (19 hours) and the half-life obtained for direct exposure to PFBA (1.4-hour average) by Chang et al. (2008). Russell et al. (2015) used only male rats in the 23-day

6:2 FTOH inhalation study, from which they estimated a half-life of 27.7 hours, over three times higher than the average obtained by Chang et al. (2008). The discrepancy also could be due to an underestimation of the metabolic yield from the 1-day experiments. In summary, whereas Russell et al. (2015) described measurements of PFBA in male rats from 23 days of exposure to 6:2 FTOH, the results for female rats after a single exposure are completely inconsistent with the results of (Chang et al., 2008). Therefore, the conclusions from the multiday study are considered too unreliable to be used.

The other long-term data available on internal dosimetry are from the bioassays (Butenhoff et al., 2012; Das et al., 2008; van Otterdijk, 2007). Serum concentrations in nonpregnant female mice after 17 days of exposure (24 hours after the last dose) are 2.0 ± 1.0 and 2.4 ± 1.7 μg/mL, and for pregnant mice are 3.8 ± 1.0 and 4.4 ± 0.7 μg/mL, for the 35- and 175-mg/kg dose groups, respectively (Das et al., 2008). For female mice dosed with 30- and 100-mg/kg PFBA, Chang et al. (2008) reported 4.1 ± 1.7 and 6.4 ± 3.9 μg/mL in serum 24 hours after the dose; using linear extrapolation based on the difference in dose, one might expect 4.8 and 11.2 μg/mL at 24 hours after doses of 35 and 175 mg/kg, given these data. Although the concentrations in the Das et al. (2008) study are somewhat lower than these projections, the difference, especially at the low dose, is within the range of uncertainty and precision expected for PK analysis.

Of note is that, given an average clearance of 28 mL/kg-hour obtained by Chang et al. (2008) after 10- and 30-mg/kg doses, the predicted average serum concentrations for a 35-mg/kg dose is 52 μg/mL. This average concentration reflects the much higher concentrations expected in the first few hours after each dose.

For male rats, Butenhoff et al. (2012) measured end-of-treatment serum levels of 38 ± 23 and 52 ± 25 μg/mL after 28 and 90 days, respectively, at 30 mg/kg-day; we presume these measurements were made 24 hours after the last dose. The corresponding values reported by Chang et al. (2008) for a 30-mg/kg oral dose in the dose-range and time-course studies are 16 ± 3 and 29 ± 13 μg/mL, respectively. Although again, some discrepancy is found between the short-term PK data and the bioassay measurements, the difference is that it is roughly within a factor of 2, which is acceptable for PK analysis and does not indicate a strong time dependence in the PK. One should keep in mind that the estimated clearance and half-life values are based on multiple time points at which the serum concentration is measured, while the comparisons above use only a single time point, 24 hours after dosing, when the result will be sensitive to experimental variation.

Given these data and results, the half-life or clearance of PFBA measured in single-day exposures by Chang et al. (2008) will be assumed to predict dosimetry after repeated exposures that occur in bioassays. This is a common assumption for chemicals with relatively short half-lives because pharmacokinetic studies are typically confined to a single day or less. Clearance in rats and mice might include a slower beta phase, like that observed in monkeys. If a slow clearance phase exists, internal dose from long-term exposure will be higher than is effectively estimated using the

clearance rate determined from single-day exposures, which would increase the HED compared with the current prediction. Using an animal-human ratio of clearance values to estimate the HED relies only on the assumptions that the average serum concentration (C_{AVG}) is predictive of systemic effects in adults and that the relationship between C_{AVG} and dose rate is linear with the proportionality determined by the clearance values estimated here (i.e., the clearance from single-day experiments is predictive of bioassay conditions).

The human half-life estimates were from subjects who had been occupationally exposed to PFBA, with the duration of the PK observation 7–10 days. Thus, those results are reasonably expected to represent clearance under (subsequent to) chronic exposure conditions. The primary uncertainty in predicting human clearance comes from assuming a volume of distribution equal to that estimated for monkeys, which is thought modest given the physiological similarity between monkeys and humans. Thus, the overall uncertainty from using the animal-human clearance ratio to predict the HED for systemic effects in adults appears modest, especially compared to the case where PK data such as used here are not available.

Because developmental effects are usually presumed to depend on peak concentration rather than average concentration, it must be noted that use of the clearance ratio to estimate HEDs for those endpoints also involves an assumption that the absorption rate in humans is similar to that of animals. For PFBA, the absorption rate in mice and rats is fairly rapid, with the peak concentration occurring 0.6–4 hours after bolus oral doses (Chang et al., 2008). That absorption in humans would be faster than in rodents seems unlikely, and exposures are more likely spread out over the day than in the animal bioassays. Therefore, the most likely case is that the peak concentration in humans exposed at the HED will be lower than the peak concentration in mice or rats at the corresponding dose rate. Thus, although this assumption creates uncertainty in the dose extrapolation, the result is not expected to underpredict human health risks.

C.2. MIXED MODELING TO ESTIMATE HALF-LIFE IN HUMANS

A linear mixed-effects model was additionally used to estimate a $t_{1/2}$ for PFBA according to methods described in Li et al. (2018). Briefly, linear mixed-effect models are extensions of simple linear models that use the best linear unbiased prediction estimator to estimate random and fixed effects for clustered data. One important consequence of clustering is that measurements of serum PFBA units within the same person (cluster) are more similar than measurements on serum PFBA in different people (i.e., other clusters). Failure to account for the intracluster correlation would result in misleading inferences. Each individual in Chang et al. (2008) was assumed to have been selected randomly from a larger population. Below is the mixed model formula used for estimating the half-life of serum PFBA:

$$\text{Serum PFBA}_{ij} = (\alpha_{\text{pop}} + \alpha_i) + (k_{\text{pop}} + k_i) \times t_{ij} + \varepsilon_{ij} \quad \text{(C-1)}$$

where $PFBA_{ij}$ is the natural logarithm of the serum PFBA concentrations measured at the *jth* time point for the *ith* subject, α_{pop} is the population mean (also known as the fixed intercept for the population); $\alpha_i \sim N(0, \sigma^2_\alpha)$ is a random intercept for the *ith* subject; k_{pop} is the fixed slope for the population (also known as the average excretion rate constant for serum PFBA for the whole population); $k_i \sim N(0, \sigma^2_k)$ is the random slope for the *ith* subject that allows the excretion rate to vary by individuals; t_{ij} represents the observation time for the *jth* measurement of serum PFBA for *ith* subject; and $\varepsilon_{ij} \sim N(0, \sigma^2_\varepsilon)$ is the random-error effect (residual) for *jth* measurement of *ith* subject. Of note, the small sample sizes (due to the exclusion of the only two subjects identified as females) limited our ability to draw clear conclusions in gender-stratified comparisons.

The half-life of serum PFBA for the study population ($t_{1/2,\,pop}$) then was estimated as:

$$t_{1/2,pop} = \left|\frac{\ln(2)}{k_{pop}}\right| \qquad \text{(C-2)}$$

The mixed-effects model estimated k_{pop} to be −0.010, therefore resulting in an estimated $t_{1/2}$ of 67.9 hours. This value matches very closely to the median value calculated when not taking clustering into account, and therefore was used in estimation of clearance in humans.

APPENDIX D. BENCHMARK DOSE MODELING RESULTS

D.1. BENCHMARK DOSE MODELING APPROACHES

As discussed in Section 5 of the body of the Toxicological Review, the endpoints selected for benchmark dose (BMD) modeling were relative liver weight, liver hypertrophy, total T4, and thyroid follicular hypertrophy incidence from Butenhoff et al. (2012) and relative liver weight, full litter resorption, delayed eye opening, delayed vaginal opening, and delayed preputial separation from Das et al. (2008). 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 clearance values; the modeling results are presented in this appendix.

D.1.1. Modeling Procedure for Dichotomous Noncancer Data

BMD modeling of dichotomous noncancer data was conducted using EPA's Benchmark Dose Software (BMDS, version 3.1.2). For these data, the Gamma, Logistic, Log-Logistic, Log-Probit, Multistage, Probit, Weibull, and Dichotomous Hill models available within the software were fit using a benchmark response (BMR) of 10% extra risk (see Toxicological Review, Section 4.2.1 for justification of selected BMRs). The Multistage model is run for all polynomial degrees up to $n - 2$, where n is the number of dose groups including control. Adequacy of model fit was judged on the basis of χ^2 goodness-of-fit p-value ($p > 0.1$), scaled residuals at the data point (except the control) closest to the predefined benchmark response (absolute value <2.0), and visual inspection of the model fit. In the cases where no best model was found to fit to the data, a reduced data set without the high-dose group was further attempted for modeling and the result presented with that of the full data set. In cases where a model with several parameters equal to the number of dose groups was fit to the data set, all parameters were estimated, and no p-value was calculated, that model was not considered for estimating a point of departure (POD) *unless* no other model provided adequate fit. Among all models providing adequate fit, the benchmark dose lower confidence limit (BMDL) from the model with the lowest Akaike's information criterion (AIC) was selected as a potential POD when BMDL values were sufficiently close (within threefold). Otherwise, the lowest BMDL was selected as a potential POD.

D.1.2. Modeling Procedure for Continuous Noncancer Data

BMD modeling of continuous noncancer data was conducted using EPA's Benchmark Dose Software (BMDS, version 3.1.2). For these data, the Exponential, Hill, Polynomial, and Power models available within the software are fit using a BMR of 1 standard deviation (SD) when no

toxicological information was available to determine an adverse level of response. When toxicological information was available, the BMR was based on relative deviation, as outlined in the Benchmark Dose Technical Guidance (U.S. EPA, 2012) (see Toxicological Review, Section 5.2.1 for justification for using BMRs); when a BMR based on relative deviation was used, modeling results using BMRs based on SD are included for reference. An adequate fit is judged on the basis of χ^2 goodness-of-fit *p*-value ($p > 0.1$), scaled residuals at the data point (except the control) closest to the predefined benchmark response (absolute value <2.0), and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination is made on whether the variance across dose groups is homogeneous. If a homogeneous variance model is deemed appropriate based on the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated from a homogeneous variance model. If the test for homogeneity of variance is rejected ($p < 0.05$), the model is run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model does not adequately fit the data (i.e., Test 3; $p < 0.05$), alternative approaches were assessed on a case-by-case basis. For example, in cases where neither variance model fit, or constant variance did not fit (with adequate Test-4 *p*-value) and nonconstant variance did fit (with inadequate Test-4 *p*-value), the log-normal distribution was attempted.

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

D.1.3. Modeling Procedure for Continuous Noncancer Developmental Toxicity Data

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

D.1.4. 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.

D.1.5. Data Used for Modeling

The source of the data used for modeling is provided in Table D-1. For endpoints from the Das et al. (2008) study, the study authors kindly provided individual dam-level data to facilitate modeling and to provide corrected data where needed. These data also are included in full in the tables below.

Table D-1. Sources of data used in benchmark dose modeling of PFBA endpoints

Endpoint/Reference	Reference	Location	HAWC link
Relative liver weight	Butenhoff et al. (2012)	Appendix 1, page 37 (van Otterdijk, 2007)	https://hawcprd.epa.gov/ani/endpoint/100507453/
Relative liver weight	Das et al. (2008)	Figure 2, page 175	https://hawcprd.epa.gov/ani/endpoint/100507508/
Liver hypertrophy	Butenhoff et al. (2012)	Table 9, page 523	https://hawcprd.epa.gov/ani/endpoint/100507383/
Total T4	Butenhoff et al. (2012)	Table 8, page 522	https://hawcprd.epa.gov/ani/endpoint/100507375/
Full litter resorption	Das et al. (2008)	Table D-2	
Fetal mortality (full litter resorptions combined with fetal death from litters without full litter resorptions)	Das et al. (2008)	Table D-3	
Eyes opening	Das et al. (2008)	Table D-4	
Vaginal opening	Das et al. (2008)	Table D-5	
Preputial separation	Das et al. (2008)	Table D-6	

Table D-2. Data received from study authors for Das et al. (2008) on full litter resorptions (FLR)

Dose (mg/kg-day)	Number of implants FLR
0	8
0	18
35	2
175	2
175	2
175	9
175	5
350	3
350	2
350	13
350	13
350	3
350	14
350	13

Table D-3. Data received from study authors for Das et al. (2008) on fetal death (litters without full litter resorptions) combined with full litter resorptions

Dose (mg/kg-day)	Number of implants	Number of dead	Dam weight on GD1 (litter-specific covariate)
0	16	1	30
0	16	1	28.2
0	11	2	27.7
0	11	0	27.4
0	12	3	25.9
0	11	0	24.1
0	15	0	29.2
0	14	1	28
0	12	3	27.1
0	14	0	26.8
0	16	1	26.6
0	13	2	25.1
0	17	3	30.1
0	14	0	29
0	6	0	27.5
0	9	2	28.1
0	6	0	26.9
0	13	1	26.7
0	11	0	23.3
0	8	8	25.8
0	18	18	31.4
35	15	3	28.1
35	13	0	29.3
35	13	0	27.4
35	14	1	27
35	15	2	26.9
35	13	2	25.7
35	12	4	31.6

Dose (mg/kg-day)	Number of implants	Number of dead	Dam weight on GD1 (litter-specific covariate)
35	13	0	29.2
35	14	1	27.7
35	16	0	27.5
35	13	2	28.1
35	7	3	25.5
35	15	1	30.3
35	13	0	27.5
35	14	1	28.1
35	13	1	27.9
35	11	0	26.4
35	10	1	27.4
35	13	1	27.9
35	13	0	26.1
35	13	1	24.8
35	12	1	24.8
35	2	2	23.1
175	14	1	28.1
175	15	0	27.5
175	14	0	27.4
175	14	1	27.5
175	15	2	29.4
175	14	1	27.5
175	15	0	26
175	16	2	26.2
175	11	0	23.4
175	16	3	29.1
175	11	0	28.2
175	13	0	25.8
175	11	2	26.8
175	15	1	26.9
175	14	1	25

Dose (mg/kg-day)	Number of implants	Number of dead	Dam weight on GD1 (litter-specific covariate)
175	13	1	26.7
175	2	2	25.5
175	2	2	25.4
175	9	9	29
175	5	5	25
350	7	2	29.2
350	12	1	26.3
350	16	3	27.4
350	11	0	25.1
350	14	2	25.3
350	12	1	29.5
350	16	2	28.8
350	17	2	26.2
350	12	2	26.2
350	16	0	27.3
350	9	3	27.6
350	13	0	27.7
350	13	0	27.4
350	13	1	26.4
350	7	1	24.6
350	3	3	21.5
350	2	2	23
350	13	13	25.8
350	13	13	24.6
350	3	3	25.1
350	14	14	28.2
350	13	13	29.2
350	1	1	25.4

Table D-4. Data received from study authors for Das et al. (2008) on delayed eye opening

Dose (mg/kg-day)	Average day of eye opening
0	16.27
0	15.57
0	15.22
0	15.27
0	14.55
0	14.91
0	17.64
0	15.69
0	15.00
0	17.57
0	17.71
0	14.91
0	16.50
0	17.58
0	16.50
0	16.25
0	15.20
0	17.25
0	18.00
0	18.00
35	16.00
35	17.31
35	18.00
35	17.23
35	17.23
35	16.82
35	18.78
35	17.31
35	17.57
35	17.53
35	18.00

Dose (mg/kg-day)	Average day of eye opening
35	15.25
35	17.00
35	17.82
35	18.09
35	17.70
35	16.11
35	18.29
35	17.50
35	17.55
35	17.60
35	17.78
175	17.69
175	17.67
175	15.71
175	17.77
175	16.91
175	18.00
175	17.69
175	17.27
175	17.17
175	17.64
175	18.00
175	18.00
175	18.09
175	18.88
175	18.00
175	18.00
175	18.20
350	15.00
350	18.64
350	17.85
350	17.64
350	18.00

Dose (mg/kg-day)	Average day of eye opening
350	17.36
350	17.85
350	17.93
350	18.00
350	18.00
350	18.00
350	18.60
350	18.00
350	18.09
350	18.00

Table D-5. Data received from study authors for Das et al. (2008) on delayed vaginal opening

Dose (mg/kg-day)	Average day of vaginal opening
0	32.40
0	27.00
0	30.80
0	30.20
0	34.17
0	33.67
0	30.33
0	28.00
0	30.14
0	33.67
0	28.00
0	31.90
0	32.50
0	34.00
0	29.25
0	28.00
0	29.33
0	35.57
0	34.83
35	28.20
35	34.00
35	37.25
35	34.00
35	31.00
35	31.20
35	35.67
35	34.25
35	35.38
35	30.00
35	31.50
35	31.20

Dose (mg/kg-day)	Average day of vaginal opening
35	33.50
35	32.50
35	37.67
35	35.00
35	35.20
35	33.00
35	34.50
35	38.50
35	34.30
175	31.60
175	29.40
175	33.67
175	31.67
175	34.20
175	34.50
175	37.00
175	32.22
175	38.00
175	34.50
175	34.33
175	34.67
175	37.86
175	33.00
175	36.50
175	35.33
175	39.25
350	35.00
350	36.00
350	33.80
350	33.00
350	32.00
350	31.17
350	33.57

Dose (mg/kg-day)	Average day of vaginal opening
350	34.10
350	33.33
350	38.70
350	36.33
350	36.00
350	37.25
350	35.00
350	38.50

Table D-6. Data received from study authors for Das et al. (2008) on delayed preputial separation

Dose (mg/kg-day)	Average day of preputial separation
0	29.00
0	28.20
0	28.20
0	28.00
0	31.80
0	29.20
0	28.71
0	30.00
0	31.00
0	28.29
0	30.00
0	29.80
0	31.00
0	29.50
0	29.00
0	31.00
0	29.67
35	27.40
35	33.40
35	28.20
35	31.80
35	30.00
35	31.33
35	35.50
35	30.22
35	33.17
35	30.00
35	29.00
35	30.14
35	30.29
35	29.80

Dose (mg/kg-day)	Average day of preputial separation
35	30.43
35	30.00
35	27.50
35	28.20
35	28.57
35	29.25
35	30.17
175	26.60
175	28.80
175	30.50
175	31.71
175	31.11
175	32.33
175	28.00
175	31.00
175	35.00
175	30.60
175	30.13
175	29.50
175	30.00
175	31.60
175	31.00
175	30.17
175	31.50
350	28.00
350	31.80
350	31.50
350	32.40
350	31.83
350	30.80
350	31.17
350	33.80
350	34.00

Dose (mg/kg-day)	Average day of preputial separation
350	30.33
350	30.00
350	33.17
350	32.00
350	32.80

D.2. RELATIVE LIVER WEIGHT—MALE RATS (Butenhoff et al., 2012; van Otterdijk, 2007)[1]

Table D-7. Dose-response data for relative liver weight in male rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Dose (mg/kg-day)	*n*	Mean	SD
0	10	2.11	0.13
1.2	10	2.29	0.14
6	10	2.26	0.16
30	10	2.8	0.32

[1]Throughout this document, if a model was selected as appropriately fitting the modeled data, that model's entries in the tables are in green shaded cells and the text is bolded.

Table D-8. Benchmark dose results for relative liver weight in male rats—constant variance, BMR = 10% relative deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction[a]	10% Relative deviation		*p*-Value	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	11.3634	9.4685	0.1720	−8.8244	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response SD >|1.5| actual response SD
Exponential 3 (CV—normal)	Restricted	11.3634	9.4572	0.1720	−8.8244	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response SD >|1.5| actual response SD
Exponential 4 (CV—normal)	Restricted	10.4110	4.8569	0.0584	−6.7628	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Exponential 5 (CV—normal)	Restricted	10.4033	4.8563	0.0584	−6.7621	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Hill (CV—normal)	Restricted	6.6152	6.0656	NA	−4.1913	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response SD >|1.5| actual response SD df = 0, saturated model (goodness-of-fit p-value cannot be calculated)

Models	Restriction[a]	10% Relative deviation		*p*-Value	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				
Constant variance							
Polynomial (3 degree) (CV—normal)	Restricted	12.8952	8.4671	0.0624	−6.8714	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Polynomial (2 degree) (CV—normal)	Restricted	12.1463	8.4560	0.0611	−6.8370	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Power (CV—normal)	Restricted	10.4151	8.4328	0.1668	−8.7631	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response SD >|1.5| actual response SD
Linear (CV—normal)	Unrestricted	10.4151	8.4328	0.1668	−8.7631	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Modeled control response SD >|1.5| actual response SD

[a]"Restriction" column denotes the restriction status of applied models.

[b]"Classification" column denotes whether a model can be considered for model selection purposes. See BMDS User Guide: https://www.epa.gov/bmds.

Table D-9. Benchmark dose results for relative liver weight in male rats—nonconstant variance, BMR = 10% relative deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV—normal)	Restricted	11.3982	9.0908	0.0362	−15.2001	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 3 (NCV—normal)	Restricted	11.3962	9.0911	0.0362	−15.2001	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 4 (NCV—normal)	Restricted	10.5179	5.2058	0.0096	−13.1325	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 5 (NCV—normal)	Restricted	10.5091	5.2055	0.0096	−13.1313	Questionable	Goodness-of-fit *p*-value < 0.1
Hill (NCV—normal)	Restricted	11.1854	7.9783	0.0090	−13.0126	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (3 degree) (NCV—normal)	Restricted	12.7313	8.1751	0.0104	−13.2674	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (NCV—normal)	Restricted	11.9089	8.1513	0.0100	−13.2065	Questionable	Goodness-of-fit *p*-value < 0.1
Power (NCV—normal)	Restricted	10.5174	8.1228	0.0350	−15.1326	Questionable	Goodness-of-fit *p*-value < 0.1
Linear (NCV—normal)	Unrestricted	10.5179	8.1236	0.0350	−15.1326	Questionable	Goodness-of-fit *p*-value < 0.1

Table D-10. Benchmark dose results for relative liver weight in male rats—log-normal distribution, constant variance, BMR = 10% relative deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models[a]	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Log-normal distribution, constant variance							
Exponential 2 (CV—log-normal)	Restricted	11.5672	9.5455	0.1004	−14.1752	Viable—Alternate	Modeled control response SD >|1.5| actual response SD
Exponential 3 (CV—log-normal)	**Restricted**	**11.5672**	**9.6019**	**0.1004**	**−14.1752**	**Viable—Recommended**	**Lowest AIC Modeled control response SD >|1.5| actual response SD**
Exponential 4 (CV—log-normal)	Restricted	10.6449	5.1404	0.0311	−12.1242	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Exponential 5 (CV—log-normal)	Restricted	10.6419	5.1401	0.0311	−12.1239	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Hill (CV—log-normal)	Restricted	10.5728	4.9799	0.0976	−14.1178	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Polynomial (3 degree) (CV—log-normal)	Restricted	12.6948	8.5635	0.0328	−12.2144	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Polynomial (2 degree) (CV—log-normal)	Restricted	11.9903	8.5515	0.0321	−12.1783	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD

Models[a]	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Log-normal distribution, constant variance							
Power (CV—log-normal)	Restricted	10.6452	8.5334	0.0979	−14.1242	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Linear (CV—log-normal)	Unrestricted	10.6452	8.5334	0.0979	−14.1242	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD

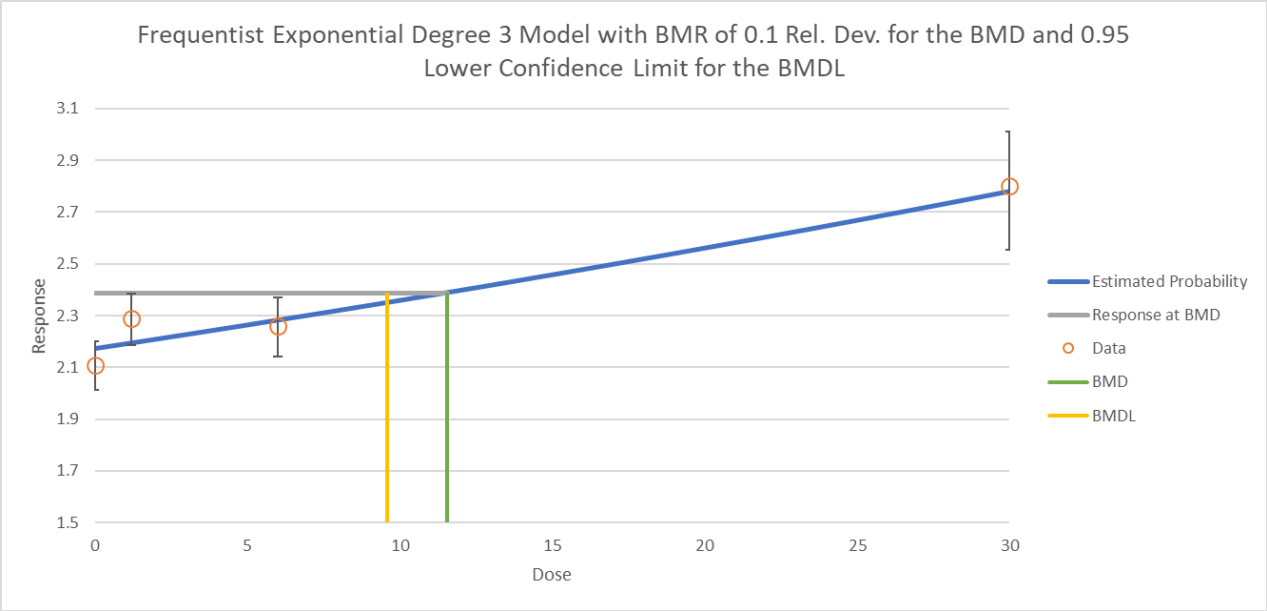

Figure D-1. Dose-response curve for the Exponential M3 model fit to relative liver weight in male rats (Butenhoff et al., 2012; van Otterdijk, 2007).

User Input

Info	
Model	frequentist Exponential degree 3 v1.1
Dataset Name	Butenhoff_90_Lweight_rel
User notes	[Add user notes here]
Dose-Response Model	M[dose] = a * exp(±1 * (b * dose)^d)
Variance Model	Var[i] = alpha

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

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

Model Results

Benchmark Dose	
BMD	11.56718731
BMDL	9.60187006
BMDU	14.67526197
AIC	-14.17517344
Test 4 P-value	0.100441772
D.O.F.	2

Model Parameters	
# of Parameters	4
Variable	Estimate
a	2.171112769
b	0.0082397
d	Bounded
log-alpha	-5.045994496

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated GSD	Calc'd GSD	Observed SD	Scaled Residual
0	10	2.171112769	2.10600663	2.11	1.08352413	1.063487	0.13	-0.17835832
1.2	10	2.192686432	2.28573248	2.29	1.08352413	1.062982	0.14	0.284010771
6	10	2.281146197	2.25435749	2.26	1.08352413	1.073268	0.16	-0.061715421
30	10	2.779944166	2.78189148	2.8	1.08352413	1.120657	0.32	0.058533184

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	12.38576382	5	-14.771528
A2	15.32442666	8	-14.648853
A3	12.38576382	5	-14.771528
fitted	10.08758672	3	-14.175173
R	-8.71328445	2	21.4265689

* Includes additive constant of -70.8323. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	48.07542222	6	<0.0001
2	5.877325671	3	0.11773355
3	5.877325671	3	0.11773355
4	4.596354207	2	0.10044177

Table D-11. Benchmark dose results for relative liver weight in male rats—log-normal distribution, constant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 Standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Log-normal distribution, constant variance							
Exponential 2 (CV—log-normal)	Restricted	9.7357	7.6047	0.1004	−14.1752	Viable—Alternate	Modeled control response SD >|1.5| actual response SD
Exponential 3 (CV—log-normal)	**Restricted**	**9.7356**	**7.6049**	**0.1004**	**−14.1752**	**Viable—Recommended**	**Lowest AIC Modeled control response SD >|1.5| actual response SD**
Exponential 4 (CV—log-normal)	Restricted	8.8962	0.0000	0.0311	−12.1242	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Exponential 5 (CV—log-normal)	Restricted	8.8943	6.9746	0.0311	−12.1239	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Hill (CV—log-normal)	Restricted	8.8323	4.0523	0.0976	−14.1178	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Polynomial (3 degree) (CV—log-normal)	Restricted	10.7197	6.8148	0.0328	−12.2144	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Polynomial (2 degree) (CV—log-normal)	Restricted	10.1369	6.8036	0.0321	−12.1783	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD
Power (CV—log-normal)	Restricted	8.8972	6.7871	0.0979	−14.1242	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD

Models	Restriction	1 Standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Log-normal distribution, constant variance							
Linear (CV—log-normal)	Unrestricted	8.8972	6.7871	0.0979	−14.1242	Questionable	Goodness-of-fit *p*-value < 0.1 Modeled control response SD >|1.5| actual response SD

D.3. RELATIVE LIVER WEIGHT—P_0 MICE (Das et al., 2008)

Table D-12. Dose-response data for relative liver weight in pregnant mice (Das et al., 2008)

Dose (mg/kg-day)	*n*	Mean	SD
0	6	8.04	0.66
35	6	8.76	1.37
175	7	10.28	0.75
350	6	10.65	0.62

Table D-13. Benchmark dose results for relative liver weight in pregnant mice—constant variance, BMR = 10% relative deviation (Das et al., 2008)

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	130.2877	98.9543	0.0486	73.1479	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 3 (CV—normal)	Restricted	130.2877	99.1362	0.0486	73.1479	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 4 (CV—normal)	**Restricted**	**36.1911**	**15.1545**	**0.8612**	**69.1285**	**Viable—recommended**	**Lowest AIC**
Exponential 5 (CV—normal)	Restricted	39.4346	15.2398	NA	71.0979	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Hill (CV—normal)	Restricted	38.7873	12.3846	NA	71.0979	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Polynomial (3 degree) (CV—normal)	Restricted	115.5880	84.4884	0.0736	72.3159	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (CV—normal)	Restricted	115.5878	84.4883	0.0736	72.3159	Questionable	Goodness-of-fit *p*-value < 0.1
Power (CV—normal)	Restricted	115.5870	84.4876	0.0736	72.3159	Questionable	Goodness-of-fit *p*-value < 0.1
Linear (CV—normal)	Unrestricted	115.5882	84.4875	0.0736	72.3159	Questionable	Goodness-of-fit *p*-value < 0.1

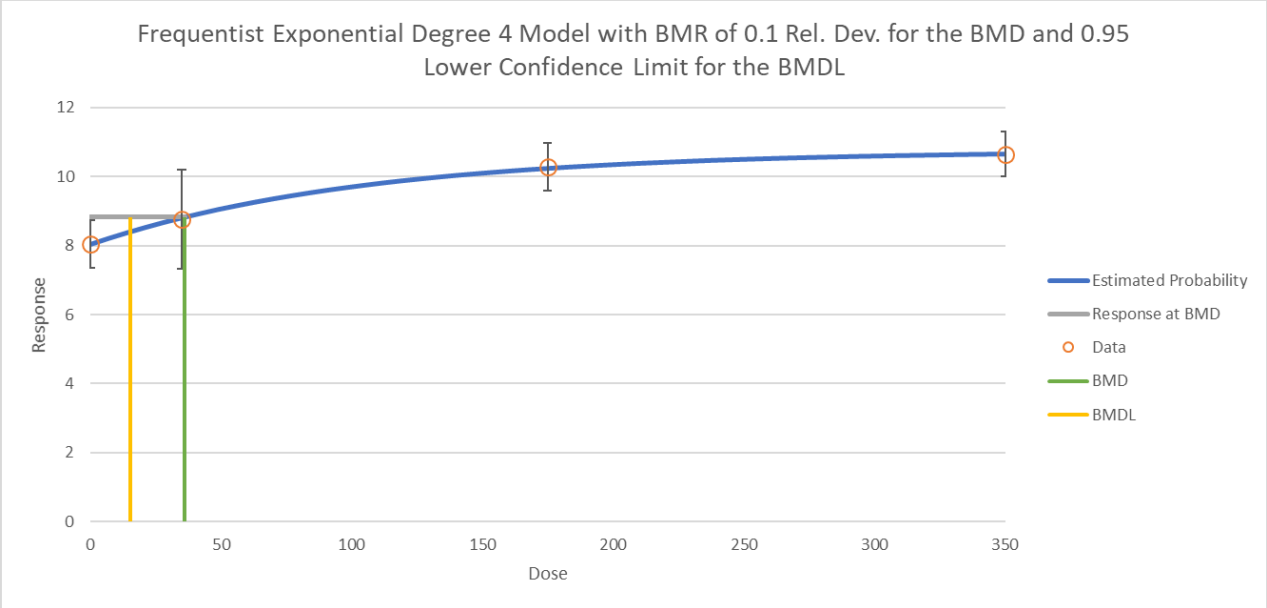

Figure D-2. Dose-response curve for the Exponential M4 model fit to relative liver weight in pregnant mice (Das et al., 2008).

User Input

Info	
Model	frequentist Exponential degree 4 v1.1
Dataset Name	Das_p_Lweight_rel
User notes	[Add user notes here]
Dose-Response Model	M[dose] = a * [c-(c-1) * exp(-b * dose)]
Variance Model	Var[i] = alpha

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

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

#NAME?

Benchmark Dose	
BMD	36.19110286
BMDL	15.15446485
BMDU	87.70968183
AIC	69.12846157
Test 4 P-value	0.861196136
D.O.F.	1

Model Parameters	
# of Parameters	4
Variable	Estimate
a	8.018710905
b	0.009531749
c	1.342753894
log-alpha	-0.39273843

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	6	8.018710905	8.04	8.04	0.82170879	0.66	0.66	0.063462168
35	6	8.798356028	8.76	8.76	0.82170879	1.37	1.37	-0.114338192
175	7	10.24876199	10.28	10.28	0.82170879	0.75	0.75	0.100580637
350	6	10.66937939	10.65	10.65	0.82170879	0.62	0.62	-0.057769406

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-30.54894422	5	71.0978884
A2	-27.8068244	8	71.6136488
A3	-30.54894422	5	71.0978884
fitted	-30.56423079	4	69.1284616
R	-42.8486201	2	89.6972402

* Includes additive constant of -22.97346. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	30.08359139	6	<0.0001
2	5.484239634	3	0.13958431
3	5.484239634	3	0.13958431
4	0.030573129	1	0.86119614

Table D-14. Benchmark dose results for relative liver weight in pregnant mice—constant variance, BMR = 1 standard deviation (Das et al., 2008)

Models	Restriction	1 Standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	141.5518	104.9937	0.0524	73.6332	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 3 (CV—normal)	Restricted	141.5511	104.9942	0.0524	73.6331	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 4 (CV—normal)	**Restricted**	**37.2658**	**16.6945**	**0.5517**	**70.0879**	**Viable—recommended**	**Lowest AIC**
Exponential 5 (CV—normal)	Restricted	40.3641	16.7699	NA	71.7337	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Hill (CV—normal)	Restricted	39.5789	13.8731	NA	71.7337	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Polynomial (3 degree) (CV—normal)	Restricted	124.9178	90.1236	0.0725	72.9822	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (CV—normal)	Restricted	124.9176	90.1235	0.0725	72.9822	Questionable	Goodness-of-fit *p*-value < 0.1
Power (CV—normal)	Restricted	124.9169	90.1256	0.0725	72.9822	Questionable	Goodness-of-fit *p*-value < 0.1
Linear (CV—normal)	Unrestricted	124.9180	90.1238	0.0725	72.9822	Questionable	Goodness-of-fit *p*-value < 0.1

D.4. LIVER HYPERTROPHY—MALE RAT (Butenhoff et al., 2012; van Otterdijk, 2007)

Table D-15. Dose-response data liver hypertrophy in male rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Dose (mg/kg-day)	*n*	Incidence
0	10	0
1.2	10	0
6	10	0
30	10	9

Table D-16. Benchmark dose results for liver hypertrophy in rats—BMR = 10% extra risk (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	10% Extra risk		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Gamma	Restricted	16.2946	5.3859	1.0000	8.5017	Viable—alternate	
Log-logistic	Restricted	23.5001	5.4486	1.0000	10.5017	Viable—alternate	
Multistage 3rd	Restricted	10.8404	5.0184	0.9796	8.8673	Viable—alternate	
Multistage 2nd	Restricted	6.8934	3.6966	0.8078	10.2814	Viable—alternate	
Multistage 1st	Restricted	2.4428	1.4091	0.0817	18.5672	Questionable	Goodness-of-fit *p*-value < 0.1
Weibull	**Restricted**	**25.2757**	**5.3801**	**1.0000**	**8.5017**	**Viable—recommended**	**Lowest AIC**
Dichotomous Hill	Unrestricted	23.4994	5.8336	0.9995	12.5017	Viable—alternate	
Logistic	Unrestricted	23.4727	8.4278	1.0000	8.5017	Viable—alternate	
Log-probit	Unrestricted	20.1374	5.4722	1.0000	10.5017	Viable—alternate	
Probit	Unrestricted	21.2661	7.6123	1.0000	10.5017	Viable—alternate	

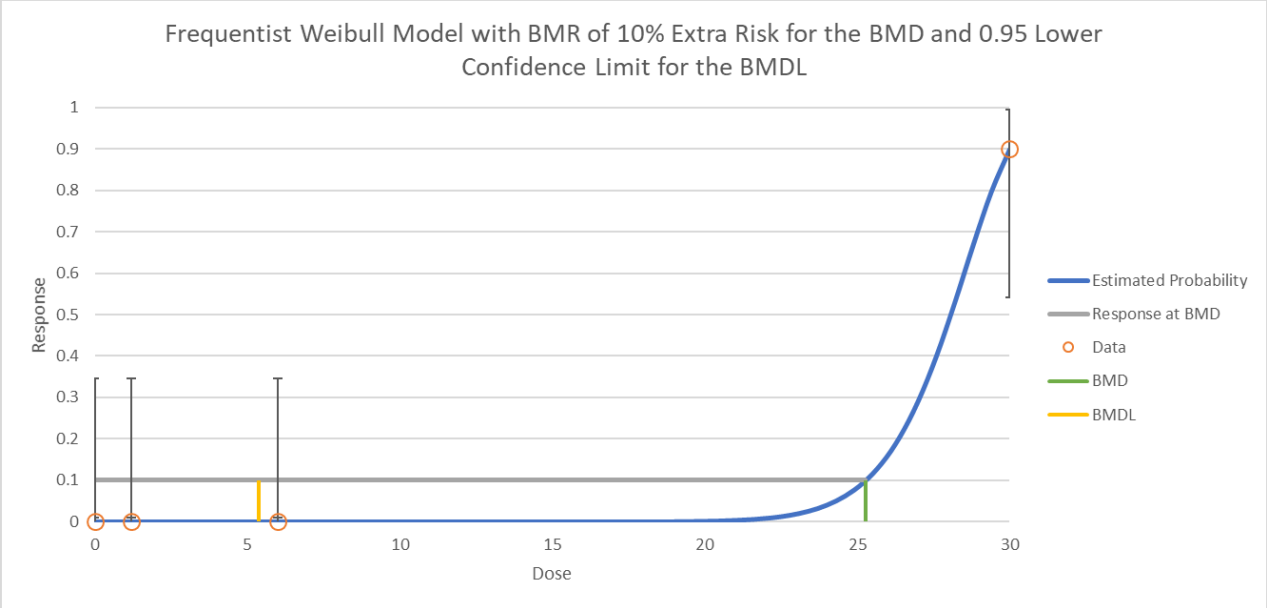

Figure D-3. Dose-response curve for the Weibull model fit to liver hypertrophy in male rats (Butenhoff et al., 2012; van Otterdijk, 2007).

User Input

Info	
Model	frequentist Weibull v1.1
Dataset Name	Butenhoff_90_Lhypertrophy
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g + (1-g)*[1-exp(-b*dose^a)]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	Dose
Independent Variable	Incidence
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	25.27565904
BMDL	5.380065202
BMDU	26.31774355
AIC	8.501660382
P-value	1
D.O.F.	3
Chi^2	4.56905E-07

Model Parameters	
# of Parameters	3
Variable	Estimate
g	Bounded
a	Bounded
b	5.94337E-27

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.523E-07	0	10	-0.00039
1.2	1.523E-08	1.523E-07	0	10	-0.00039
6	1.52306E-08	1.52306E-07	0	10	-0.00039
30	0.899999999	8.999999992	9	10	8.003E-09

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-3.250829734	4	-	-	-
Fitted Model	-3.250830191	1	9.1381E-07	3	1
Reduced Model	-21.32655363	1	36.1514478	3	<0.0001

Table D-17. Dose-response data for liver hypertrophy (slight severity lesions) in male rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Dose (mg/kg-day)	*n*	Incidence
0	10	0
1.2	10	0
6	10	0
30	10	4

Table D-18. Benchmark dose results for liver hypertrophy (slight severity lesions) in male rats—BMR = 10% extra risk (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	10% Extra risk		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Gamma	Restricted	23.1357	5.6717	1.0000	15.4602	Viable—alternate	
Log-logistic	Restricted	27.1575	5.5461	1.0000	17.4602	Viable—alternate	
Multistage 3rd	Restricted	17.7871	5.5407	0.9978	15.5422	Viable—alternate	
Multistage 2nd	Restricted	13.9892	5.1121	0.8984	17.8741	Viable—alternate	
Multistage 1st	**Restricted**	**8.1158**	**3.9098**	**0.5376**	**19.5942**	**Viable—recommended**	**Lowest BMDL**
Weibull	Restricted	27.4811	5.6718	1.0000	17.4602	Viable—alternate	
Dichotomous Hill	Unrestricted	27.1562	5.2830	0.9995	19.4602	Viable—alternate	BMD:BMDL ratio > 5
Logistic	Unrestricted	26.9449	13.6106	1.0000	15.4602	Viable—alternate	
Log-Probit	Unrestricted	24.8237	5.3131	1.0000	17.4602	Viable—alternate	
Probit	Unrestricted	25.5166	12.1561	1.0000	17.4602	Viable—alternate	

D.5. TOTAL T4—MALE RAT (Butenhoff et al., 2012; van Otterdijk, 2007)

Table D-19. Dose-response data for total T4 levels in male rats (Butenhoff et al., 2012; van Otterdijk, 2007)

Dose (mg/kg-day)	*n*	Mean	SD
0	10	5.27	0.71
1.2	10	5.97	1.08
6	9	4.46	0.88
30	9	3.23	0.55

Table D-20. Benchmark dose results for total T4 levels in male rats—constant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 Standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	9.2322	6.5166	0.0138	104.3816	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 3 (CV—normal)	Restricted	9.2324	6.5166	0.0138	104.3816	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 4 (CV—normal)	Restricted	4.9496	2.5239	0.0075	104.9572	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 5 (CV—normal)	Restricted	5.7655	3.5138	NA	103.5642	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Hill (CV—normal)	Restricted	5.5394	3.2999	NA	103.5644	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Polynomial (3 degree) (CV—normal)	Restricted	11.5906	8.7704	0.0090	105.2374	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (CV—normal)	Restricted	11.5906	8.7704	0.0090	105.2374	Questionable	Goodness-of-fit *p*-value < 0.1
Power (CV—normal)	Restricted	11.5906	8.7706	0.0090	105.2374	Questionable	Goodness-of-fit *p*-value < 0.1
Linear (CV—normal)	Unrestricted	11.5906	8.7704	0.0090	105.2374	Questionable	Goodness-of-fit *p*-value < 0.1

Table D-21. Benchmark dose results for total T4 levels in male rats—nonconstant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 Standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV—normal)	Restricted	11.3786	7.8978	0.0182	102.5921	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 3 (NCV—normal)	Restricted	11.3789	7.8977	0.0182	102.5921	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 4 (NCV—normal)	Restricted	5.8707	2.9606	0.0104	103.1558	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 5 (NCV—normal)	Restricted	5.8297	3.9098	NA	102.1810	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Hill (NCV—normal)	Restricted	5.8562	3.7033	NA	102.1809	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Polynomial (3 degree) (NCV—normal)	Restricted	13.7327	10.1890	0.0130	103.2666	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (NCV—normal)	Restricted	13.7329	10.1889	0.0130	103.2666	Questionable	Goodness-of-fit *p*-value < 0.1
Power (NCV—normal)	Restricted	13.7325	10.1890	0.0130	103.2666	Questionable	Goodness-of-fit *p*-value < 0.1
Linear (NCV—normal)	Unrestricted	13.7332	10.1889	0.0130	103.2666	Questionable	Goodness-of-fit *p*-value < 0.1

Table D-22. Benchmark dose results for total T4 levels in male rats—log-normal distribution, constant variance, BMR = 1 standard deviation (Butenhoff et al., 2012; van Otterdijk, 2007)

Models	Restriction	1 Standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Log-normal distribution, constant variance							
Exponential 2 (CV—log-normal)	Restricted	12.0074	7.6347	0.0223	98.5676	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 3 (CV—log-normal)	Restricted	12.0074	7.6347	0.0223	98.5676	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 4 (CV—log-normal)	Restricted	5.7060	2.5325	0.0200	98.3698	Questionable	Goodness-of-fit *p*-value < 0.1
Exponential 5 (CV—log-normal)	Restricted	5.9263	3.4425	NA	97.5382	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Hill (CV—log-normal)	Restricted	-	-	-	-	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Polynomial (3 degree) (CV—log-normal)	Restricted	-	-	-	-	Questionable	Goodness-of-fit *p*-value < 0.1
Polynomial (2 degree) (CV—log-normal)	Restricted	-	-	-	-	Questionable	Goodness-of-fit *p*-value < 0.1
Power (CV—log-normal)	Restricted	-	-	-	-	Questionable	Goodness-of-fit *p*-value < 0.1
Linear (CV—log-normal)	Unrestricted	-	-	-	-	Questionable	Goodness-of-fit *p*-value < 0.1

D.6. INCREASED FETAL MORTALITY – MALE AND FEMALE F_1 MICE (Das et al., 2008)

Table D-23. Dose-response data for increased fetal mortality (Das et al., 2008)

Dose (mg/kg-day)	*n* (No. of implants)	No. of dead fetuses/neonates by PND 21	Litter-specific covariate (Maternal weight on GD1)
0	16	1	30
0	16	1	28.2
0	11	2	27.7
0	11	0	27.4
0	12	3	25.9
0	11	0	24.1
0	15	0	29.2
0	14	1	28
0	12	3	27.1
0	14	0	26.8
0	16	1	26.6
0	13	2	25.1
0	17	3	30.1
0	14	0	29
0	6	0	27.5
0	9	2	28.1
0	6	0	26.9
0	13	1	26.7
0	11	0	23.3
0	8	8	25.8
0	18	18	31.4
35	15	3	28.1
35	13	0	29.3
35	13	0	27.4
35	14	1	27
35	15	2	26.9
35	13	2	25.7
35	12	4	31.6

Dose (mg/kg-day)	*n* (No. of implants)	No. of dead fetuses/neonates by PND 21	Litter-specific covariate (Maternal weight on GD1)
35	13	0	29.2
35	14	1	27.7
35	16	0	27.5
35	13	2	28.1
35	7	3	25.5
35	15	1	30.3
35	13	0	27.5
35	14	1	28.1
35	13	1	27.9
35	11	0	26.4
35	10	1	27.4
35	13	1	27.9
35	13	0	26.1
35	13	1	24.8
35	12	1	24.8
35	2	2	23.1
175	14	1	28.1
175	15	0	27.5
175	14	0	27.4
175	14	1	27.5
175	15	2	29.4
175	14	1	27.5
175	15	0	26
175	16	2	26.2
175	11	0	23.4
175	16	3	29.1
175	11	0	28.2
175	13	0	25.8
175	11	2	26.8
175	15	1	26.9
175	14	1	25

Dose (mg/kg-day)	*n* (No. of implants)	No. of dead fetuses/neonates by PND 21	Litter-specific covariate (Maternal weight on GD1)
175	13	1	26.7
175	2	2	25.5
175	2	2	25.4
175	9	9	29
175	5	5	25
350	7	2	29.2
350	12	1	26.3
350	16	3	27.4
350	11	0	25.1
350	14	2	25.3
350	12	1	29.5
350	16	2	28.8
350	17	2	26.2
350	12	2	26.2
350	16	0	27.3
350	9	3	27.6
350	13	0	27.7
350	13	0	27.4
350	13	1	26.4
350	7	1	24.6
350	3	3	21.5
350	2	2	23
350	13	13	25.8
350	13	13	24.6
350	3	3	25.1
350	14	14	28.2
350	13	13	29.2

Table D-24. Benchmark dose results for increased fetal mortality (male and female mice)—BMR = 1% extra risk (Das et al., 2008)

Models	Restriction	1% Extra risk	*p*-Value	AIC		BMDS notes

		BMD	BMDL			BMDS classification	
Nested logistic (lsc+ilc+)	**Restricted**	**19.5989**	**5.7383**	**Infinity**	**0.2633**	**Viable—Recommended**	**Lowest AIC BMDL 3× lower than lowest non-zero dose**
Nested logistic (lsc+ilc−)	Restricted	326.9633	170.7455	Infinity	<0.0001	Questionable	Goodness of fit p-value < 0.1
Nested logistic (lsc−ilc+)	Restricted	50.4014	10.1822	Infinity	0.0833	Questionable	Goodness of fit p-value < 0.1 BMDL 3× lower than lowest non-zero dose
Nested logistic (lsc−ilc−)	Restricted	191.2272	81.9934	Infinity	<0.0001	Questionable	Goodness of fit p-value < 0.1

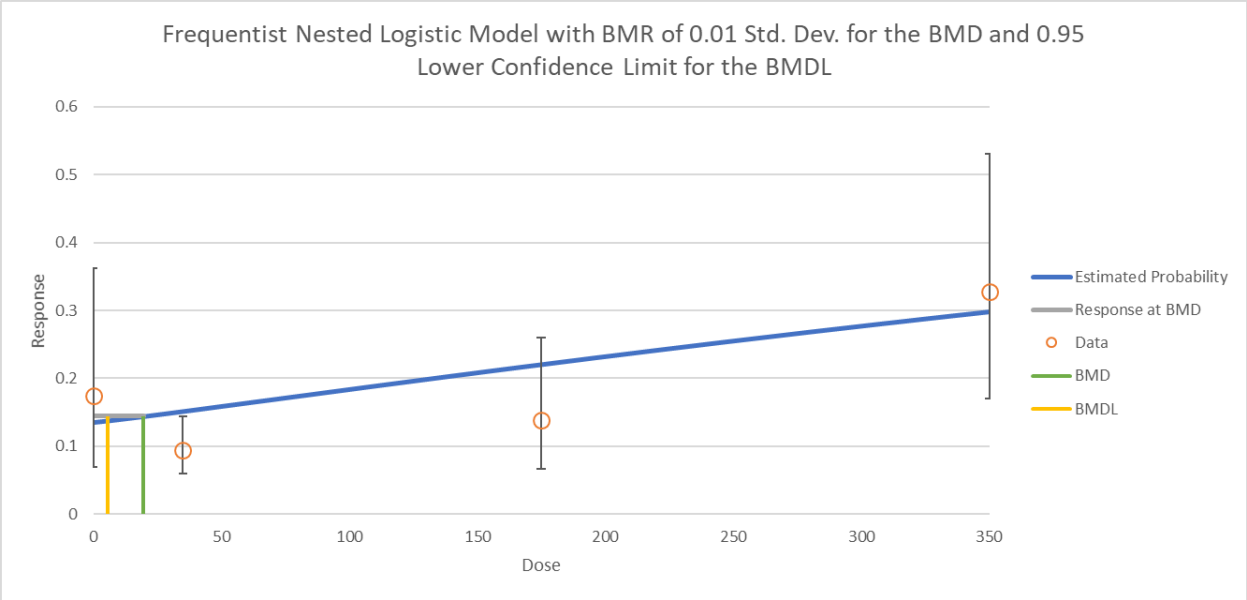

Figure D-4. Dose-response curve for the Nested-Logistic model fit to increased fetal mortality in male and female mice (Das et al., 2008).

User Input

Info	
Model	frequentist Nested Logistic_lsc+ilc+_ v2.2
Dataset Name	Das_FLR_Fetal_Death
User notes	[Add user notes here]
Dose-Response Model	P[dose] = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/[1+exp(-beta-theta2*Rij-rho*log(dose))]

Model Options	
Risk Type	Extra Risk
BMR	0.01
Confidence Level	0.95
Litter Specific Covariate	Overall Mean
Intralitter Correlation	Estimate
Background	Estimate

Model Data	
Dependent Variable	Dose
Independent Variable	Incidence
Total # of Observations	87

Model Results

Benchmark Dose	
BMD	19.59891366
BMDL	5.738265629
BMDU	-
AIC	688.92042
P-value	0.263333333
D.O.F.	78
Chi^2	96.74138773

Model Parameters	
# of Parameters	9
Variable	Estimate
alpha	-0.5312932
beta	16.8290783
theta1	0.024711312
theta2	-0.913645475
rho	1.08467654
phi1	0.368252856
phi2	0.135465621
phi3	0.509745798
phi4	0.576861839

Bootstrap Results	
# Iterations	1000
Bootstrap Seed	1599045577
Log-likelihood	-335.46021
Observed Chi-square	96.74138773
Combined P-value	0.263333333

Bootstrap Runs

Run	Bootstrap Chi-square Percentiles				
	P-Value	50th	90th	95th	99th
1	0.285	85.65851617	109.848694	117.6	134.18995
2	0.258	85.12942257	110.722914	119.0851	131.33939
3	0.247	85.05473338	108.751327	115.9296	137.48443
Combined	0.263333333	85.30000651	109.644757	117.9646	135.17128

Scaled Residuals	
Minimum scaled residual for dose group nearest the BMD	-0.50395
Minimum ABS(scaled residual) for dose group nearest the BMD	0.503952
Average Scaled residual for dose group nearest the BMD	-0.50395
Average ABS(scaled residual) for dose group nearest the BMD	0.503952
Maximum scaled residual for dose group nearest the BMD	-0.50395
Maximum ABS(scaled residual) for dose group nearest the BMD	0.503952

Litter Data						
Dose	Lit. Spec. Cov.	Est. Prob.	Litter Size	Expected	Observed	Scaled Residual
0	23.3	0.044480361	11	0.489284	0	-0.330689547
0	24.1	0.06424941	11	0.706744	0	-0.401615664
0	25.1	0.088960722	13	1.156489	2	0.353012617
0	25.8	0.10625864	8	0.850069	8	4.33673202
0	25.9	0.108729771	12	1.304757	3	0.699492477
0	26.6	0.126027689	16	2.016443	1	-0.299772055
0	26.7	0.12849882	13	1.670485	1	-0.238711127
0	26.8	0.130969952	14	1.833579	0	-0.603802545
0	26.9	0.133441083	6	0.800646	0	-0.570250323
0	27.1	0.138383345	12	1.6606	3	0.498243205
0	27.4	0.145796738	11	1.603764	0	-0.633212408
0	27.5	0.14826787	6	0.889607	0	-0.606305933
0	27.7	0.153210132	11	1.685311	2	0.121734257
0	28	0.160623525	14	2.248729	1	-0.377819018
0	28.1	0.163094657	9	1.467852	2	0.241698202
0	28.2	0.165565788	16	2.649053	1	-0.434253259
0	29	0.185334837	14	2.594688	0	-0.741848906
0	29.2	0.190277099	15	2.854156	0	-0.756724162
0	30	0.210046149	16	3.360738	1	-0.567256611
0	30.1	0.21251728	17	3.612794	3	-0.138387912
0	31.4	0.244641985	18	4.403556	18	2.76675012
35	23.1	0.420439493	2	0.840879	2	1.558208765
35	24.8	0.193667312	12	2.324008	1	-0.612920728
35	24.8	0.193667312	13	2.517675	1	-0.657368032
35	25.5	0.160429819	7	1.123009	3	1.435714172
35	25.7	0.15539473	13	2.020131	2	-0.009511434
35	26.1	0.149721705	13	1.946382	0	-0.933727752
35	26.4	0.148530963	11	1.633841	0	-0.902727468
35	26.9	0.150776303	15	2.261645	2	-0.110930327
35	27	0.151716296	14	2.124028	1	-0.503951928
35	27.4	0.156698447	13	2.03708	0	-0.959178264
35	27.4	0.156698447	10	1.566984	1	-0.331093052
35	27.5	0.158199979	16	2.5312	0	-0.995853056
35	27.5	0.158199979	13	2.0566	0	-0.964622031
35	27.7	0.16145156	14	2.260322	1	-0.550928027
35	27.9	0.164988496	13	2.14485	1	-0.527947549
35	27.9	0.164988496	13	2.14485	1	-0.527947549
35	28.1	0.168763344	15	2.53145	3	0.189788804
35	28.1	0.168763344	14	2.362687	1	-0.585185094
35	28.1	0.168763344	13	2.193923	2	-0.088622513
35	29.2	0.192293335	13	2.499813	0	-1.08570969
35	29.3	0.194583201	13	2.529582	0	-1.093706422
35	30.3	0.218173919	15	3.272609	1	-0.834803748
35	31.6	0.249793258	12	2.997519	4	0.423637452
175	23.4	0.753292803	11	8.286221	0	-2.346999161
175	25	0.450913899	14	6.312795	1	-1.033293673
175	25	0.450913899	5	2.254569	5	1.415449135
175	25.4	0.381299381	2	0.762599	2	1.466122666
175	25.5	0.365523168	2	0.731046	2	1.516399081
175	25.8	0.322690467	13	4.194976	0	-0.932876612
175	26	0.298046899	15	4.470703	0	-0.884741991
175	26.2	0.276550941	16	4.424815	2	-0.460908554
175	26.7	0.235805287	13	3.065469	1	-0.505849379
175	26.8	0.229690589	11	2.526596	2	-0.152863219
175	26.9	0.2241858	15	3.362787	1	-0.512836334
175	27.4	0.204707536	14	2.865906	0	-0.687383811
175	27.5	0.202204115	14	2.830858	1	-0.441145101
175	27.5	0.202204115	14	2.830858	1	-0.441145101
175	27.5	0.202204115	15	3.033062	0	-0.683561326
175	28.1	0.194568014	14	2.723952	1	-0.421446879
175	28.2	0.194300585	11	2.137306	0	-0.659587572
175	29	0.199087513	9	1.791788	9	2.670218904
175	29.1	0.200338227	16	3.205412	3	-0.043633258
175	29.4	0.204695235	15	3.070429	2	-0.240145327
350	21.5	0.971795484	3	2.915386	3	0.201065485
350	23	0.901250165	2	1.8025	2	0.372789514
350	24.6	0.695111669	13	9.036452	13	0.848375256
350	24.6	0.695111669	7	4.865782	1	-1.502678856
350	25.1	0.601246578	3	1.80374	3	0.961150815
350	25.1	0.601246578	11	6.613712	0	-1.565382323
350	25.3	0.562306321	14	7.872288	2	-1.085132125
350	25.4	0.542866281	1	0.542866	1	0.91764604
350	25.8	0.467290016	13	6.07477	13	1.367719528
350	26.2	0.398842995	12	4.786116	2	-0.606043119
350	26.2	0.398842995	17	6.780331	2	-0.740295677
350	26.3	0.383300844	12	4.59961	1	-0.788584398
350	26.4	0.368470315	13	4.790114	1	-0.774204472
350	27.3	0.269132881	16	4.306126	0	-0.781258285
350	27.4	0.261775084	13	3.403076	0	-0.762807027
350	27.4	0.261775084	16	4.188401	3	-0.217527974
350	27.6	0.249017755	9	2.24116	3	0.246847242
350	27.7	0.243559594	13	3.166275	0	-0.726875687
350	28.2	0.224244443	14	3.139422	14	2.387132337
350	28.8	0.214724836	16	3.435597	2	-0.281313821
350	29.2	0.214117934	13	2.783533	13	2.454127084
350	29.2	0.214117934	7	1.498826	2	0.218630258
350	29.5	0.215777472	12	2.58933	1	-0.411519634

D.7. DELAYED EYE OPENING—F_1 MALE AND FEMALE MICE (Das et al., 2008)

Table D-25. Dose-response data for delayed eye opening in male and female mice (Das et al., 2008)

Dose (mg/kg-day)	*n*	Mean	SD
0	20	16.28	1.19
35	22	17.38	0.79
175	17	17.69	0.68
350	15	17.8	0.83

Table D-26. Benchmark dose results for delayed eye opening in male and female mice—constant variance, BMR = 5% relative deviation (Das et al., 2008)

Models	Restriction	5% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	252.3387	178.6688	0.0008	211.1176	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Exponential 3 (CV—normal)	Restricted	252.3380	178.7347	0.0008	211.1176	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Exponential 4 (CV—normal)	Restricted	20.4436	0.0000	0.7270	198.8811	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated
Exponential 5 (CV—normal)	Restricted	175.5239	0.0000	NA	215.6060	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated |Residual at control| >2 df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Hill (CV—normal)	**Restricted**	**16.1508**	**4.8878**	**0.8659**	**198.7878**	**Viable—recommended**	**Lowest AIC BMDL 3× lower than lowest nonzero dose**
Polynomial (3 degree) (CV—normal)	Restricted	247.2477	172.9292	0.0008	210.9441	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Polynomial (2 degree) (CV—normal)	Restricted	247.2476	172.9292	0.0008	210.9441	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Power (CV—normal)	Restricted	247.2483	172.9366	0.0008	210.9441	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Linear (CV—normal)	Unrestricted	247.2471	172.9288	0.0008	210.9441	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2

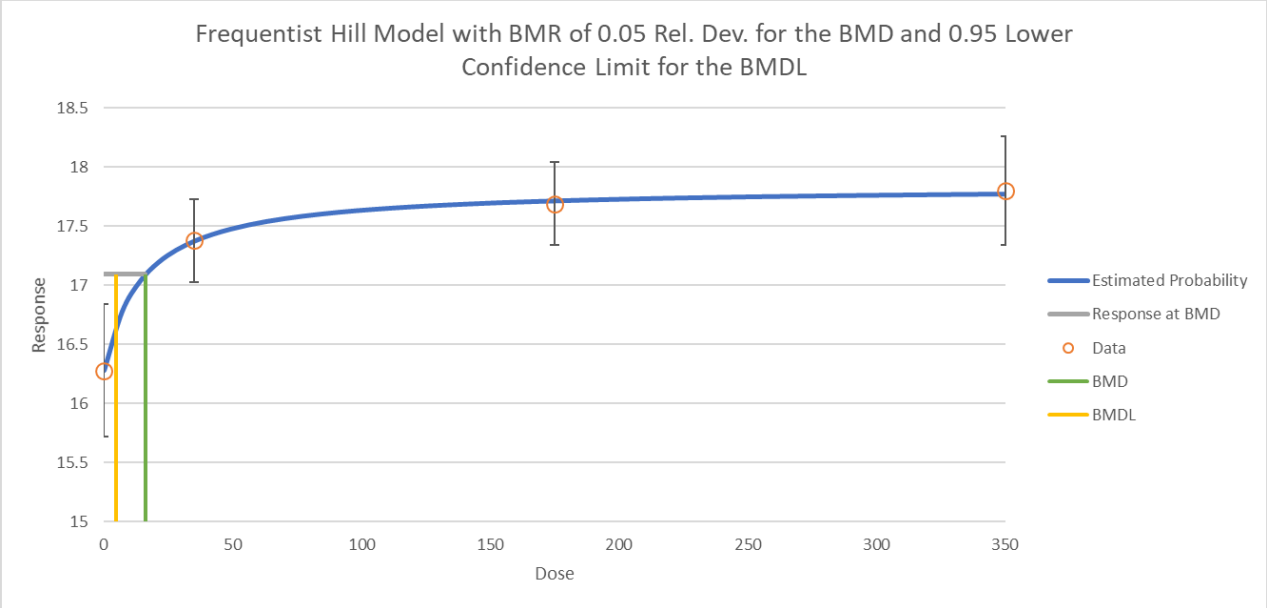

Figure D-5. Dose-response curve for the Hill model fit to delayed eye opening in male and female mice (Das et al., 2008).

User Input

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

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

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

Model Results

Benchmark Dose	
BMD	16.15084927
BMDL	4.88775303
BMDU	58.67497527
AIC	198.7877861
Test 4 P-value	0.865852068
D.O.F.	1

Model Parameters	
# of Parameters	5
Variable	Estimate
g	16.28027637
v	1.557732828
k	14.75612987
n	Bounded
alpha	0.771309051

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	20	16.28027637	16.28	16.28	0.87824202	1.19	1.19	-0.001407337
35	22	17.3760338	17.38	17.38	0.87824202	0.79	0.79	0.021182211
175	17	17.71687421	17.69	17.69	0.87824202	0.68	0.68	-0.126167037
350	15	17.77499146	17.8	17.8	0.87824202	0.83	0.83	0.110285841

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-95.37962446	5	200.759249
A2	-91.88601151	8	199.772023
A3	-95.37962446	5	200.759249
fitted	-95.39389305	4	198.787786
R	-109.7197233	2	223.439447

* Includes additive constant of -68.00145. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	35.6674235	6	<0.0001
2	6.987225901	3	0.07230604
3	6.987225901	3	0.07230604
4	0.028537187	1	0.86585207

Table D-27. Benchmark dose results for delayed eye opening in male and female mice—constant variance, BMR = 1 standard deviation (Das et al., 2008)

Models	Restriction	1 Standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	289.0417	204.0632	0.0008	211.1176	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Exponential 3 (CV—normal)	Restricted	289.0397	204.0631	0.0008	211.1176	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Exponential 4 (CV—normal)	**Restricted**	**23.0895**	**12.5328**	**0.7270**	**198.8811**	**Viable—recommended**	**Lowest AIC**
Exponential 5 (CV—normal)	Restricted	−9,999.0000	0.0000	NA	215.6060	Unusable	BMD computation failed BMD not estimated BMDL not estimated |Residual at control| >2 df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Hill (CV—normal)	Restricted	19.0723	0.0000	0.8659	198.7878	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated
Polynomial (3 degree) (CV—normal)	Restricted	284.0211	198.2059	0.0008	210.9441	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Polynomial (2 degree) (CV—normal)	Restricted	284.0211	198.2059	0.0008	210.9441	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Power (CV—normal)	Restricted	284.0218	198.2009	0.0008	210.9441	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Linear (CV—normal)	Unrestricted	284.0204	198.2054	0.0008	210.9441	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2

D.8. VAGINAL OPENING—F_1 FEMALE MICE (Das et al., 2008)

Table D-28. Dose-response data for delayed vaginal opening in female mice (Das et al., 2008)

Dose (mg/kg-day)	*n*	Mean	SD
0	83	31.59	5.386
35	97	33.598	5.715
175	89	34.292	5.714
350	87	35.023	5.188

Table D-29. Benchmark dose results for delayed vaginal opening in female mice—constant variance, 5% relative deviation (Das et al., 2008)

Models	Restriction	5% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	199.6149	137.1410	0.0106	348.8761	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Exponential 3 (CV—normal)	Restricted	199.6216	137.1431	0.0106	348.8761	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Exponential 4 (CV—normal)	Restricted	17.1139	0.0000	0.6944	341.9320	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated
Exponential 5 (CV—normal)	Restricted	30.5201	0.0000	NA	343.9392	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Hill (CV—normal)	**Restricted**	**13.5161**	**3.7929**	**0.8401**	**341.8184**	**Viable—recommended**	**Lowest AIC BMDL 3× lower than lowest nonzero dose**
Polynomial (3 degree) (CV—normal)	Restricted	193.4400	130.5619	0.0115	348.7113	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Polynomial (2 degree) (CV—normal)	Restricted	193.4443	130.5615	0.0115	348.7113	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Power (CV—normal)	Restricted	193.4434	130.5626	0.0115	348.7113	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Linear (CV—normal)	Unrestricted	193.4436	130.5610	0.0115	348.7113	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2

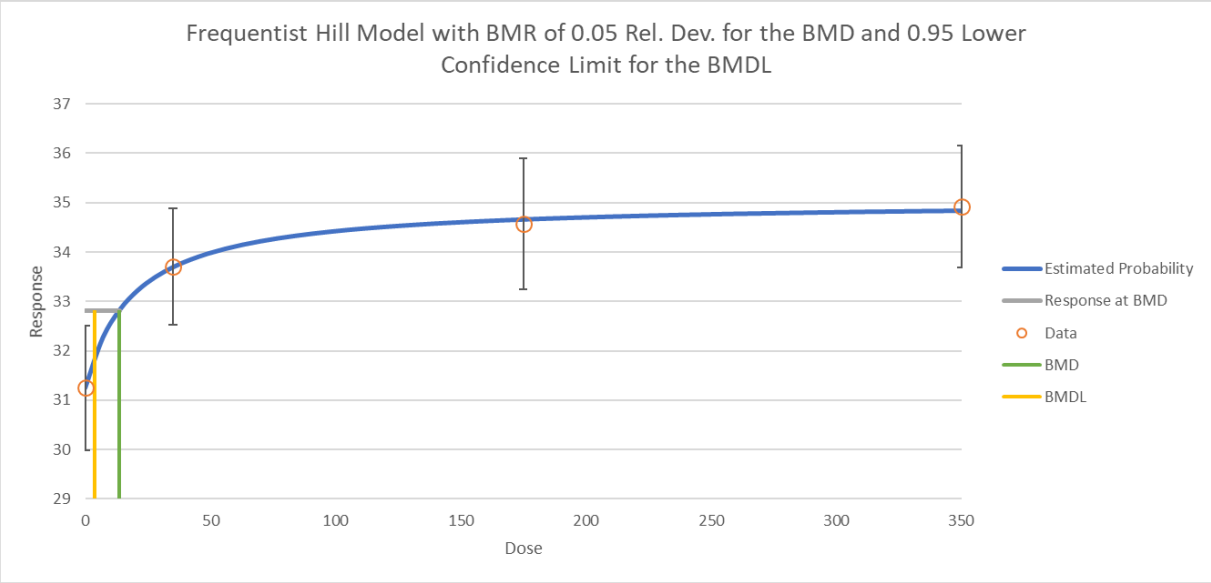

Figure D-6. Dose-response curve for the Hill model fit to delayed vaginal opening in female mice (Das et al., 2008).

User Input

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

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

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

Model Results

Benchmark Dose	
BMD	13.51609885
BMDL	3.792905489
BMDU	58.81907947
AIC	341.8183924
Test 4 P-value	0.840124836
D.O.F.	1

Model Parameters	
# of Parameters	5
Variable	Estimate
g	31.25160173
v	3.782877454
k	19.2052612
n	Bounded
alpha	6.040525655

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	19	31.25160173	31.25	31.25	2.45774809	2.62	2.62	-0.002840717
35	21	33.69418217	33.71	33.71	2.45774809	2.59	2.59	0.029493016
175	17	34.66038453	34.57	34.57	2.45774809	2.59	2.59	-0.151628625
350	15	34.83770206	34.92	34.92	2.45774809	2.23	2.23	0.129687238

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-166.8888479	5	343.777696
A2	-166.5982185	8	349.196437
A3	-166.8888479	5	343.777696
fitted	-166.9091962	4	341.818392
R	-177.364099	2	358.728198

* Includes additive constant of -66.16357. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	21.53176107	6	0.00147157
2	0.581258883	3	0.900709
3	0.581258883	3	0.900709
4	0.040696527	1	0.84012484

Table D-28. Benchmark dose results for delayed vaginal opening in female mice—constant variance, 1 standard deviation (Das et al., 2008)

Models	Restriction	1 Standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	316.9350	218.4320	0.0106	348.8761	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Exponential 3 (CV—normal)	Restricted	316.9457	218.4320	0.0106	348.8761	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Exponential 4 (CV—normal)	**Restricted**	**35.1705**	**15.4720**	**0.6944**	**341.9320**	**Viable—recommended**	**Lowest AIC**
Exponential 5 (CV—normal)	Restricted	34.9991	15.4632	NA	343.9392	Questionable	df = 0, saturated model (goodness-of-fit p-value cannot be calculated)
Hill (CV—normal)	Restricted	35.6204	0.0000	0.8401	341.8184	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated
Polynomial (3 degree) (CV—normal)	Restricted	311.4806	211.1287	0.0115	348.7113	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Polynomial (2 degree) (CV—normal)	Restricted	311.4877	211.1313	0.0115	348.7113	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Power (CV—normal)	Restricted	311.4864	211.1303	0.0115	348.7113	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2
Linear (CV—normal)	Unrestricted	311.4866	211.1307	0.0115	348.7113	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2

D.9. PREPUTIAL SEPARATION—F_1 MALE MICE (Das et al., 2008)

Table D-29. Dose-response data for delayed preputial separation in male mice (Das et al., 2008)

Dose (mg/kg-day)	*n*	Mean	SD
0	17	29.55	1.14
35	21	30.21	1.99
175	17	30.56	1.84
350	15	31.88	1.72

Table D-30. Benchmark dose results for delayed preputial separation in male mice—constant variance, BMR = 5% relative deviation (Das et al., 2008)

Models	Restriction	5% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	254.8183	179.1436	0.6004	277.5960	Viable—alternate	
Exponential 3 (CV—normal)	**Restricted**	**254.8005**	**179.1431**	**0.6004**	**277.5960**	**Viable—recommended**	**Lowest AIC**
Exponential 4 (CV—normal)	Restricted	252.8480	102.0115	0.3080	279.6149	Viable—alternate	
Exponential 5 (CV—normal)	Restricted	252.5410	101.9527	0.3076	279.6166	Viable—alternate	
Hill (CV—normal)	Restricted	194.2094	175.4639	0.2286	280.0252	Viable—alternate	
Polynomial (3 degree) (CV—normal)	Restricted	276.4524	176.5648	0.3427	279.4759	Viable—alternate	
Polynomial (2 degree) (CV—normal)	Restricted	269.5337	175.9153	0.3268	279.5372	Viable—alternate	
Power (CV—normal)	Restricted	252.7648	175.1179	0.5950	277.6140	Viable—alternate	
Linear (CV—normal)	Unrestricted	252.7653	175.1182	0.5950	277.6140	Viable—alternate	

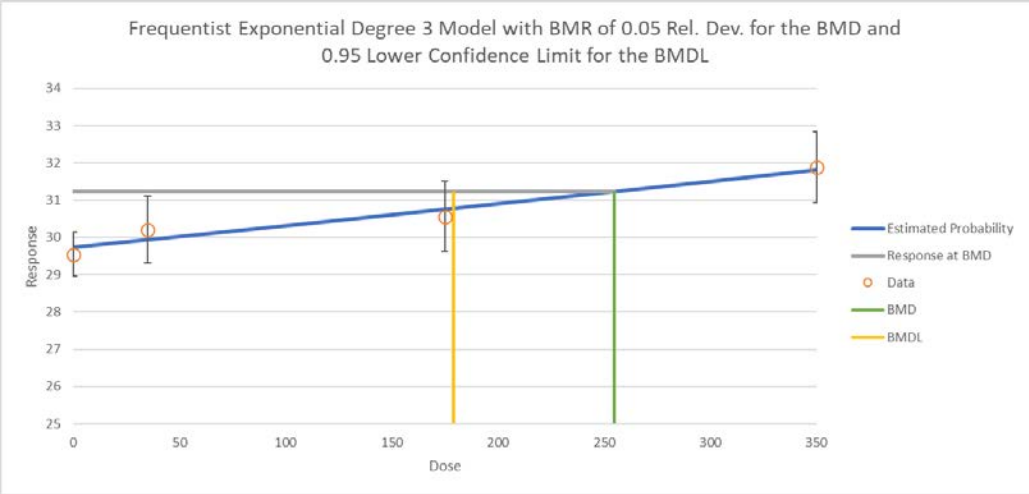

Figure D-7. Dose-response curve for the Exponential 3 model fit to delayed preputial separation in male mice (Das et al., 2008).

User Input

Info	
Model	frequentist Exponential degree 3 v1.1
Dataset Name	Das_PS_litter_SDs
User notes	[Add user notes here]
Dose-Response Model	M[dose] = a * exp(±1 * (b * dose)^d)
Variance Model	Var[i] = alpha

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

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

Model Results

Benchmark Dose	
BMD	254.8005164
BMDL	179.1431485
BMDU	443.2041287
AIC	277.5960319
Test 4 P-value	0.600364435
D.O.F.	2

Model Parameters	
# of Parameters	4
Variable	Estimate
a	29.74458616
b	0.000191484
d	Bounded
log-alpha	1.042066246

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	17	29.74458616	29.55	29.55	1.68376629	1.14	1.14	-0.47649088
35	21	29.94460185	30.21	30.21	1.68376629	1.99	1.99	0.722313504
175	17	30.75820529	30.56	30.56	1.68376629	1.84	1.84	-0.485353184
350	15	31.80636595	31.88	31.88	1.68376629	1.72	1.72	0.169372344

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-135.2877975	5	280.575595
A2	-132.4445224	8	280.889045
A3	-135.2877975	5	280.575595
fitted	-135.7980159	3	277.596032
R	-142.6419354	2	289.283871

* Includes additive constant of -64.3257. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	20.39482594	6	0.00235492
2	5.686550161	3	0.12789698
3	5.686550161	3	0.12789698
4	1.020436835	2	0.60036443

Table D-31. Benchmark dose results for delayed preputial separation in male mice—constant variance, BMR = 1 standard deviation (Das et al., 2008)

Models	Restriction	1 Standard deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
Exponential 2 (CV—normal)	Restricted	287.5467	201.6707	0.6004	277.5960	Viable—alternate	
Exponential 3 (CV—normal)	**Restricted**	**287.5612**	**201.6697**	**0.6004**	**277.5960**	**Viable—recommended**	**Lowest AIC**
Exponential 4 (CV—normal)	Restricted	286.3951	198.7931	0.3080	279.6149	Viable—alternate	
Exponential 5 (CV—normal)	Restricted	286.1679	197.6553	0.3076	279.6166	Viable—alternate	
Hill (CV—normal)	Restricted	201.3711	94.7311	0.2286	280.0252	Viable—alternate	
Polynomial (3 degree) (CV—normal)	Restricted	302.3780	199.5688	0.3427	279.4759	Viable—alternate	
Polynomial (2 degree) (CV—normal)	Restricted	297.6581	198.8516	0.3268	279.5372	Viable—alternate	
Power (CV—normal)	Restricted	286.2526	197.9759	0.5950	277.6140	Viable—alternate	
Linear (CV—normal)	Unrestricted	286.2531	197.9763	0.5950	277.6140	Viable—alternate	

D.10. RELATIVE LIVER WEIGHT—MALE HUMANIZED PPARα MICE (Foreman et al., 2009)

Table D-32. Dose-response data for relative liver weight in male humanized PPARα mice (Foreman et al., 2009)

Dose (mg/kg-day)	*n*	Mean	SD
0	10	4.07	0.261
35	10	5.62	0.719
175	10	6.65	0.784
350	10	7.38	0.719

Table D-33. Benchmark dose results for delayed preputial separation in male mice—nonconstant variance, BMR = 10% relative deviation (Das et al., 2008)

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Exponential 2 (NCV—normal)	Restricted	77.3820	62.7400	<0.0001	107.4138	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2 Modeled control response SD >|1.5| actual response SD
Exponential 3 (NCV—normal)	Restricted	77.3912	62.7399	<0.0001	107.4138	Questionable	Goodness-of-fit *p*-value < 0.1 |Residual at control| >2 Modeled control response SD >|1.5| actual response SD
Exponential 4 (NCV—normal)	Restricted	6.7656	4.8076	0.0951	80.0462	Questionable	Goodness-of-fit *p*-value < 0.1 BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose
Exponential 5 (NCV—normal)	Restricted	6.7678	4.8076	0.0951	80.0462	Questionable	Goodness-of-fit *p*-value < 0.1 BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose
Hill (NCV—normal)	**Restricted**	**5.4945**	**4.4070**	**0.2883**	**78.3878**	**Viable—recommended**	**Lowest AIC BMD 3× lower than lowest nonzero dose BMDL 3× lower than lowest nonzero dose**
Polynomial (3 degree) (NCV—normal)	Restricted	59.5695	46.0032	<0.0001	104.4698	Questionable	Goodness-of-fit *p*-value < 0.1 |residual for dose group near BMD| >2 |residual at control| >2 Modeled control response SD >|1.5| actual response SD

Models	Restriction	10% Relative deviation		*p*-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nonconstant variance							
Polynomial (2 degree) (NCV—normal)	Restricted	59.5723	46.0033	<0.0001	104.4698	Questionable	Goodness-of-fit *p*-value < 0.1 |residual for dose group near BMD| >2 |residual at control| >2 Modeled control response SD >|1.5| actual response SD
Power (NCV—normal)	Restricted	59.5691	46.0034	<0.0001	104.4698	Questionable	Goodness-of-fit *p*-value < 0.1 |residual for dose group near BMD| >2 |residual at control| >2 Modeled control response SD >|1.5| actual response SD
Linear (NCV—normal)	Unrestricted	59.5725	46.0031	<0.0001	104.4698	Questionable	Goodness-of-fit *p*-value < 0.1 |residual for dose group near BMD| >2 |residual at control| >2 Modeled control response SD >|1.5| actual response SD

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

APPENDIX F. QUALITY ASSURANCE FOR THE IRIS TOXICOLOGICAL REVIEW OF PERFLUOROBUTANOIC ACID AND RELATED COMPOUND AMMONIUM PERFLUOROBUTANOIC ACID

This assessment was 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 policy, and that policy is 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)*. An 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 Perfluorobutanoic Acid and Related Compound Ammonium Perfluorobutanoic Acid* has been designated as Highly Influential Scientific Information (HISA)/Influential Scientific Information (ISI) and is classified as QA Category A. Category A designations require reporting of all critical QA activities, including audits. IRIS assessments are developed through a seven-step process. Documentation of this process is available on the IRIS website: https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#process.

Specific management of quality assurance within the IRIS Program is documented in a Programmatic Quality Assurance Project Plan (PQAPP). A PQAPP was developed using the EPA *Guidance for Quality Assurance Project Plans (QA/G-5),* and the latest approved version is dated March 2020. All IRIS assessments follow the IRIS PQAPP, and all assessment leads and team members are required to receive QA training on the IRIS PQAPP. During assessment development, additional QAPPs may be applied for quality assurance management. They include:

Title	Document Number	Date
Program Quality Assurance Project Plan (PQAPP) for the Integrated Risk Information System (IRIS) Program	L-CPAD-0030729-QP-1-4	April 2021
An Umbrella Quality Assurance Project Plan (QAPP) for Dosimetry and Mechanism-Based Models (PBPK)	L-CPAD-0032188-QP-1-2	December 2020
Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS)	L-HEEAD-0032189-QP-1-2	September 2020
Umbrella Quality Assurance Project Plan for CPHEA PFAS Toxicity Assessments	L-CPAD-0031652-QP-1-3	October 2020

During assessment development, this project underwent two quality audits during assessment development including:

Date	Type of audit	Major findings	Actions taken
August 2020	Technical System Audit	No findings	None
August 2019	Technical System Audit	No findings	None

During **Step 3** of the IRIS Process, the IRIS Toxicological Review was subjected to external reviews by other federal agency partners including the Executive Offices of the White House. Comments during these IRIS Process steps are available in the Docket [EPA-HQ-ORD-2020-0675] on regulations.gov.

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