⊕EPA

Toxicological Review of Perfluorobutanoic Acid and Related Compound Ammonium Perfluorobutanoic Acid

(CASRN 375-22-4
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Supplemental Information—Appendices B, C, D, and E

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

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FIGURES

ABBREVIATIONS AND ACRONYMS

AIC	Akaike's information criterion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service registry number
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	extra risk
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GLP	good laboratory practices
GSH	glutathione
GST	glutathione-S-transferase
HBCD	hexabromocyclododecane
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
i.v.	intravenous
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose

NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RD	relative deviation
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
TSCATS	Toxic Substances Control Act Test Submissions
TWA	time-weighted average
UF	uncertainty factor
UF_C	composite uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOS	Web of Science

APPENDIX 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

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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 a 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 and/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 and/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	

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	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 or 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.

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

	Used in title/abstract and full-text screening					Used in full text 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	• 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	• Adrenal • Sex hormones (e.g., androgen; estrogen; progesterone) • Neuroendocrine • Pituitary • Steroidogenesis • Thyroid

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| | | | | Ecotoxicity studies

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) | | chemistry measures

Hepatic, including liver measures and serum markers (e.g., ALT; AST)

Immune/ inflammation

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

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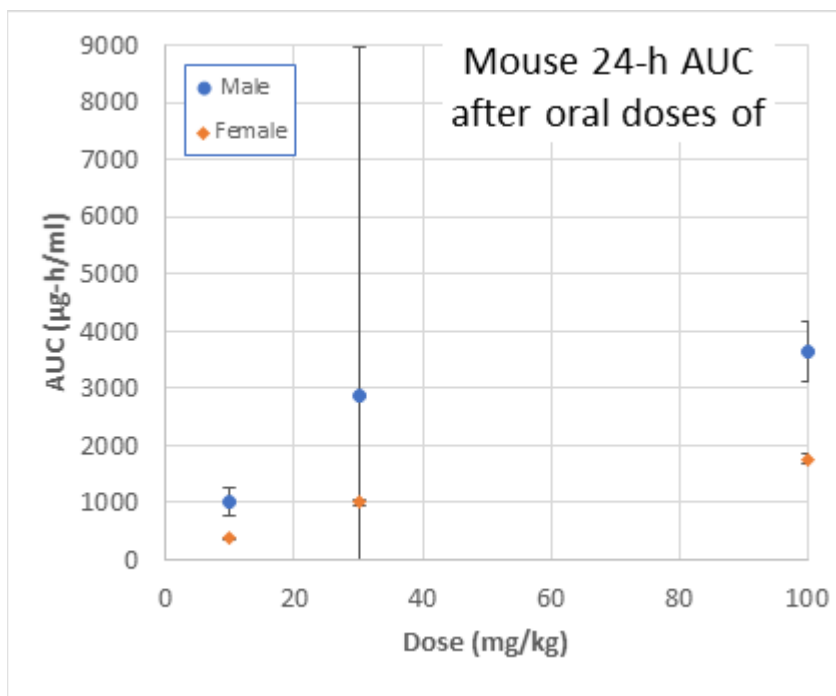
Figure C-1. Mouse AUC after oral doses of PFBA.

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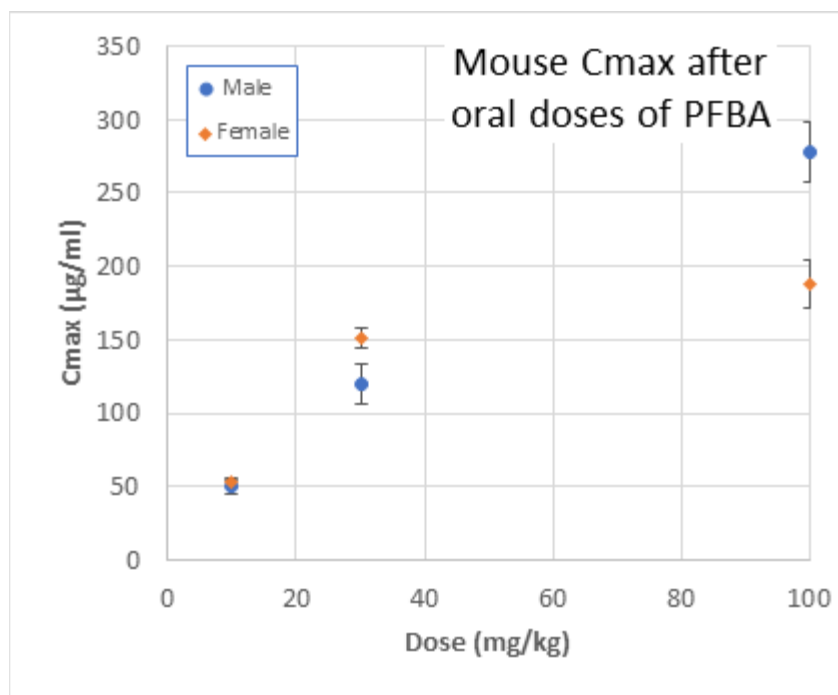
Figure C-2. Mouse C_{max} after oral doses of PFBA.

1   Chang et al. (2008) reported serum and liver concentrations in male rats and serum

2   concentrations in female rats given a 3–300 mg/kg oral dose of PFBA at 24 hours after dosing.

3   While the time point for these measurements is not ideal given the short half-life of PFBA, the data

4   indicate that the dosimetry is approximately linear up to 100 mg/kg in male rats and up to

5   30 mg/kg in female rats (see Figure C-3, Figure C-4). Tissue levels then appear to saturate or

6   decline; this may be due to incomplete absorption at higher doses and/or saturable renal

7   resorption, whereby excretion is more rapid for concentrations above the level of saturable

8   resorption in the kidney. With the half-life in female rats being ~3 hours, the female serum 24-hour

9   data are particularly subject to experimental noise, but at least provide an indication that use of the

10   half-life measured using a 30 mg/kg dose is applicable to BMD levels from bioassays at or below

11   this dose rate.

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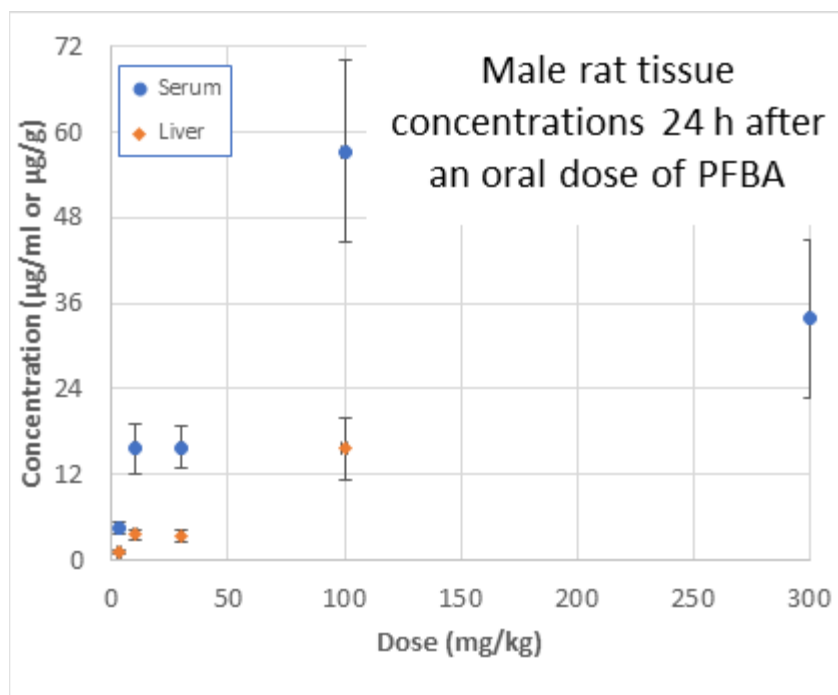
Figure C-3. Rat AUC after oral doses of PFBA.
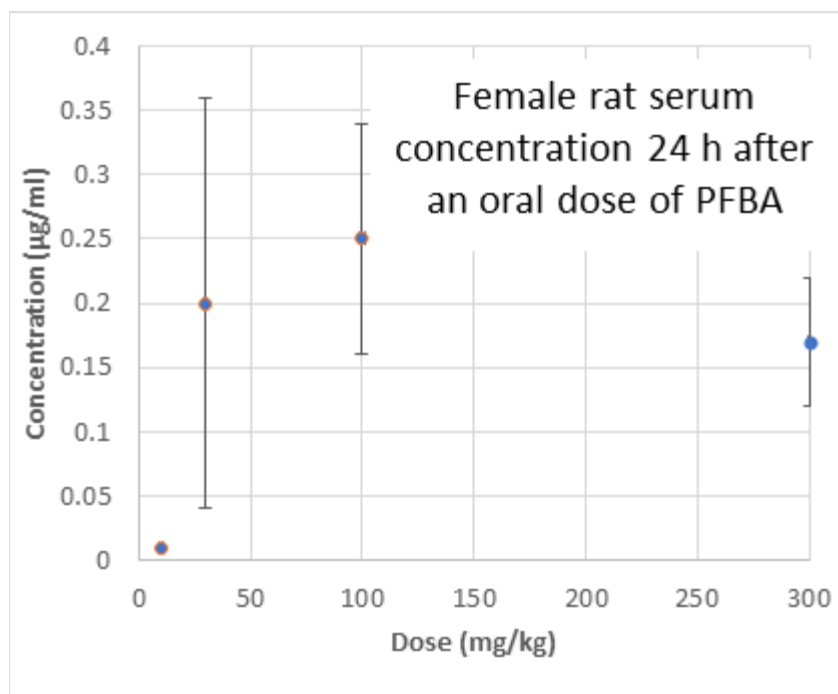
Figure C-4. Rat C_{max} after oral doses of PFBA.

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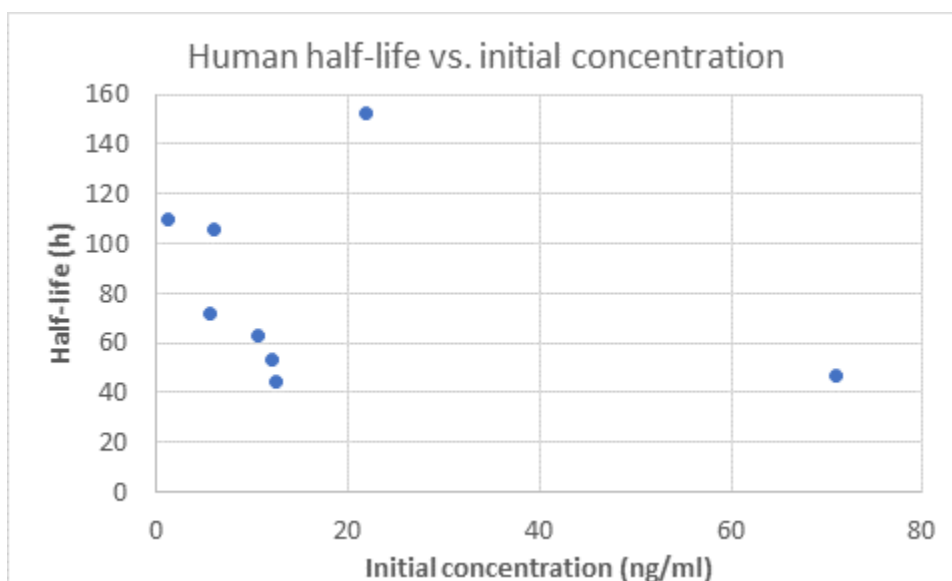
Figure C-5. Estimated human half-lives versus initial serum concentrations.

10   Chang et al. (2008) only evaluated one PFBA dose in monkeys, so it is not possible to
11 determine whether the biphasic clearance pattern is due to the classical distinction between
12 distribution and excretion phases or a nonlinearity in clearance. However, the data show linear
13 clearance from 1–7 or 10 days after the i.v. dose was given, when serum concentrations were below
14 100 ng/mL. Hence it seems reasonable to interpret these data as showing linear kinetics for serum
15 concentrations below 100 ng/mL under long-term exposure conditions. Since the highest initial
16 condition of the human subjects of Chang et al. (2008) had an initial condition of 72 ng/mL, to the
17 extent that kinetics in monkeys can be extrapolated to humans, the results for monkeys confirm the
18 conclusion that human kinetics are also reasonably assumed to be linear below ~100 ng/mL.
19 However, this is approximately 1,000-fold below the range of linearity in mice and rats, so there is
20 uncertainty as to whether the range of linear kinetics in humans and monkeys extends into the
21 range of rodent-based points of departure.

1   [Russell et al. (2015)](#) attempted to evaluate the kinetics of PFBA as a metabolite of
2   6:2 fluorotelomer alcohol (FTOH) during a 1-day inhalation study (6-hour exposure, 24-hour
3   observation) and at the end of 23 days of exposure. However, the half-life of PFBA could not be
4   estimated from the single-day data for male rats and only for the high-level exposure in female rats,
5   with yields of PFBA being 0.2% in males and not detectable or 0.02% in females. Also, there are
6   three metabolic intermediates between 6:2 FTOH and PFBA, but the model appears to have
7   assumed direct, instantaneous transformation through the first two steps. Assumptions about the
8   volume of distribution were made by [Russell et al. (2015)](#). These simplifications in the model likely
9   explains the large discrepancy between the PFBA half-life determined from the single-day exposure
10 6:2 FTOH for female rats (19 hours) and the half-life obtained for direct exposure to PFBA (1.4-hour
11 average) by [Chang et al. (2008)](#). [Russell et al. (2015)](#) only used male rats in the 23-day 6:2 FTOH
12 inhalation study, from which they estimated a half-life of 27.7 hours, over three times higher than
13 the average obtained by [Chang et al. (2008)](#). The discrepancy could also occur because of an
14 under-estimation of the metabolic yield from the 1-day experiments. In summary, while [Russell et](#)
15 [al. (2015)](#) described measurements of PFBA in male rats from 23 days of exposure to 6:2 FTOH, the
16 results for female rats after a single exposure are completely inconsistent with the results of ([Chang](#)
17 [et al., 2008](#)). Hence the conclusions from the multiday study are considered too unreliable to be
18 used.
19 The other long-term data available on internal dosimetry are from the bioassays ([Butenhoff](#)
20 [et al., 2012](#); [Das et al., 2008](#); [van Otterdijk, 2007](#)). For serum concentrations in nonpregnant female
21 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
22 for pregnant mice are 3.8 ± 1.0 and 4.4 ± 0.7 µg/mL, for the 35- and 175-mg/kg dose groups,
23 respectively ([Das et al., 2008](#)). For female mice dosed with 30 and 100 mg/kg PFBA, [Chang et al.](#)
24 [(2008)](#) reported 4.1 ± 1.7 and 6.4 ± 3.9 µg/mL in serum 24 hours after the dose; using linear
25 extrapolation based on the difference in dose, one might expect 4.8 and 11.2 µg/mL at 24 hours
26 after doses of 35 and 175 mg/kg, given these data. Though the concentrations in the [Das et al.](#)
27 [(2008)](#) study are somewhat lower than these projections, the difference, especially at the low dose,
28 is within the range of uncertainty and precision expected for PK analysis.
29 It should be noted that, given an average clearance of 28 mL/kg-hour obtained by [Chang et](#)
30 [al. (2008)](#) after 10- and 30-mg/kg doses, the predicted average serum concentrations for a
31 35-mg/kg dose is 52 µg/mL. This average concentration reflects the much higher concentrations
32 expected in the first few hours after each dose.
33 For male rats, [Butenhoff et al. (2012)](#) measured end-of-treatment serum levels of 38 ± 23
34 and 52 ± 25 µg/mL after 28 and 90 days, respectively, at 30 mg/kg-day; we presume these
35 measurements were made 24 hours after the last dose. The corresponding values reported by
36 [Chang et al. (2008)](#) for a 30-mg/kg oral dose in the dose-range and time-course studies are 16 ± 3
37 and 29 ± 13 µg/mL, respectively. While there is again some discrepancy between the short-term
38 PK data and the bioassay measurements, the difference is it is roughly within a factor of 2, which is

1 acceptable for PK analysis and does not indicate a strong time dependence in the PK. One should
2 keep in mind that the estimated clearance and half-life values are based on multiple time points at
3 which the serum concentration is measured, while the comparisons above use only a single time
4 point, 24 hours after dosing, when the result will be sensitive to experimental variation.

5 Given these data and results, the half-life or clearance of PFBA measured in single-day
6 exposures by Chang et al. (2008) will be assumed to predict dosimetry after repeated exposures
7 that occur in bioassays. This is a common assumption for chemicals with relatively short half-lives
8 because pharmacokinetic studies are typically confined to a single day or less. It may be that
9 clearance in rats and mice includes a slower beta phase, like that observed in monkeys. If a slow
10 clearance phase exists, internal dose from long-term exposure will be higher than is effectively
11 estimated using the clearance rate determined from single-day exposures, which would increase
12 the HED compared with the current prediction. Using animal-human ratio of clearance values to
13 estimate the HED only relies on the assumptions that the average serum concentration (C_{AVG}) is
14 predictive of systemic effects in adults, that the relationship between C_{AVG} and dose rate is linear
15 with the proportionality determined by the clearance values estimated here (i.e., the clearance from
16 single-day experiments is predictive of bioassay conditions).

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

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

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

35 A linear mixed-effects model was additionally used to estimate a $t_{1/2}$ for PFBA according to
36 methods described in Li et al. (2018). Briefly, linear mixed effect models are an extension of simple

1   linear models that use the best linear unbiased prediction estimator to estimate both random and
2   fixed effects for clustered data. One important consequence of clustering is that measurements of
3   serum PFBA units within the same person (cluster) are more similar than measurements on serum
4   PFBA in different people (i.e., other clusters). Failure to account for the intracluster correlation
5   would result in misleading inferences. It was assumed that each individual in Chang et al. (2008)
6   was selected randomly from a larger population. Below is the mixed model formula used for
7   estimating the half-life of serum PFBA:

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

9   where PFBA_{ij} is the natural logarithm of the serum PFBA concentrations measured at the jth
10   time point for the ith subject, α_{pop} is the population mean (also known as the fixed intercept for the
11   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
12   population (also known as the average excretion rate constant for serum PFBA for the whole
13   population); $k_i \sim N\,(0, \sigma^2{}_k)$ is the random slope for the ith subject that allows the excretion rate to
14   vary by individuals; t_{ij} represents the observation time for the jth measurement of serum PFBA for i^{th}
15   subject; and $\varepsilon_{ij} \sim N\,(0, \sigma^2{}_\varepsilon)$ is the random-error effect (residual) for j^{th} measurement of ith subject. Of
16   note, the small sample sizes (due to the exclusion of the only two subjects identified as females)
17   limited our ability to draw clear conclusions in gender-stratified comparisons.
18   The half-life of serum PFBA for the study population ($t_{1/2,\,\text{pop}}$) was then estimated as:

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

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

APPENDIX D. BENCHMARK DOSE MODELING RESULTS

D.1. BMD MODELING APPROACHES

1   As discussed in Section 5 of the body of the report, the endpoints selected for benchmark
2 dose (BMD) modeling were relative liver weight, liver hypertrophy, total T4, and thyroid follicular
3 hypertrophy incidence from Butenhoff et al. (2012) and relative liver weight, full-litter resorption,
4 delayed eye opening, delayed vaginal opening, and delayed preputial separation from Das et al.
5 (2008). The animal doses in the study were used in the BMD modeling and then converted to
6 human equivalent doses (HEDs) using the ratio of animal-to-human clearance values; the modeling
7 results are presented in this appendix.

8 ***Modeling Procedure for Dichotomous Noncancer Data***

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

26 ***Modeling Procedure for Continuous Noncancer Data***

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

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1   toxicological information was available to determine an adverse level of response. When

2   toxicological information was available, the BMR was based on relative deviation, as outlined in the

3   Benchmark Dose Technical Guidance (U.S. EPA, 2012) (see Toxicological Review, Section 4.2.1 for

4   justification of BMRs). An adequate fit is judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$),

5   scaled residuals at the data point (except the control) closest to the predefined benchmark

6   response (absolute value <2.0), and visual inspection of the model fit. In addition to these three

7   criteria for judging adequacy of model fit, a determination is made as to whether the variance

8   across dose groups is homogeneous. If a homogeneous variance model is deemed appropriate

9   based on the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated

10   from a homogeneous variance model. If the test for homogeneity of variance is rejected ($p < 0.05$),

11   the model is run again while modeling the variance as a power function of the mean to account for

12   this nonhomogeneous variance. If this nonhomogeneous variance model does not adequately fit

13   the data (i.e., Test 3; $p < 0.05$), then alternative approaches were assessed on a case-by-case basis.

14   For example, in cases where neither variance model fit, or constant variance did not fit (with

15   adequate Test-4 p-value) and nonconstant variance did fit (with inadequate Test-4 p-value), then

16   the log-normal distribution was attempted.

17   In cases where a model with a number of parameters equal to the number of dose groups

18   was fit to the data set and all parameters were estimated and no p-value was calculated, that model

19   was not considered for estimation of a point of departure (POD) *unless* no other model provided

20   adequate fit. . Among all models providing adequate fit, the BMDL from the model with the lowest

21   AIC was selected as a potential POD when BMDL estimates differed by less than threefold. When

22   BMDL estimates differed by greater than threefold, the model with the lowest BMDL was selected

23   to account for model uncertainty.

24   ***Modeling Procedure for Continuous Noncancer Data***

25   For continuous developmental toxicity data, individual animal data was requested from the

26   study authors when possible. The use of individual animal data allows for the correct measure of

27   variance to be calculated. When a biological rationale for selecting a benchmark response level is

28   lacking, a BMR equal to 0.5 SD was used. The use of 1 SD for the BMR for continuous endpoints is

29   based on the observation that shifting the distribution of the control group by 1 SD results in ~10%

30   of animal data points falling beyond an adversity cutoff defined at the ~1.5 percentile ([Crump,

31   1995]). This approximates the 10% extra risk that is commonly used as the BMR for dichotomous

32   endpoints. Thus, the use of 0.5 SD for continuous developmental toxicity endpoints approximates

33   the extra risk commonly used for dichotomous developmental toxicity endpoints.

34   ***Modeling Procedure for Continuous Noncancer Data***

35   For dichotomous developmental toxicity data, individual animal data was requested from

36   the study authors when possible. This allowed the use of the nested logistic model, which

1   statistically accounts for intralitter similarity (the propensity of littermates to respond more alike

2   one another than pups from another litter) by estimating intralitter correlation and using

3   litter-specific covariates. Judging model fit for this model is identical to the procedure used for

4   regular dichotomous models.

5   *Data Used for Modeling*

6   The source of the data used for modeling is provided in the section below. For endpoints

7   from the Das et al. (2008) study, the study authors kindly provided individual dam-level data to

8   facilitate modeling and to provide corrected data where needed. These data are also included in full

9   in the section below.

10

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/neonatal death	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	

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

Dose	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

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Table D-3. Data received from study authors for [Das et al. (2008)](#) on fetal and neonatal death (decreased survival to PND 21)

Dose	Number of implants	Number of dead
0	16	1
0	16	2
0	11	2
0	11	0
0	12	3
0	11	0
0	15	0
0	14	1
0	12	3
0	14	0
0	16	1
0	13	2
0	15	3
0	12	0
0	4	0
0	7	2
0	4	0
0	11	1
0	9	0
35	15	3
35	13	0
35	13	3
35	14	1
35	15	2
35	13	2
35	12	4
35	13	0
35	14	1
35	16	0
35	13	2
35	7	3

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Dose	Number of implants	Number of dead
35	13	1
35	11	0
35	12	1
35	11	1
35	9	0
35	8	1
35	11	1
35	11	0
35	11	1
35	10	1
175	14	1
175	15	0
175	14	7
175	14	1
175	15	2
175	14	1
175	15	0
175	16	2
175	11	0
175	14	3
175	9	0
175	11	0
175	9	2
175	13	1
175	12	1
175	11	1
350	7	2
350	12	1
350	16	3
350	11	0
350	14	2
350	12	1
350	16	3

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Dose	Number of implants	Number of dead
350	17	2
350	12	3
350	14	0
350	7	3
350	11	1
350	11	0
350	11	1
350	5	1

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

Dose	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

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Dose	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

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Dose	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

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

Dose	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

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Dose	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

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Dose	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	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

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Dose	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	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.

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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 test cannot be calculated)

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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

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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

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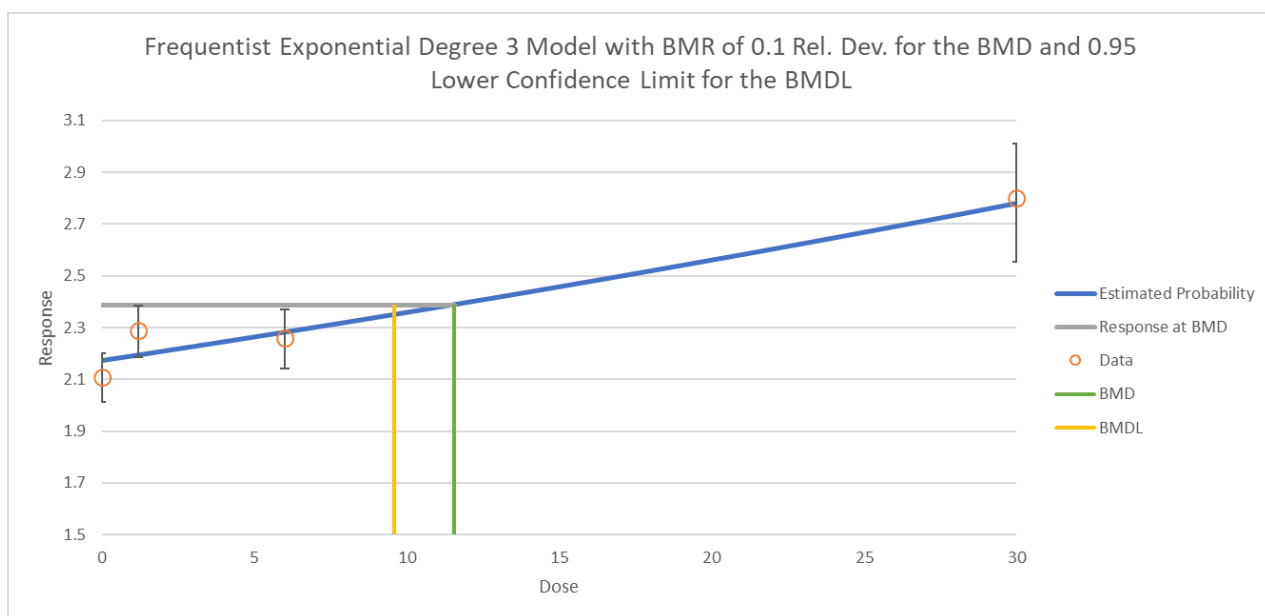
Figure D-1. Dose-response curve for the Exponential M3 model fit to relative liver weight in male rats (Butenhoff et al., 2012; van Otterdijk, 2007).

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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

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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

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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			AIC	BMDS classification	BMDS notes
		BMD	BMDL	*p*-Value			
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

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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

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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 test cannot be calculated)
Hill (CV—normal)	Restricted	38.7873	12.3846	NA	71.0979	Questionable	df = 0, saturated model (goodness-of-fit test 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

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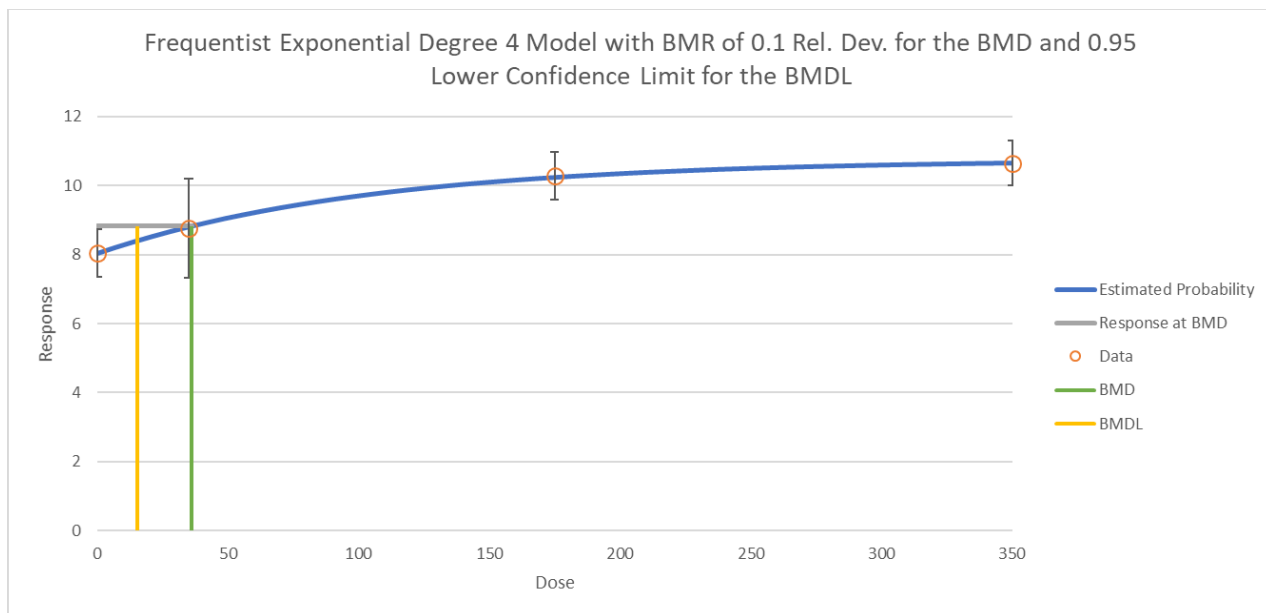
Figure D-2. Dose-response curve for the Exponential M4 model fit to relative liver weight in pregnant mice ([Das et al., 2008](#)).

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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

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#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

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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 test cannot be calculated)
Hill (CV—normal)	Restricted	39.5789	13.8731	NA	71.7337	Questionable	df = 0, saturated model (goodness-of-fit test 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

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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	

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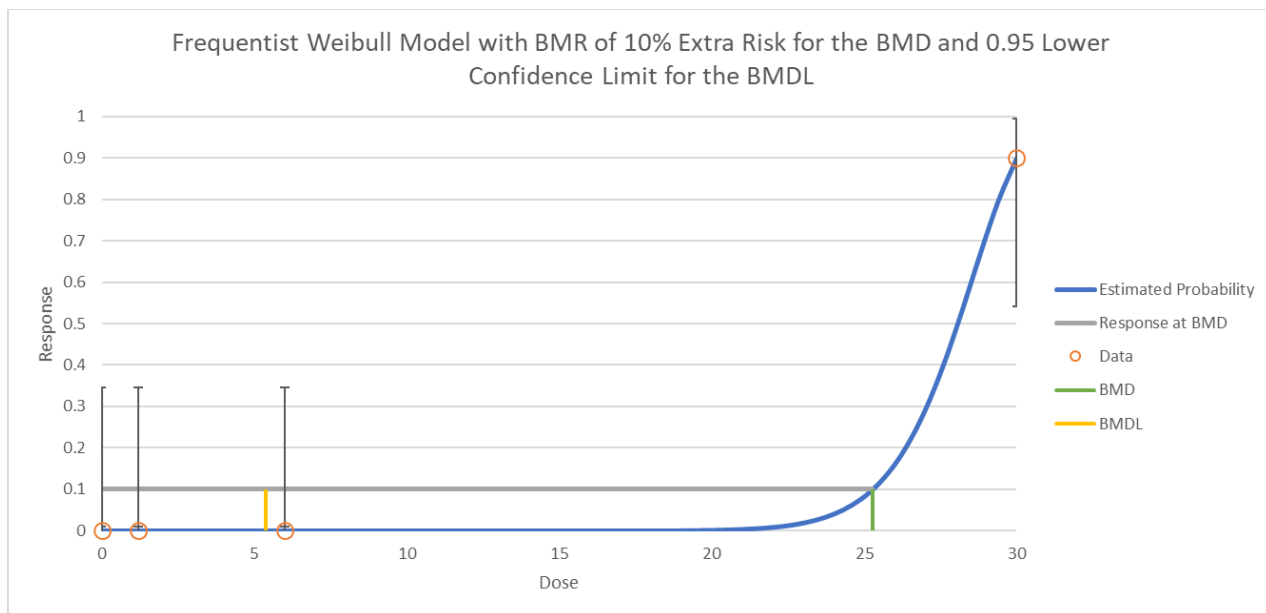
Figure D-3. **Dose-response curve for the Weibull model fit to liver hypertrophy in male rats ([Butenhoff et al., 2012](); [van Otterdijk, 2007]()).**

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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

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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 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

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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 BMD	BMDL	*p*-Value	AIC	BMDS classification	BMDS notes
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

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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 test cannot be calculated)
Hill (CV—normal)	Restricted	5.5394	3.2999	NA	103.5644	Questionable	df = 0, saturated model (goodness-of-fit test 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

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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 test cannot be calculated)
Hill (NCV—normal)	Restricted	5.8562	3.7033	NA	102.1809	Questionable	df = 0, saturated model (goodness-of-fit test 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

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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 BMD	BMDL	*p*-Value	AIC	BMDS classification	BMDS notes
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 test cannot be calculated)
Hill (CV—log-normal)	Restricted	-	-	-	-	Questionable	df = 0, saturated model (goodness-of-fit test 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

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D.6. FULL-LITTER RESORPTION—P$_0$ MICE ([Das et al., 2008](#))

Table D-23. Dose-response data full-litter resorption in pregnant mice ([Das et al., 2008](#))

Dose (mg/kg-day)	n	Incidence
0	29	2
35	29	1
175	28	4
350	29	8

Table D-24. Benchmark dose results for full-litter resorption in pregnant mice—BMR = 10% extra risk ([Das et al., 2008](#))

Models	Restriction	10% Extra risk		p-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Gamma	Restricted	129.1841	44.7442	0.4932	86.8779	Viable—alternate	
Log-logistic	Restricted	127.8222	40.0187	0.4909	86.8834	Viable—alternate	
Multistage 3rd	Restricted	138.9282	44.3498	0.4590	86.9663	Viable—alternate	
Multistage 2nd	Restricted	138.9320	44.3496	0.4590	86.9663	Viable—alternate	
Multistage 1st	Restricted	73.4834	42.3774	0.6063	85.4560	Viable—alternate	
Weibull	Restricted	127.8028	44.6144	0.4818	86.9068	Viable—alternate	
Dichotomous Hill	Unrestricted	156.8426	20.2203	NA	88.7417	Questionable	BMD:BMDL ratio > 5 df = 0, saturated model (goodness-of-fit test cannot be calculated)
Logistic	Unrestricted	124.9030	90.4959	0.7318	85.0591	Viable—alternate	
Log-probit	**Unrestricted**	**127.1407**	**21.2133**	**0.5263**	**86.8003**	**Viable— recommended**	**Lowest BMDL BMD:BMDL ratio > 5**
Probit	Unrestricted	115.5983	82.8726	0.7314	85.0520	Viable—alternate	

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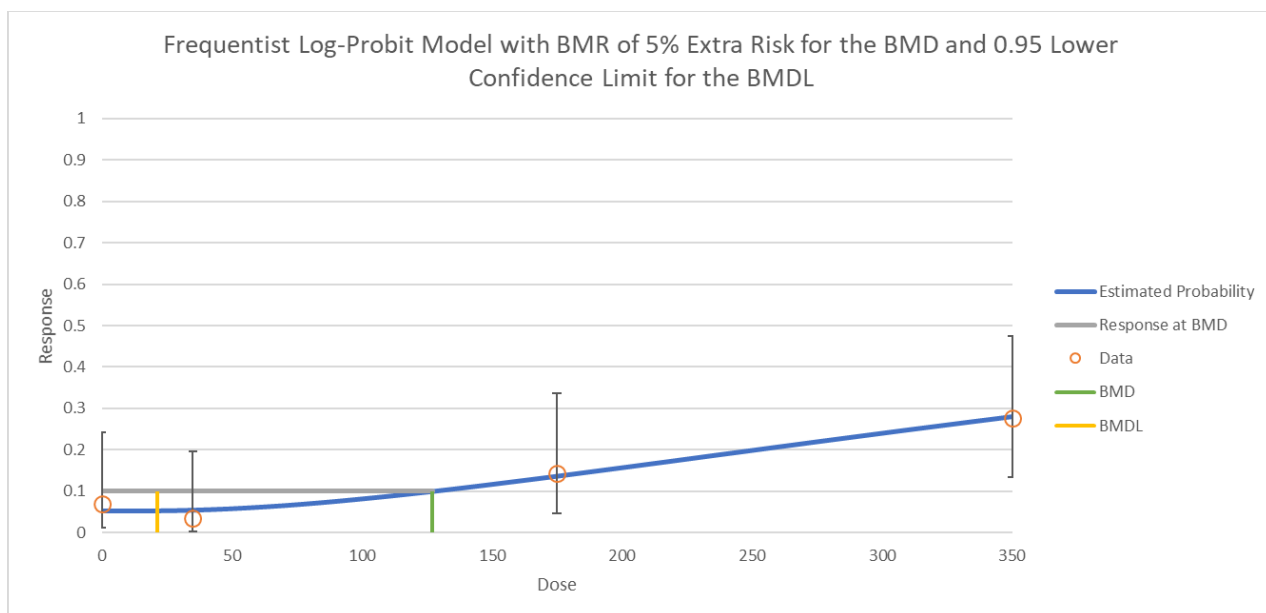
Figure D-4. Dose-response curve for the Log-Probit model fit to full-litter resorption in pregnant mice (Das et al., 2008).

User Input

Info	
Model	frequentist Log-Probit v1.1
Dataset Name	Das_FLR
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g+(1-g) * CumNorm(a+b*Log(Dose))

Model Options	
Risk Type	Extra Risk
BMR	0.05
Confidence Level	0.95
Background	Estimated

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

Model Results

Benchmark Dose	
BMD	127.1407067
BMDL	21.21334273
BMDU	342.236715
AIC	86.80025182
P-value	0.526335491
D.O.F.	1
Chi^2	0.401461403

Model Parameters	
# of Parameters	3
Variable	Estimate
g	0.051917349
a	-6.142891209
b	0.928331121

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.051917349	1.505603111	2	29	0.4138066
35	0.054040208	1.567166041	1	29	-0.465818
175	0.136095182	3.810665089	4	28	0.1043512
350	0.279903357	8.117197346	8	29	-0.048475

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-40.19187457	4	-	-	-
Fitted Model	-40.40012591	3	0.41650268	1	0.5186873
Reduced Model	-44.52942315	1	8.67509716	3	0.0339376

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D.7. INCREASED FETAL/NEONATAL DEATH (Das et al., 2008)

Table D-25. Dose-response data for increased fetal/neonatal death (Das et al., 2008)

Dose (mg/kg-day)	n (No. of implants)	No. of dead fetuses/neonates by PND 21	Litter-specific covariate (No. of implants)
0	16	1	16
0	16	2	16
0	11	2	11
0	11	0	11
0	12	3	12
0	11	0	11
0	15	0	15
0	14	1	14
0	12	3	12
0	14	0	14
0	16	1	16
0	13	2	13
0	15	3	15
0	12	0	12
0	4	0	4
0	7	2	7
0	4	0	4
0	11	1	11
0	9	0	9
35	15	3	15
35	13	0	13
35	13	3	13
35	14	1	14
35	15	2	15
35	13	2	13
35	12	4	12
35	13	0	13
35	14	1	14
35	16	0	16
35	13	2	13

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Dose (mg/kg-day)	n (No. of implants)	No. of dead fetuses/neonates by PND 21	Litter-specific covariate (No. of implants)
35	7	3	7
35	13	1	13
35	11	0	11
35	12	1	12
35	11	1	11
35	9	0	9
35	8	1	8
35	11	1	11
35	11	0	11
35	11	1	11
35	10	1	10
175	14	1	14
175	15	0	15
175	14	7	14
175	14	1	14
175	15	2	15
175	14	1	14
175	15	0	15
175	16	2	16
175	11	0	11
175	14	3	14
175	9	0	9
175	11	0	11
175	9	2	9
175	13	1	13
175	12	1	12
175	11	1	11
350	7	2	7
350	12	1	12
350	16	3	16
350	11	0	11
350	14	2	14
350	12	1	12
350	16	3	16

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Dose (mg/kg-day)	n (No. of implants)	No. of dead fetuses/neonates by PND 21	Litter-specific covariate (No. of implants)
350	17	2	17
350	12	3	12
350	14	0	14
350	7	3	7
350	11	1	11
350	11	0	11
350	11	1	11
350	5	1	5

Table D-26. Benchmark dose results for increased fetal/neonatal deaths (male and female mice)—BMR = 5% extra risk (Das et al., 2008)

Models	Restriction	5% Extra risk		p-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Nested logistic (lsc+ilc+)	Restricted	651.0725	240.5239	0.4233	602.7296	Viable—alternate	BMD higher than maximum dose
Nested logistic (lsc+ilc−)	Restricted	387.2376	260.0529	0.0383	602.1221	Questionable	Goodness-of-fit p-value < 0.1 BMD higher than maximum dose
Nested logistic (lsc−ilc+)	**Restricted**	**423.4064**	**184.9996**	**0.3473**	**601.4715**	**Viable—recommended**	**Lowest AIC BMD higher than maximum dose**
Nested logistic (lsc−ilc−)	Restricted	422.6433	196.9360	0.0243	600.8256	Questionable	Goodness-of-fit p-value < 0.1 BMD higher than maximum dose

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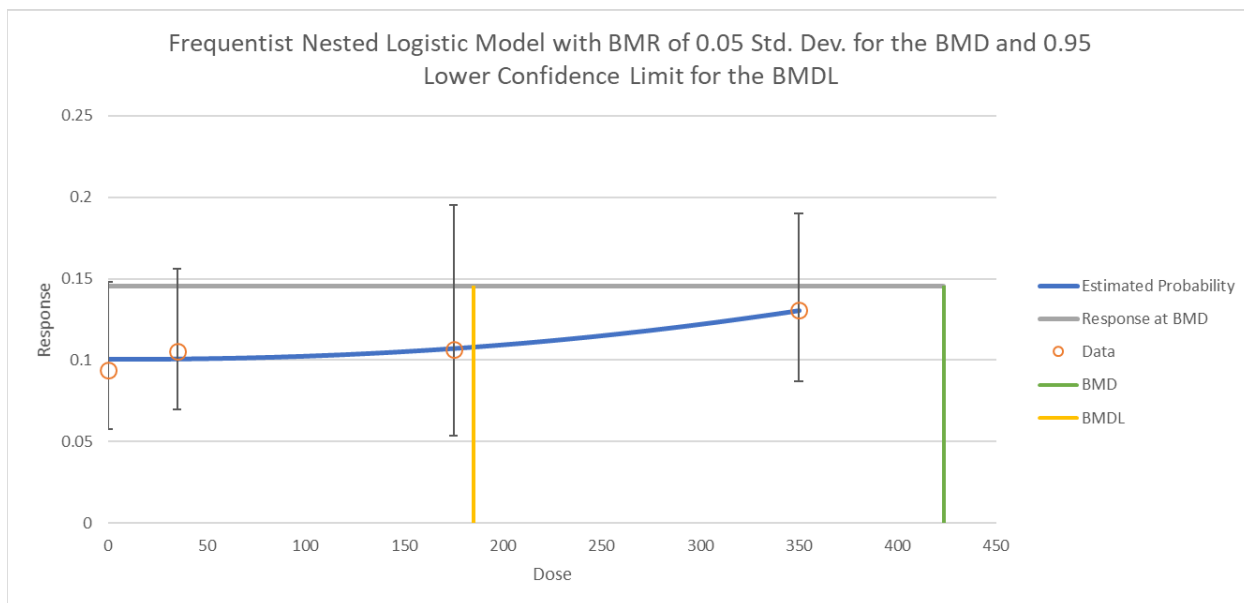
Figure D-5. Dose-response curve for the Nested-Logistic model fit to increased fetal/neonatal deaths in male and female mice ([Das et al., 2008](#)).

User Input

Info	
Model	frequentist Nested Logistic_lsc-ilc+_ v2.2
Dataset Name	Das_Fetal_Neonatal_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	0.05
Confidence Level	0.95
Litter Specific Covariate	Not used
Intralitter Correlation	Estimate
Background	Estimate

Model Data	
Dependent Variable	Dose
Independent Variable	Incidence
Total # of Observations	72

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Model Results

Benchmark Dose	
BMD	423.4064302
BMDL	184.9995764
BMDU	-
AIC	601.4714968
P-value	0.347333333
D.O.F.	66
Chi^2	76.79997014

Model Parameters	
# of Parameters	9
Variable	Estimate
alpha	0.100476405
beta	-16.2845194
theta1	0
theta2	0
rho	2.205579856
phi1	0.020885196
phi2	0.015603884
phi3	0.067890272
phi4	0

Bootstrap Results	
# Iterations	1000
Bootstrap Seed	1586275718
Log-likelihood	-294.7357484
Observed Chi-square	76.79997014
Combined P-value	0.347333333

Bootstrap Runs					
		Bootstrap Chi-square Percentiles			
Run	P-Value	50th	90th	95th	99th
1	0.353	71.32565706	92.424738	100.3736	114.82313
2	0.34	70.62605739	92.7840964	99.33441	116.40601
3	0.349	70.993585	91.2707375	98.52652	113.31872
Combined	0.347333333	70.92745637	92.2361697	99.00129	114.82313

Scaled Residuals	
Minimum scaled residual for dose group nearest the BMD	-0.48544
Minimum ABS(scaled residual) for dose group nearest the BMD	0.485442
Average Scaled residual for dose group nearest the BMD	0.085792
Average ABS(scaled residual) for dose group nearest the BMD	0.085792
Maximum scaled residual for dose group nearest the BMD	1.228261
Maximum ABS(scaled residual) for dose group nearest the BMD	1.228261

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	Litter Data					
Dose	Lit. Spec. Cov.	Est. Prob.	Litter Size	Expected	Observed	Scaled Residual
0	4	0.100476405	4	0.401906	0	-0.648424618
0	4	0.100476405	4	0.401906	0	-0.648424618
0	7	0.100476405	7	0.703335	2	1.536753401
0	9	0.100476405	9	0.904288	0	-0.928103507
0	11	0.100476405	11	1.10524	1	-0.095997848
0	11	0.100476405	11	1.10524	0	-1.008174126
0	11	0.100476405	11	1.10524	2	0.816178429
0	11	0.100476405	11	1.10524	0	-1.008174126
0	12	0.100476405	12	1.205717	3	1.553659628
0	12	0.100476405	12	1.205717	3	1.553659628
0	12	0.100476405	12	1.205717	0	-1.044023416
0	13	0.100476405	13	1.306193	2	0.572354915
0	14	0.100476405	14	1.40667	1	-0.320612147
0	14	0.100476405	14	1.40667	0	-1.108996845
0	15	0.100476405	15	1.507146	0	-1.138607757
0	15	0.100476405	15	1.507146	3	1.127810423
0	16	0.100476405	16	1.607622	1	-0.440917356
0	16	0.100476405	16	1.607622	1	-0.440917356
0	16	0.100476405	16	1.607622	2	0.284726228
35	7	0.10067014	7	0.704691	3	2.757073997
35	8	0.10067014	8	0.805361	1	0.217152338
35	9	0.10067014	9	0.906031	0	-0.94638627
35	10	0.10067014	10	1.006701	1	-0.006595085
35	11	0.10067014	11	1.107372	1	-0.100068359
35	11	0.10067014	11	1.107372	1	-0.100068359
35	11	0.10067014	11	1.107372	1	-0.100068359
35	11	0.10067014	11	1.107372	0	-1.032050523
35	11	0.10067014	11	1.107372	0	-1.032050523
35	12	0.10067014	12	1.208042	1	-0.184396334
35	12	0.10067014	12	1.208042	4	2.474633365
35	13	0.10067014	13	1.308712	2	0.584799703
35	13	0.10067014	13	1.308712	2	0.584799703
35	13	0.10067014	13	1.308712	3	1.430756163
35	13	0.10067014	13	1.308712	0	-1.107113218
35	13	0.10067014	13	1.308712	0	-1.107113218
35	13	0.10067014	13	1.308712	1	-0.261156757
35	14	0.10067014	14	1.409382	1	-0.331549985
35	14	0.10067014	14	1.409382	1	-0.331549985
35	15	0.10067014	15	1.510052	2	0.380881424
35	15	0.10067014	15	1.510052	3	1.158273108
35	16	0.10067014	16	1.610722	0	-1.204711311
175	9	0.107170489	9	0.964534	2	0.898239995
175	9	0.107170489	9	0.964534	0	-0.836708992
175	11	0.107170489	11	1.178875	1	-0.134561118
175	11	0.107170489	11	1.178875	0	-0.88682293
175	11	0.107170489	11	1.178875	0	-0.88682293
175	12	0.107170489	12	1.286046	1	-0.201977253
175	13	0.107170489	13	1.393216	1	-0.26172042
175	14	0.107170489	14	1.500387	3	0.944316054
175	14	0.107170489	14	1.500387	1	-0.315096819
175	14	0.107170489	14	1.500387	1	-0.315096819
175	14	0.107170489	14	1.500387	7	3.463141798
175	14	0.107170489	14	1.500387	1	-0.315096819
175	15	0.107170489	15	1.607557	0	-0.960793417
175	15	0.107170489	15	1.607557	2	0.234552335
175	15	0.107170489	15	1.607557	0	-0.960793417
175	16	0.107170489	16	1.714728	2	0.162285182
350	5	0.130545153	5	0.652726	1	0.460981681
350	7	0.130545153	7	0.913816	2	1.218569937
350	7	0.130545153	7	0.913816	3	2.340451698
350	11	0.130545153	11	1.435997	0	-1.285148171
350	11	0.130545153	11	1.435997	0	-1.285148171
350	11	0.130545153	11	1.435997	1	-0.390196125
350	11	0.130545153	11	1.435997	1	-0.390196125
350	12	0.130545153	12	1.566542	1	-0.485442138
350	12	0.130545153	12	1.566542	1	-0.485442138
350	12	0.130545153	12	1.566542	3	1.228260558
350	14	0.130545153	14	1.827632	2	0.13673773
350	14	0.130545153	14	1.827632	0	-1.449842671
350	16	0.130545153	16	2.088722	3	0.676218093
350	16	0.130545153	16	2.088722	3	0.676218093
350	17	0.130545153	17	2.219268	2	-0.157850557

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D.8. DELAYED EYE OPENING—F$_1$ MALE AND FEMALE MICE ([Das et al., 2008](#))

Table D-27. 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

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Table D-28. 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 test 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

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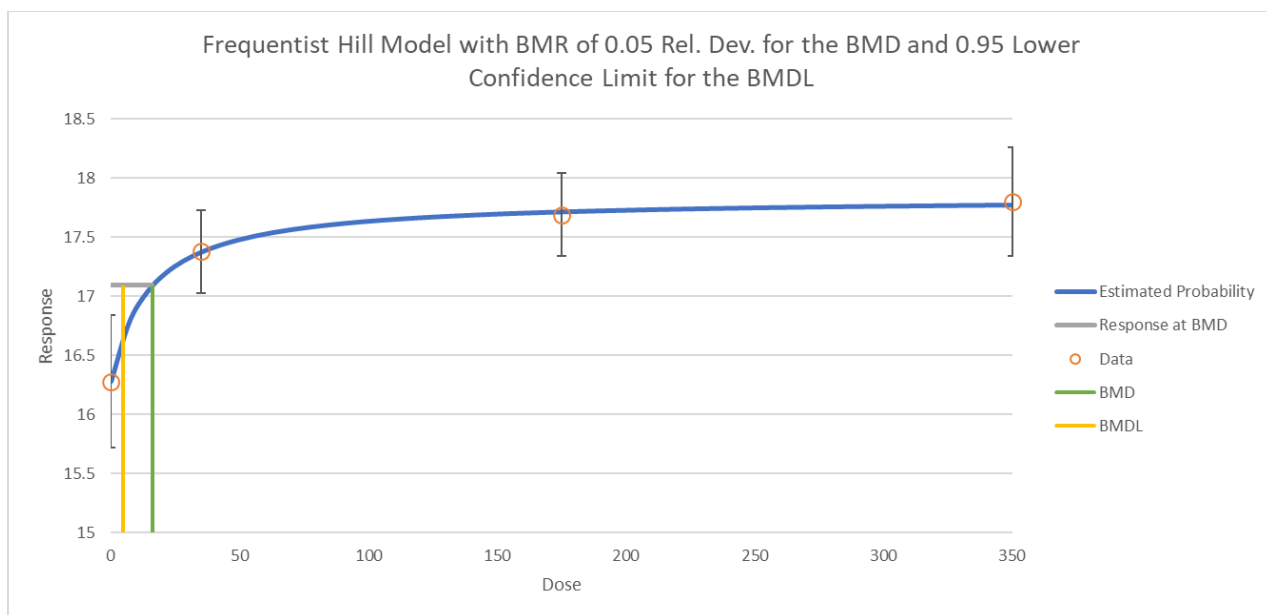
Figure E-6. Dose-response curve for the Hill model fit to delayed eye opening in male and female mice ([Das et al., 2008](#)).

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

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Table D-29. 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 test 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

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Models	Restriction	1 Standard deviation		p-Value	AIC	BMDS classification	BMDS notes
		BMD	BMDL				
Constant variance							
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.9. VAGINAL OPENING—F₁ FEMALE MICE (Das et al., 2008)

Table D-30. 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

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Table D-31. 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 test 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
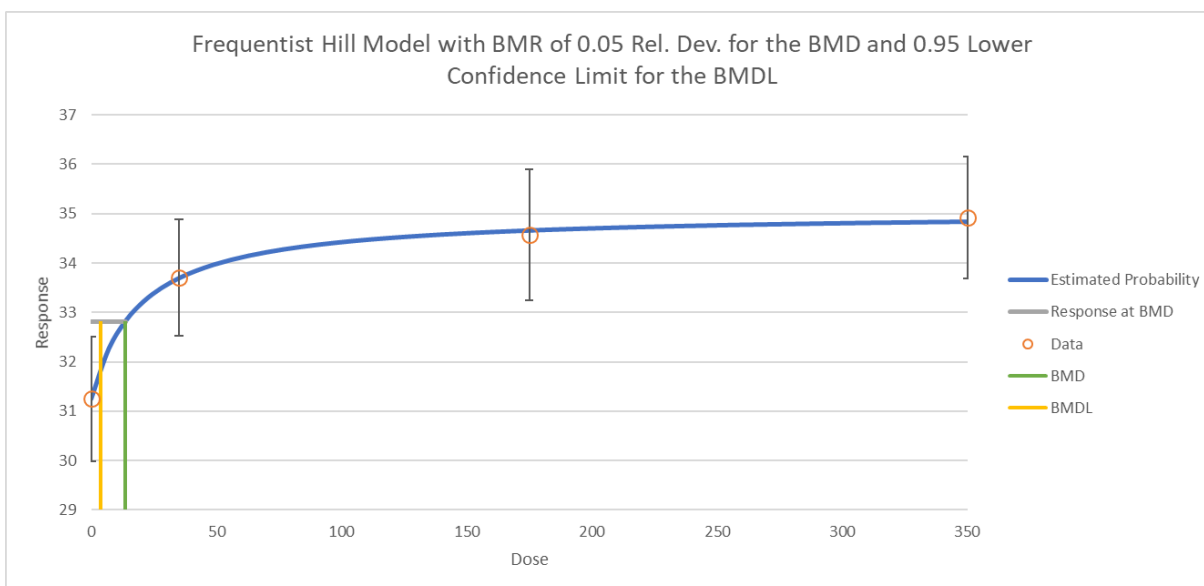
Figure D-7. Dose-response curve for the Hill model fit to delayed vaginal opening in female mice ([Das et al., 2008](#)).

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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

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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

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Table D-32. 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 test 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

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D.10. PREPUTIAL SEPARATION—F₁ MALE MICE (Das et al., 2008)

Table D-33. 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-34. 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 BMD	5% Relative deviation BMDL	*p*-Value	AIC	BMDS classification	BMDS notes
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	

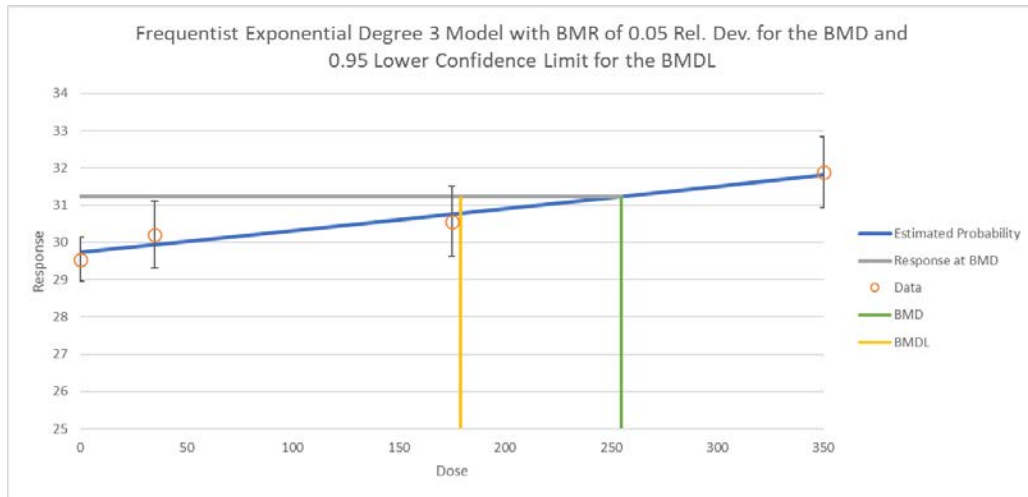This document is a draft for review purposes only and does not constitute Agency policy.

Figure D-8. Dose-response curve for the Exponential 3 model fit to delayed preputial separation in male mice (Das et al., 2008).

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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

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Table D-35. 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.11. RELATIVE LIVER WEIGHT—MALE HUMANIZED PPARα MICE (Foreman et al., 2009)

Table D-36. 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

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Table D-37. 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

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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

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

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