

**Benchmark Dose (BMD) Analysis of Laryngeal Metaplasia
Incidences in Rats Following Inhalation Exposure to
Pyrethrins**

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Limitations

The purpose of this report is to perform a Benchmark Dose (BMD) analysis for the compound Pyrethrins as requested by the European Food Safety Authority (EFSA). This report is based on procedures recommended in the EFSA (2022) BMD guidance. Exponent makes no claims regarding any BMD estimate that EFSA may develop. The responsible use of the BMD estimates calculated herein remains fully with EFSA. These conclusions are based on currently available information and may change if new or substantially different information becomes available.

Executive Summary

The European Food Safety Authority (EFSA) requested a benchmark dose (BMD) analysis to be conducted for the laryngeal squamous metaplasia incidence data reported in a study of rats exposed by inhalation to Pyrethrins for 90-days (CA 5.3.3/01; ██████████ 1992). Following EFSA (2022) guidance for BMD analysis, the datasets (Mucosa-male, Mucosa-female, Ventral-male, Ventral-female) were evaluated for their usability for estimating BMD. The data usability evaluation found that the Mucosa-male and Mucosa-female datasets lacked sufficient information to provide reliable BMD estimates, while the Ventral-male and Ventral-female dataset had sufficient information for reliable BMD estimates. Dose-response analysis using EFSA BMD app for the Mucosa-male and Mucosa-female datasets resulted in model average BMD estimates that failed EFSA (2022) diagnostic ratio criteria for reliable BMD estimates. EFSA BMD app model average estimates for the BMDL for Ventral-male = 8.57 mg/m³ and for the Ventral-female = 33.8 mg/m³, and they fulfill EFSA (2022) diagnostic ratio criteria. A preliminary analysis of the Mucosa-male dataset using newly released BMDS Version 3.3 resulted in model average BMD estimates that failed EFSA (2022) diagnostic criteria. Additional dose-response analysis using EPA BMDS Version 3.2 resulted in model average estimates of the BMDL for Mucosa-male = 1.15 mg/m³, Ventral-male = 20.11 mg/m³, and Ventral-female = 41.85 mg/m³, and they fulfill EFSA (2022) diagnostic criteria. The results of the data usability and dose-response analyses led to the conclusion that reliable BMD estimates could not be obtained for the Mucosa-male and Mucosa-female datasets.

Introduction

The European Food Safety Authority (EFSA) requested a Benchmark Dose (BMD) analysis to be conducted for the incidence of squamous metaplasia of the larynx reported in a 90-day inhalation study of rats exposed to Pyrethrins.¹ Exponent conducted a BMD analysis of the reported laryngeal squamous metaplasia data following EFSA BMD guidance (EFSA, 2022). This report documents the data evaluation, procedures involved in the BMD analysis, and the results of the analysis in a format recommended in the EFSA guidance.

¹ Reporting tables No. 2(39).

Data Description

Incidences of squamous metaplasia of the larynx reported in a study of rats exposed by inhalation to Pyrethrins for 90-days (CA 5.3.3/01; ██████ 1992) is the focus of this BMD analysis. Specifically, the reported findings were incidences of squamous/squamoid metaplasia/hyperplasia in the larynx mucosa pseudostratified columnar epithelium, and in the larynx ventral diverticulum cuboidal/columnar epithelium, in both male and female rats. These datasets are referred to herein this report as Mucosa-male, Mucosa-female, Ventral-male, and Ventral-female, respectively. Tables 1 and 2 summarize the reported incidences.

Table 1. Incidences of squamous/squamoid metaplasia/hyperplasia in the larynx mucosa pseudostratified columnar epithelium reported in ██████ (1992; CA 5.3.3/01)

	Dose (mg/m ³)				
	0	11	30	100	356
Male					
Number Examined	14	15	15	15	15
Severity					
1	2	0	1	2	0
2	0	7	4	5	0
3	0	3	8	3	9
4	0	0	0	5	6
Total	2	10	13	15	15
Female					
Number Examined	15	14	15	15	15
Severity					
1	0	2	0	0	0
2	0	9	7	4	3
3	0	2	5	6	10
4	0	0	0	1	2
Total	0	13	12	11	15

Table 2. Incidences of squamous/squamoid metaplasia/hyperplasia in the larynx ventral diverticulum cuboidal/columnar epithelium reported in ██████ (1992; CA 5.3.3/01)

	Dose (mg/m ³)				
	0	11	30	100	356
Male					
Number Examined	14	15	15	15	15
Severity					

	Dose (mg/m³)				
	0	11	30	100	356
1	0	1	1	5	4
2	0	1	0	0	6
3	0	0	0	0	2
Total	0	2	1	5	12
Female					
Number Examined	15	14	15	15	15
Severity					
1	0	0	1	2	7
2	0	0	0	0	1
3	0	0	0	0	0
Total	0	0	1	2	8

Suitability to estimate BMD using dose-response modeling

The total incidence of metaplasia across severity scores at each dose is evaluated in this analysis since it represents the highest, and more conservative, reported incidence at each dose. The four datasets were evaluated as recommended in the EFSA (2022) guidance for their suitability to estimate BMD using dose-response modeling. Pairwise comparisons of the reported total incidences from the five tested doses were conducted using Fisher Exact Test.

Selection of the Benchmark Response (BMR)

The endpoint of interest, squamous metaplasia in the larynx, is a binary (“quantal”) measurement (i.e., presence or absence of squamous metaplasia). This BMD analysis follows EFSA (2022) recommendation to use a BMR of 10% for this type of response dataset.

Software Used

Dose-response analysis was conducted using EFSA BMD app at <https://r4eu.efsa.europa.eu/app/bmd>, which is based on version 70.0 of PROAST. Due to partially unreliable BMD estimates resulted in the preliminary analysis, as described in the Results section, further analysis was conducted using U.S. Environmental Protection Agency's (EPA's) Benchmark Dose Software (BMDS). A preliminary analysis using one dataset was conducted using BMDS Version 3.3 PC- and Web-versions (released 2022-10-26) but, as described in the Results section, the results were uncertain. An additional BMD analysis was conducted using BMDS Version 3.2.0.1 (released 2022-03-15).

Deviation from EFSA Guidance Procedure and Assumptions

The analysis did not deviate from EFSA (2022) guidance procedure and assumptions.

Results

Data suitability for BMD analysis

Result of the pairwise comparisons using Fisher Exact Test are summarized in Table 3. Statistically-significant differences (p-value < 0.05) are indicated in bold.

Table 3. Results of pairwise comparison of reported total incidences of squamous metaplasia

Dataset	Mucosa-male ^a	Mucosa-female	Ventral-male	Ventral-female
Comparison of Incidence at Doses (mg/m ³)	Results of Fisher Exact Test (p-value)			
0, 11	7.78E-03	2.06E-07	0.48	1
11, 30	0.39	0.598	1	1
30, 100	0.483	1	0.166	1
100, 356	b	0.0996	0.0253	0.0502
^a Mucosa-male, Mucosa-female: squamous/squamoid metaplasia/hyperplasia in the larynx mucosa pseudostratified columnar epithelium in male and female rats, respectively. Ventral-male, Ventral-female: squamous/squamoid metaplasia/hyperplasia in the larynx ventral diverticulum cuboidal/columnar epithelium in male and female rats, respectively.				
^b Incidence values were identical so a statistical comparison is not needed to detect difference.				

For Mucosa-male and Mucosa-female, results of the pairwise comparison show that the metaplasia incidences of the control group was significantly different (p<0.05) from the incidences at 11 mg/m³ dose, and that there was no significant difference between the incidences at 11, 30, 100, 356 mg/m³. In contrast, for Ventral-male and Ventral-female, results of the pairwise comparison show that the metaplasia incidences of the control group and doses 11, 30, and 100 were not significantly different, and significant difference is found comparing incidences at dose 100 and 300 mg/m³. Figure 1 illustrates the results of the comparisons in a manner similar to Figures 3.1 to 3.3 in the EFSA guidance.

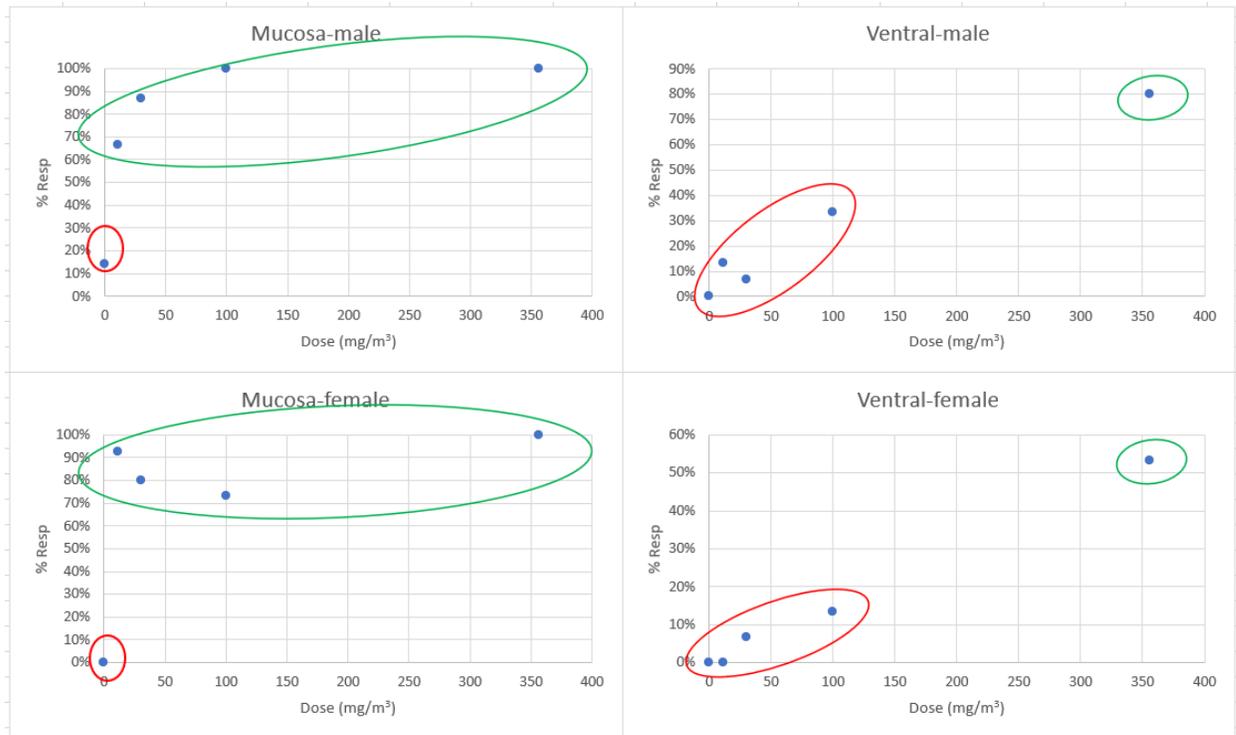


Figure 1. Illustrated results of pairwise comparison of reported total incidences of squamous metaplasia. Mucosa-male, Mucosa-female: squamous/ squamoid metaplasia/ hyperplasia in the larynx mucosa pseudostratified columnar epithelium in male and female rats, respectively. Ventral-male, Ventral-female: squamous/ squamoid metaplasia/ hyperplasia in the larynx ventral diverticulum cuboidal/ columnar epithelium in male and female rats, respectively. Data points within the same color circle are not significantly different based on Fisher Exact Test. Different color circles indicate significant difference based on Fisher Exact Test.

The results of the pairwise comparisons show that the datasets are consistent with two of the examples that EFSA (2022) described concerning data suitability. The results of the Mucosa-male and Mucosa-female are consistent with the example where only two groups of responses were significantly different (red oval enclosed data and green oval enclosed data in Figure 1), and the lowest response group contains only the control group. In this instance, EFSA (2022) cautioned that “the study might have enough information to define a dose–response curve, but it is expected that the study does not contain enough information for BMD estimation. In general, it is expected to produce small BMDL values as not enough small doses have been tested in the experiment conducted, and the BMD will certainly be estimated to be below the first dose tested with a wide credible interval. **Although the data could be modelled, the available information might not be sufficient for estimating the BMD.**”

The pairwise comparison results of the Ventral-male and Ventral-female datasets are consistent with the EFSA (2022) example where only two groups of responses were significantly different, and the lowest increasing relationship response groups contain not only the control, but also other dose groups. In this instance, EFSA (2022) noted that “the study might have enough information to estimate reliably the dose–response curve at low dose levels, and it is expected that the study does contain enough information for BMD estimation (meaning that the lower bound of the credible interval is expected to be close to the estimated BMD) as enough low dose responses are observed. **The data can be modelled, and estimation of BMD would produce BMDL values that may be considered suitable to identify a reference point.**”

Dose-response analysis using EFSA BMD app

The results of the preliminary BMD analysis conducted with EFSA BMD app found two of the four datasets analyzed were unreliable based on EFSA (2022) criteria. Table 4 summarizes the BMD estimates from the best-fit models, selected based on the lowest Akaike's Information Criterion (AIC) value, and reliability diagnostic ratios of BMD-to-BMDL, BMD-to-lowest tested dose (11 mg/m³), and BMDU-to-BMDL. Table 5 summarizes the model average BMD estimates and their respective diagnostic ratios. More detailed results from this preliminary analysis are shown in Appendix A.

The diagnostic ratios are recommended by EFSA (2022) to identify instances when BMD results are not reliable as bases for reference point (RP). EFSA (2022) recommends that the BMDL is not a reliable basis for RP when no model can sufficiently fit the data, or when the diagnostic ratios are:

- BMD/BMDL > 20, or.
- The BMD is 10 times lower than the lowest non-zero dose, or.
- BMDU/BMDL > 50.

The results that failed the diagnostic criteria are bolded in Tables 4 and 5.

Table 4. Results of best-fit model from preliminary BMD analysis using EFSA BMD app

Dataset	Best-fit Model	BMD	BMDL	BMDU	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Mucosa-male	LVM: Hill m3-	0.378	0.000586	3.92	645	0.034	6689
Mucosa-female	log.prob	0.000001	0^a	0.0992	#DIV/0!	0.0000001	#DIV/0!
Ventral-male	LVM: Expon. m3-	22.9	5.77	85.7	3.97	2.08	14.9
Ventral-female	gamma	66.8	24	158	2.78	6.07	6.58

^a The value is not truly a zero but a very small number rounded to zero according to PROAST Team at Dutch National Institute for Public Health (RIVM).

Table 5. Model average results from preliminary BMD analysis using EFSA BMD app

Dataset	BMD	BMDL	BMDU	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Mucosa-male	NA ^a	0.02	7.38	NA	NA	369
Mucosa-female	NA	0^b	0.16	NA	NA	#DIV/0!
Ventral-male	NA	8.57	81.1	NA	NA	9.46
Ventral-female	NA	33.8	268	NA	NA	7.93

^a Model average central estimate of BMD is not available from EFSA BMD app.
^b The value is not truly a zero but a very small number rounded to zero according to PROAST Team at Dutch National Institute for Public Health (RIVM).

For Mucosa-male, the best-fit model failed the diagnostic ratio criteria for BMD:BMDL (645) and BMDU:BMDL (6689), and the model average failed the criterion for BMDU:BMDL (369). And, for Mucosa-female, the best-fit model failed the diagnostic ratio criterion for BMD:lowest tested dose of 11 mg/m³ (10 million-times lower), and an unreliable BMDL estimate of zero (or a very small number rounded to zero, according to the PROAST Team at Dutch National Institute for Public Health, RIVM) from both the best-fit model and model average. These findings are not unexpected, as the data suitability evaluation reported in the previous sub-section (“Data

suitability for BMD analysis”) had identified these two datasets may have insufficient information for BMD estimates.

The results for Ventral-male and Ventral-female best-fit models fulfill the EFSA (2022) diagnostic ratio criteria. The BMDL and BMDU results from the model average analysis fulfill the EFSA (2022) diagnostic ratio criterion for BMDU:BMDL. The model average analysis does not provide the central estimate, BMD, for evaluating ratios of BMD:BMDL and BMD:lowest tested dose.

The unreliable BMD estimates for the Mucosa-male and Mucosa-female datasets led to further analysis using an alternative BMD analytical tool, BMDS.

Preliminary analysis using BMDS Version 3.3

The results of the preliminary BMD analysis for one dataset, Mucosa-male, based on BMDS Version 3.3 were found to be unreliable. Table 5 summarizes the Bayesian model average BMD estimates and the calculated diagnostic ratios, based on both PC- and Web-versions of BMDS Version 3.3. The results that failed the diagnostic criteria are bolded. More detailed results from this preliminary analysis are shown in Appendix B.

Table 6. Bayesian model average results of preliminary analysis of Mucosa-male dataset using BMDS Version 3.3 PC- and Web-Versions

BMDS 3.3 Version	Analysis Type	BMD	BMDL	BMDU	BMD : BMDL	BMD : 11 mg/m3	BMDU : BMDL
PC	Bayesian MA	1.72	0.06	5.25	27.7	0.16	84.6
Web	Bayesian MA	1.014	0.041	5.406	24.7	0.09	132

The results showed inconsistency in the Bayesian model average BMD estimates obtained from the PC- and Web-versions of the same version numbered BMDS. The BMDL estimate from the PC-version is 50% higher than the estimate from the Web-version, and the BMD estimate from the PC-version is 70% higher than the estimate from the Web-version. For the PC-version, the

diagnostic ratios BMD:BMDL (27.7) and BMDU:BMDL (84.6) fail the EFSA (2022) reliability criteria. For the Web-version, the diagnostic ratios BMD:BMDL (24.7), BMD:lowest tested dose of 11 mg/m³ (0.09), and BMDU:BMDL (132) also fail the reliability criteria. Strikingly, the Bayesian model average BMDL estimates from Version 3.3 PC-version are almost 20-times lower (and Web-version almost 30-times lower) than the estimates from Version 3.2.0.1, as reported below. An inquiry regarding the discrepancy of the BMD estimates was sent to EPA, and EPA acknowledged the discrepancy and that it will be investigated.

The uncertainty in the BMD estimates for the Mucosa-male dataset based on BMDS Version 3.3 led to the additional analysis using BMDS 3.2.0.1.

Additional analysis using BMDS Version 3.2.0.1

The result of the BMD analysis conducted with BMDS 3.2.0.1 is summarized below in Tables 7-10. Calculation of diagnostic ratios of BMD-to-BMDL, BMD-to-lowest tested dose (11 mg/m³), and BMDU-to-BMDL are also included in these tables.

Table 7. Results of BMD analysis for Dataset: Mucosa-male

Model	Analysis Type	BMD	BMDL	BMDU	P-value	AIC	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Dichotomous Hill	frequentist	2.50	0.33	6.65	0.78	49.06	7.62	0.23	20.3
Gamma	frequentist	1.49	0.97	5.71	0.94	46.74	1.54	0.14	5.91
Log-Logistic	frequentist	2.50	0.33	6.65	0.78	49.06	7.62	0.23	20.3
Multistage Degree 4	frequentist	1.50	0.97	4.51	0.82	48.74	1.55	0.14	4.66
Multistage Degree 3	frequentist	1.49	0.97	4.52	0.94	46.74	1.54	0.14	4.67
Multistage Degree 2	frequentist	1.49	0.97	4.52	0.94	46.74	1.54	0.14	4.67
Multistage Degree 1	frequentist	1.49	0.97	2.50	0.94	46.74	1.54	0.14	2.58
Weibull	frequentist	1.49	0.97	5.05	0.94	46.74	1.54	0.14	5.23
Logistic	frequentist	3.26	2.18	4.85	0.53	48.63	1.49	0.30	2.23
Log-Probit	frequentist	2.26	0.09	6.67	0.86	48.79	25.3	0.21	74.5

Model	Analysis Type	BMD	BMDL	BMDU	p-value	AIC	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Probit	frequentist	3.70	2.78	5.07	0.54	47.77	1.33	0.34	1.82
Model Average	Bayesian MA	2.31	1.15	5.31	-	--	2.00	0.21	4.61

Table 8. Results of BMD analysis for Dataset: Mucosa-female

Model	Analysis Type	BMD	BMDL	BMDU	p-value	AIC	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Dichotomous Hill	frequentist	88.86	0.00	Infinity	<0.0001	81.57	#DIV/0!	8.08	#VALUE!
Gamma	frequentist	5.63	2.92	14.80	<0.0001	75.61	1.92	0.51	5.06
Log-Logistic	frequentist	0.61	0.30	1.27	0.00	55.24	2.05	0.06	4.3
Multistage Degree 4	frequentist	5.63	2.92	17.56	<0.0001	75.61	1.92	0.51	6.01
Multistage Degree 3	frequentist	5.63	2.92	17.31	<0.0001	75.61	1.92	0.51	5.92
Multistage Degree 2	frequentist	5.63	2.92	16.32	<0.0001	75.61	1.92	0.51	5.58
Multistage Degree 1	frequentist	5.63	2.92	13.24	<0.0001	75.61	1.93	0.51	4.53
Weibull	frequentist	5.63	2.92	15.13	<0.0001	75.61	1.92	0.51	5.18
Logistic	frequentist	11.88	7.20	23.34	<0.0001	77.84	1.65	1.08	3.24
Log-Probit	frequentist	0.00	0.00	Infinity	0.06	52.53	#DIV/0!	0.00	#VALUE!
Probit	frequentist	13.13	8.27	25.63	<0.0001	77.96	1.59	1.19	3.10
Model Average	Bayesian MA	0.14	0.00	1.03	-	-	373.50	0.01	2679.90

Table 9. Results of BMD analysis for Dataset: Ventral-male

Model	Analysis Type	BMD	BMDL	BMDU	p-value	AIC	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Dichotomous Hill	frequentist	88.11	10.00	101.41	0.15	64.01	8.81	8.01	10.1
Gamma	frequentist	24.24	16.29	97.63	0.47	59.45	1.49	2.20	5.99
Log-Logistic	frequentist	20.39	10.17	40.62	0.34	60.19	2.01	1.85	4.0

Model	Analysis Type	BMD	BMDL	BMDU	p-value	AIC	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Multistage Degree 4	frequentist	26.33	16.33	95.88	0.30	61.42	1.61	2.39	5.87
Multistage Degree 3	frequentist	26.43	16.33	95.93	0.30	61.42	1.62	2.40	5.87
Multistage Degree 2	frequentist	26.26	16.31	95.87	0.30	61.44	1.61	2.39	5.88
Multistage Degree 1	frequentist	24.24	16.29	40.26	0.47	59.45	1.49	2.20	2.47
Weibull	frequentist	24.24	16.29	96.63	0.47	59.45	1.49	2.20	5.93
Logistic	frequentist	75.60	52.97	106.63	0.37	61.45	1.43	6.87	2.01
Log-Probit	frequentist	53.46	7.76	106.87	0.32	62.09	6.9	4.86	13.8
Probit	frequentist	70.40	51.24	96.99	0.40	61.16	1.37	6.40	1.89
Model Average	Bayesian MA	42.95	20.11	89.35	-	-	2.14	3.90	4.44

Table 10. Results of BMD analysis for Dataset: Ventral-female

Model	Analysis Type	BMD	BMDL	BMDU	p-value	AIC	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Dichotomous Hill	frequentist	65.33	26.66	118.91	0.43	48.55	2.45	5.94	4.5
Gamma	frequentist	66.83	35.22	119.38	0.91	44.50	1.90	6.08	3.39
Log-Logistic	frequentist	65.33	28.66	118.91	0.89	44.55	2.28	5.94	4.1
Multistage Degree 4	frequentist	69.92	35.27	197.08	0.81	46.48	1.98	6.36	5.59
Multistage Degree 3	frequentist	70.04	35.26	177.93	0.81	46.49	1.99	6.37	5.05
Multistage Degree 2	frequentist	69.63	35.23	154.94	0.79	46.50	1.98	6.33	4.40
Multistage Degree 1	frequentist	54.99	34.49	94.98	0.90	44.76	1.59	5.00	2.75
Weibull	frequentist	67.60	35.23	124.79	0.92	44.50	1.92	6.15	3.54
Logistic	frequentist	149.48	105.82	203.84	0.62	46.46	1.41	13.59	1.93
Log-Probit	frequentist	59.73	24.77	104.84	0.69	46.62	2.4	5.43	4.2
Probit	frequentist	134.86	96.31	185.72	0.66	46.15	1.40	12.26	1.93
Model Average	Bayesian MA	98.69	41.85	184.43	-	-	2.36	8.97	4.41

Bayesian model average results

The Bayesian model average results from BMDS are summarized in Tables 11-14. Calculation of diagnostic ratios of BMD-to-BMDL, BMD-to-lowest tested dose (11 mg/m³), and BMDU-to-BMDL are also included in these tables.

Table 11. Bayesian model average results for Dataset: Mucosa-male

Model	Posterior Probability	BMD	BMDL	BMDU	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Dichotomous Hill	2.80E-02	1.25	0.12	4.14	10.83	0.11	35.76
Gamma	1.61E-02	1.45	0.19	5.28	7.58	0.13	27.59
Logistic	2.52E-02	4.42	2.67	9.29	1.65	0.40	3.47
Log-Logistic	1.38E-03	0.38	0.01	2.55	28.97	0.03	194.84
Log-Probit	9.53E-03	1.35	0.18	4.51	7.39	0.12	24.61
Multistage	8.60E-05	14.97	5.51	43.84	2.72	1.36	7.96
Probit	7.39E-02	4.21	2.69	8.48	1.57	0.38	3.16
Quantal Linear	8.31E-01	2.17	1.31	4.37	1.66	0.20	3.35
Weibull	1.43E-02	0.64	0.07	2.83	8.99	0.06	39.76
Model Average		2.31	1.15	5.31	2.00	0.21	4.61

Table 12. Bayesian model average results for Dataset: Mucosa-female

Model	Posterior Probability	BMD	BMDL	BMDU	BMD : BMDL	BMD : 11 mg/m ³	BMDU : BMDL
Dichotomous Hill	9.17E-01	0.09	0.00	1.01	241.98	0.01	2623.15
Gamma	5.14E-04	0.28	0.01	7.85	39.05	0.03	1099.69
Logistic	4.52E-04	18.16	9.78	41.19	1.86	1.65	4.21
Log-Logistic	5.38E-02	0.03	0.00	0.48	185.16	0.00	2739.07
Log-Probit	2.04E-02	0.18	0.00	1.22	35.65	0.02	246.49
Multistage	3.35E-04	25.91	11.43	55.60	2.27	2.36	4.86
Probit	4.97E-04	18.12	10.15	36.25	1.79	1.65	3.57

Model	Posterior Probability	BMD	BMDL	BMDU	BMD : BMDL	BMD : 11 mg/m3	BMDU : BMDL
Quantal Linear	2.86E-03	9.38	4.49	23.12	2.09	0.85	5.15
Weibull	3.95E-03	0.09	0.00	0.83	31.43	0.01	294.76
Model Average		0.14	0.00	1.03	373.50	0.01	2679.90

Table 13. Bayesian model average results for Dataset: Ventral-male

Model	Posterior Probability	BMD	BMDL	BMDU	BMD : BMDL	BMD : 11 mg/m3	BMDU : BMDL
Dichotomous Hill	3.44E-02	47.60	15.90	97.81	2.99	4.33	6.15
Gamma	5.40E-02	43.10	16.46	87.30	2.62	3.92	5.31
Logistic	5.10E-02	75.00	53.05	105.51	1.41	6.82	1.99
Log-Logistic	7.02E-02	49.38	16.53	106.27	2.99	4.49	6.43
Log-Probit	3.35E-02	65.27	28.85	122.21	2.26	5.93	4.24
Multistage	2.62E-01	39.18	23.79	65.70	1.65	3.56	2.76
Probit	7.51E-02	70.79	51.60	97.86	1.37	6.44	1.90
Quantal Linear	3.54E-01	28.80	18.87	47.97	1.53	2.62	2.54
Weibull	6.62E-02	56.55	20.18	123.39	2.80	5.14	6.12
Model Average		42.95	20.11	89.35	2.14	3.90	4.44

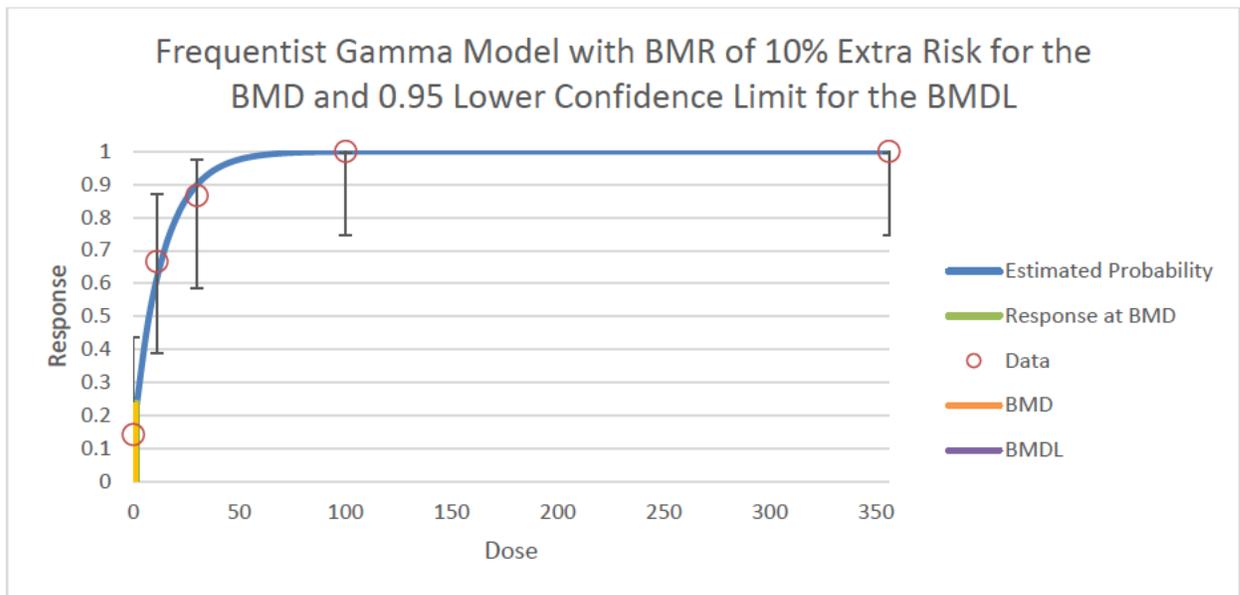
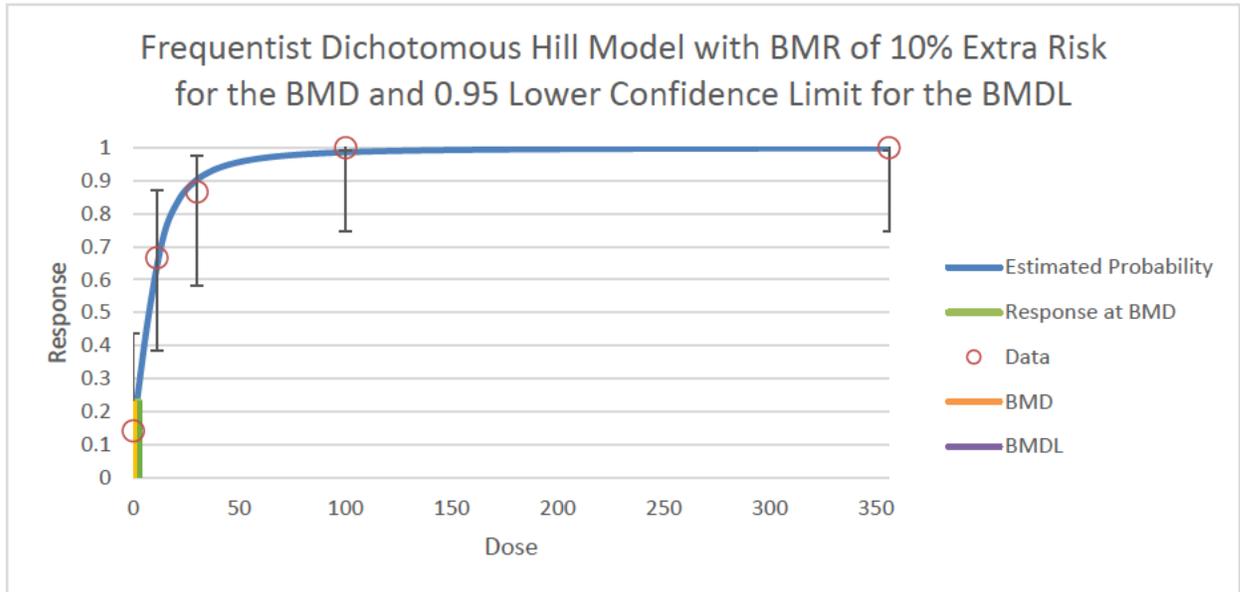
Table 14. Bayesian model average results for Dataset: Ventral-female

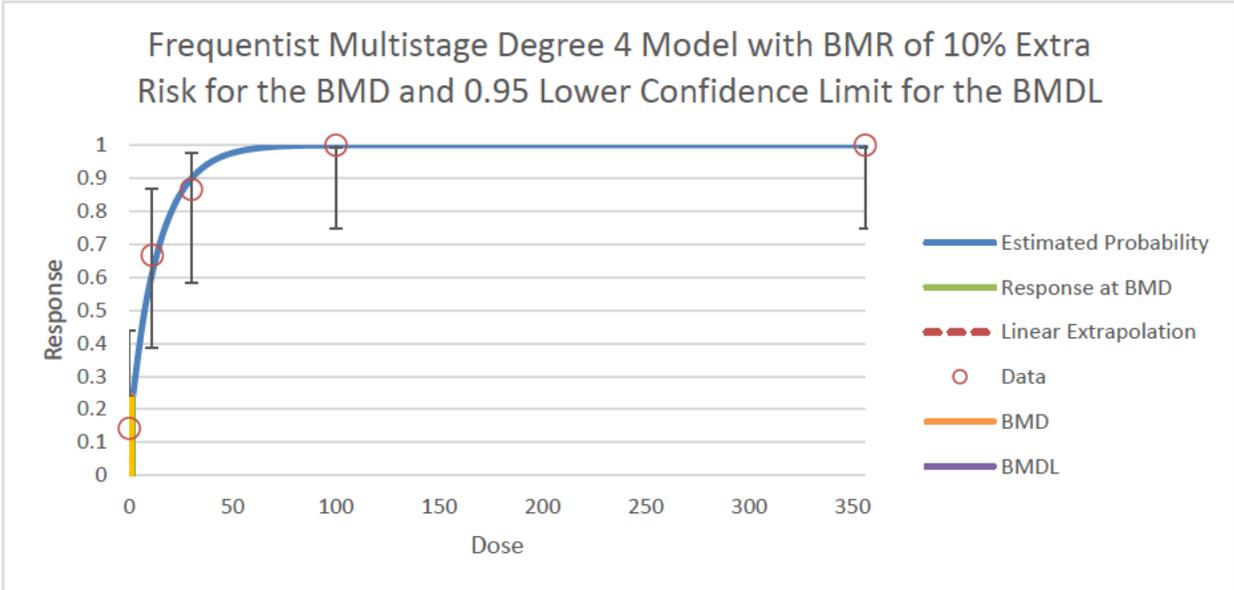
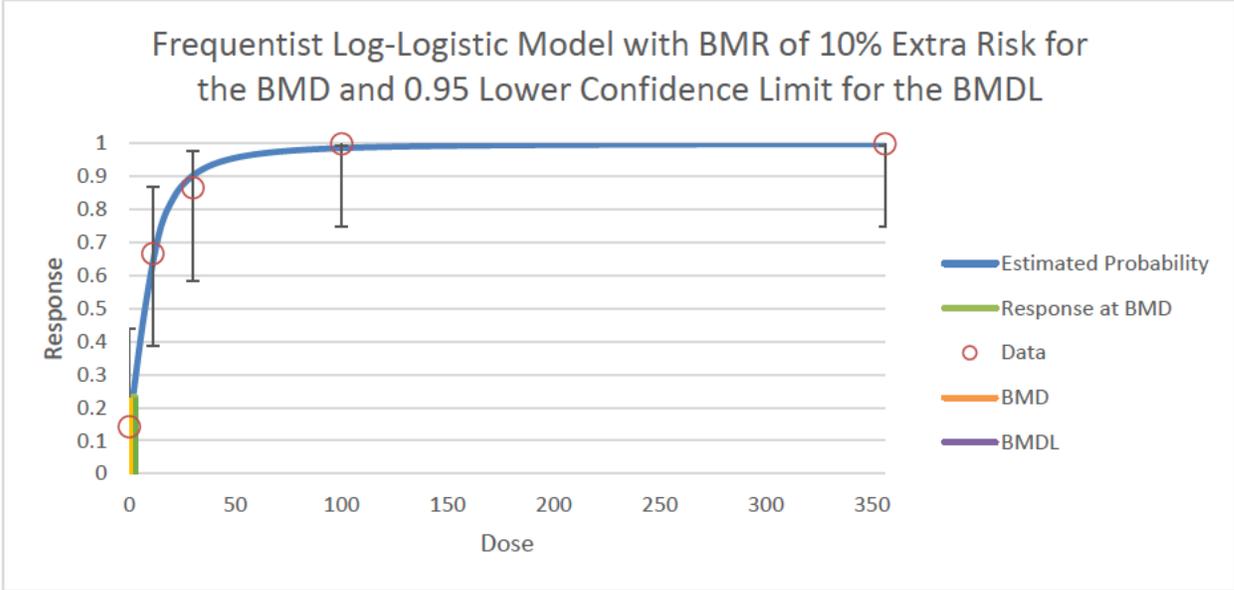
Model	Posterior Probability	BMD	BMDL	BMDU	BMD : BMDL	BMD : 11 mg/m3	BMDU : BMDL
Dichotomous Hill	9.84E-02	85.57	35.95	177.24	2.38	7.78	4.93
Gamma	7.72E-02	88.94	42.77	167.42	2.08	8.09	3.91
Logistic	8.44E-02	140.49	99.99	202.59	1.41	12.77	2.03
Log-Logistic	1.45E-01	91.20	39.04	179.66	2.34	8.29	4.60
Log-Probit	5.57E-02	118.61	52.88	238.29	2.24	10.78	4.51
Multistage	4.11E-02	63.03	40.38	97.87	1.56	5.73	2.42
Probit	1.86E-01	133.22	95.11	187.82	1.40	12.11	1.97

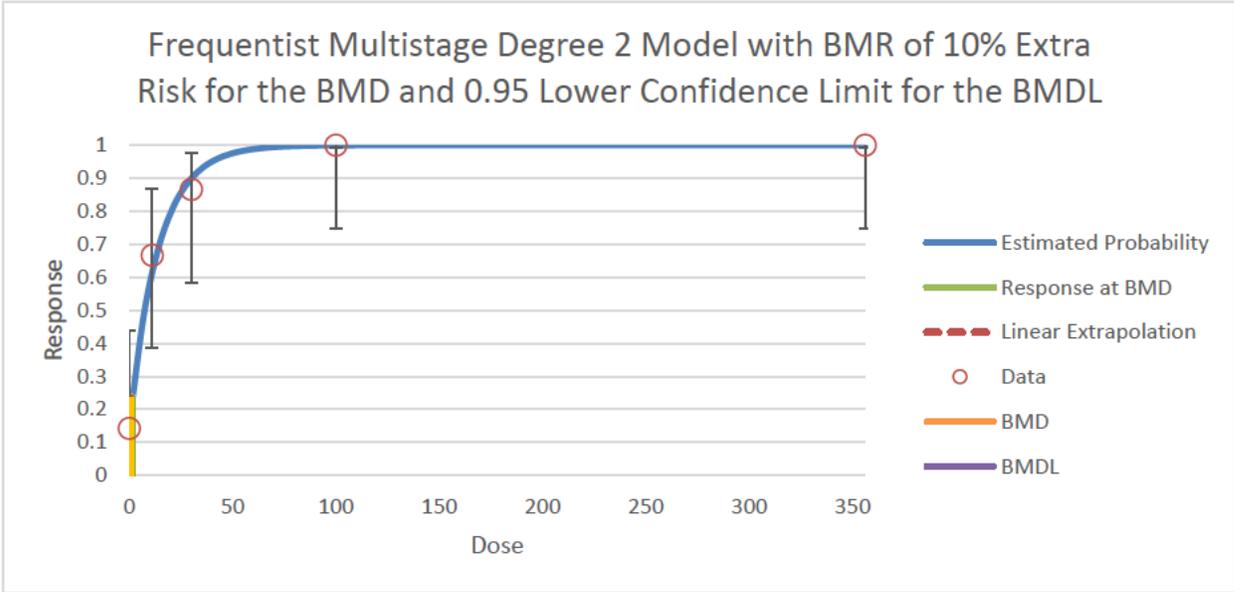
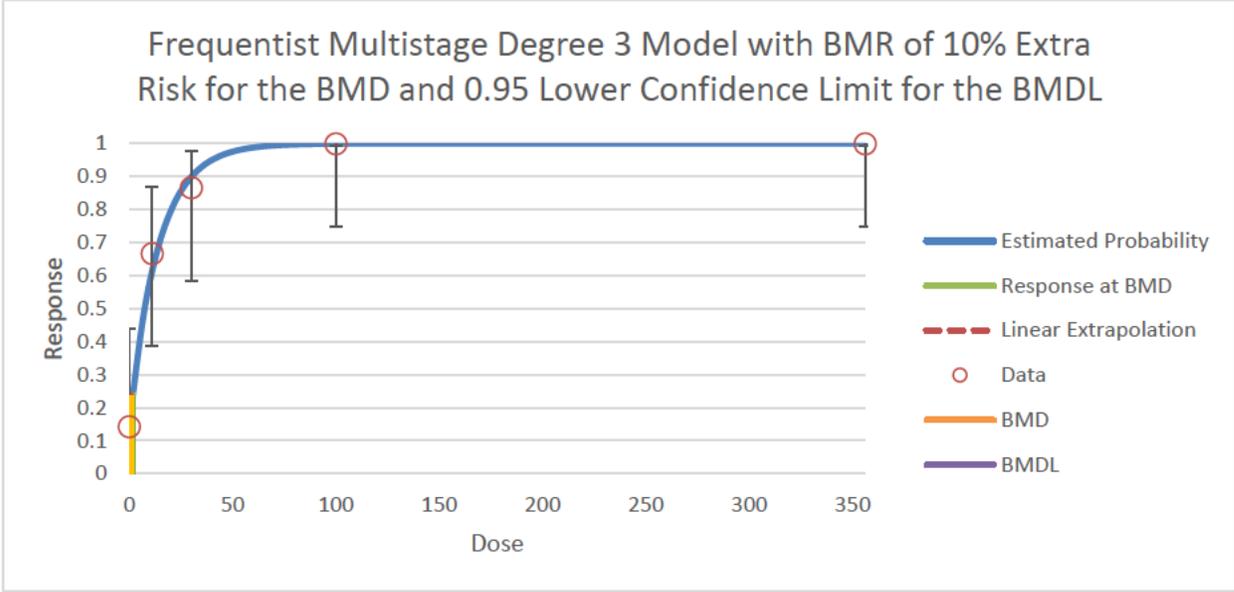
Model	Posterior Probability	BMD	BMDL	BMDU	BMD : BMDL	BMD : 11 mg/m3	BMDU : BMDL
Quantal Linear	2.00E-01	60.42	37.16	110.27	1.63	5.49	2.97
Weibull	1.12E-01	109.39	49.47	201.07	2.21	9.94	4.06
Model Average		98.69	41.85	184.43	2.36	8.97	4.41

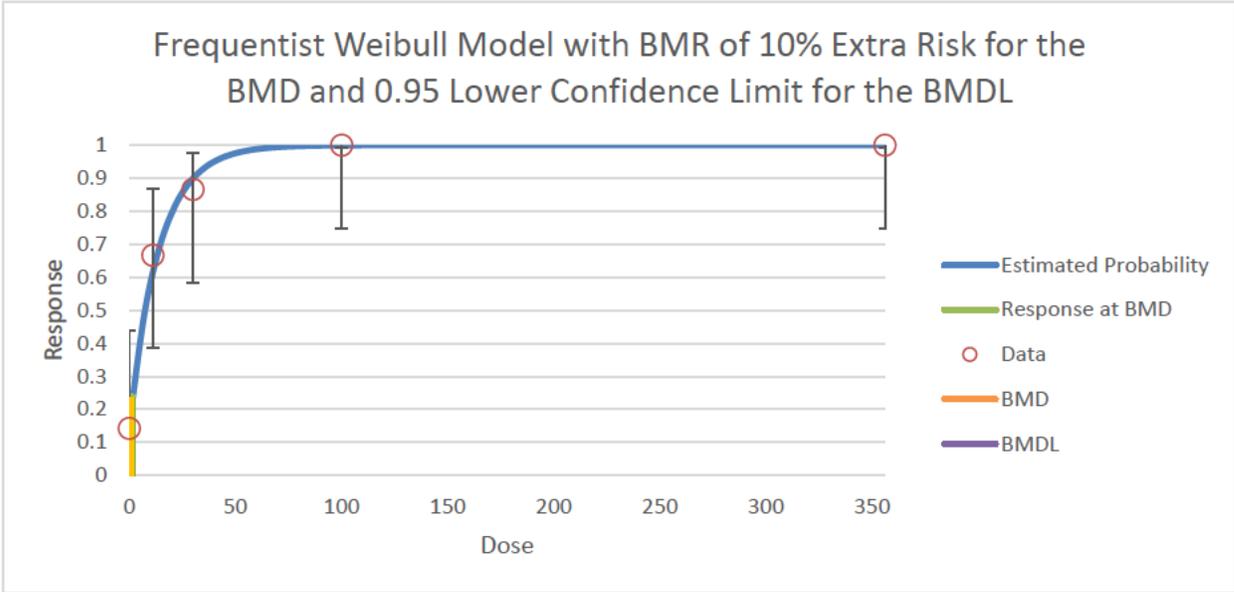
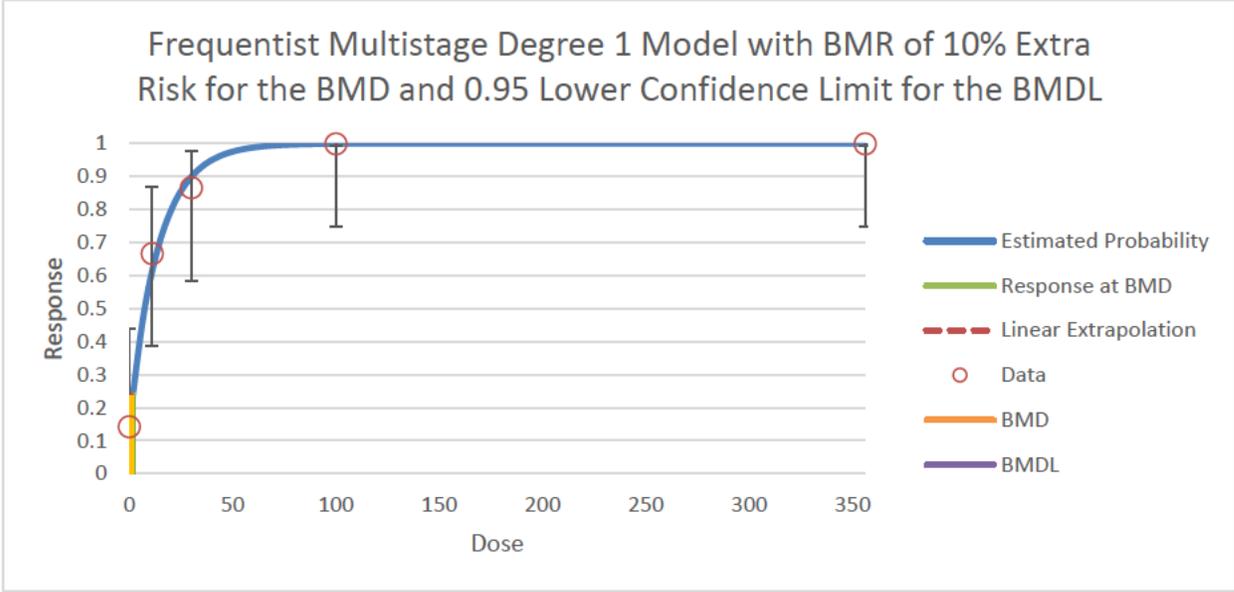
Plots of Fitted Models

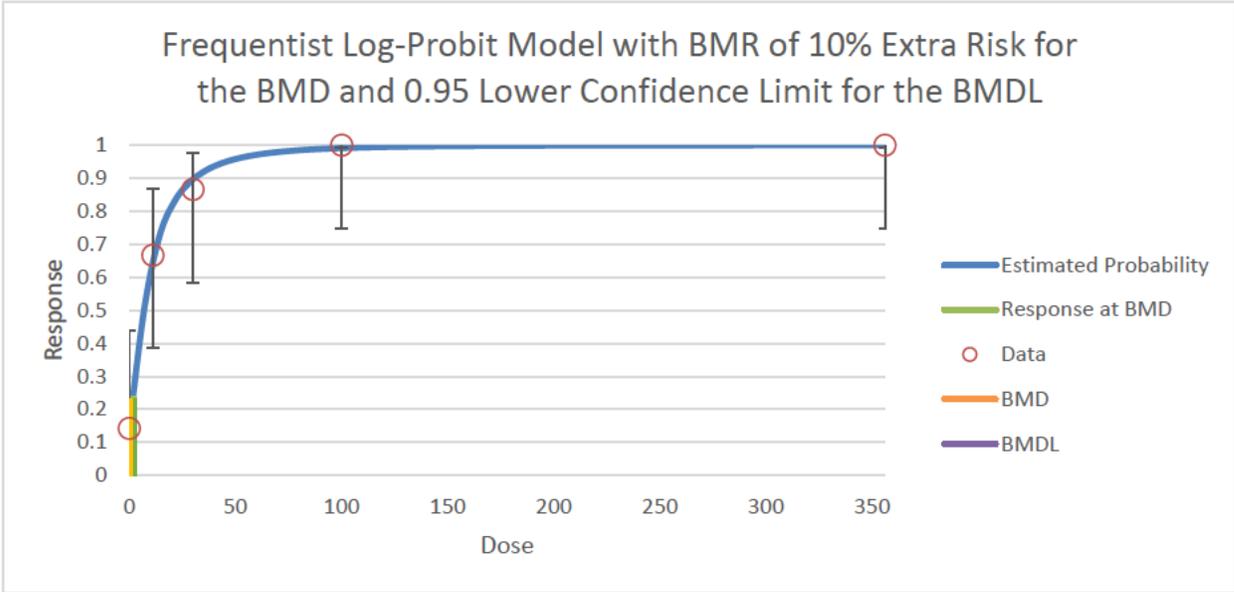
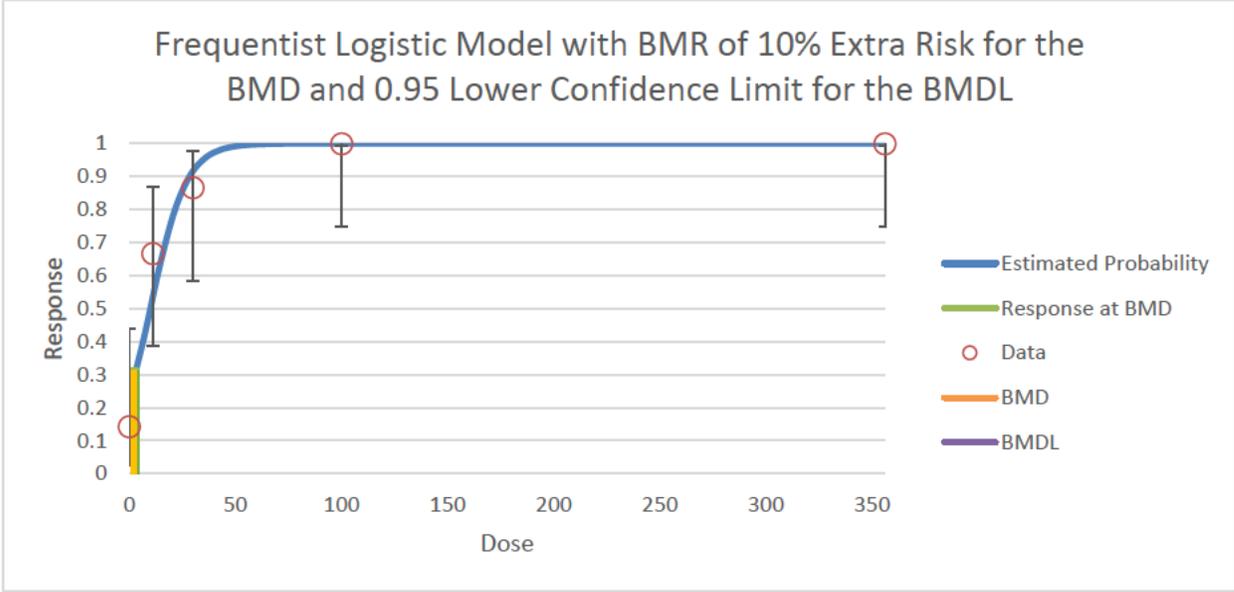
Mucosa-male

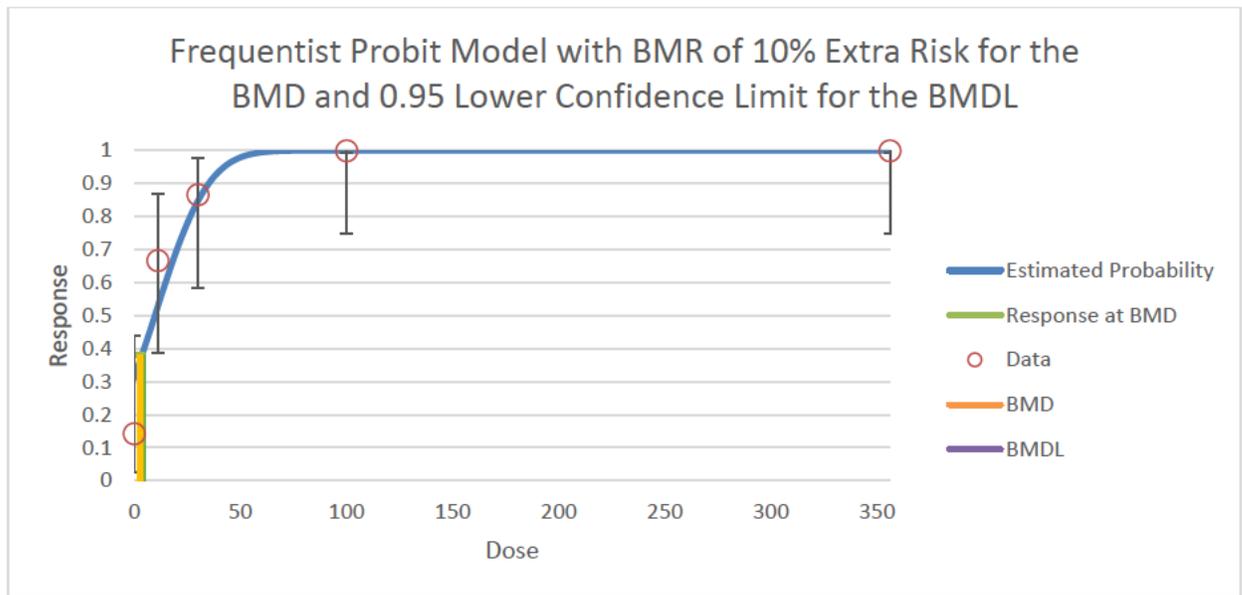






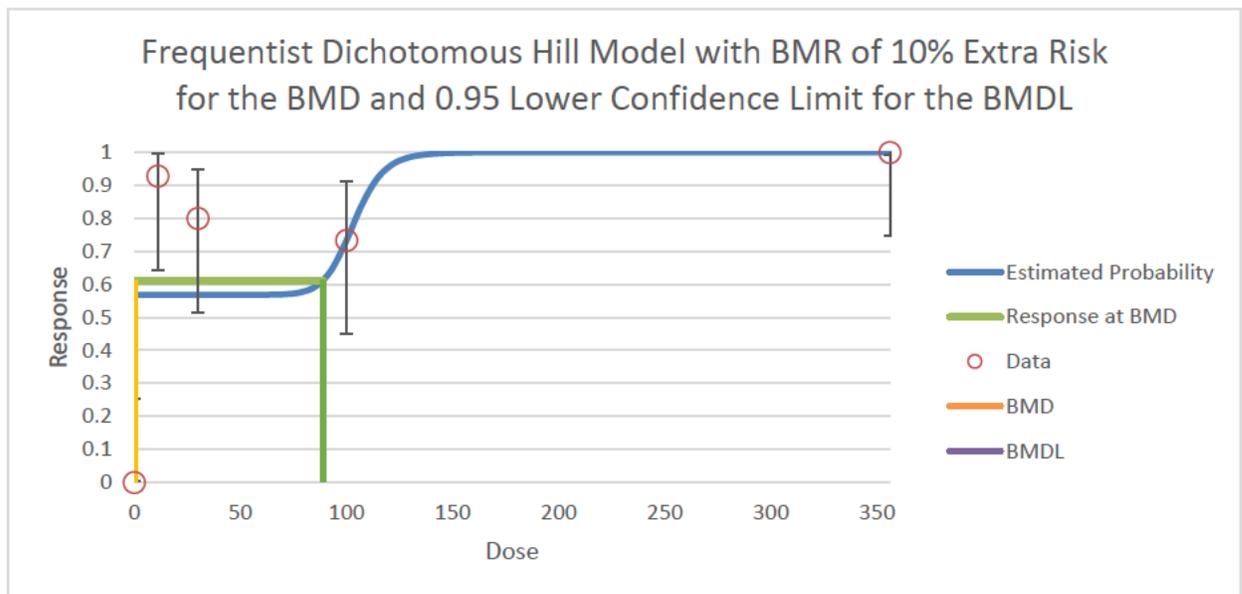


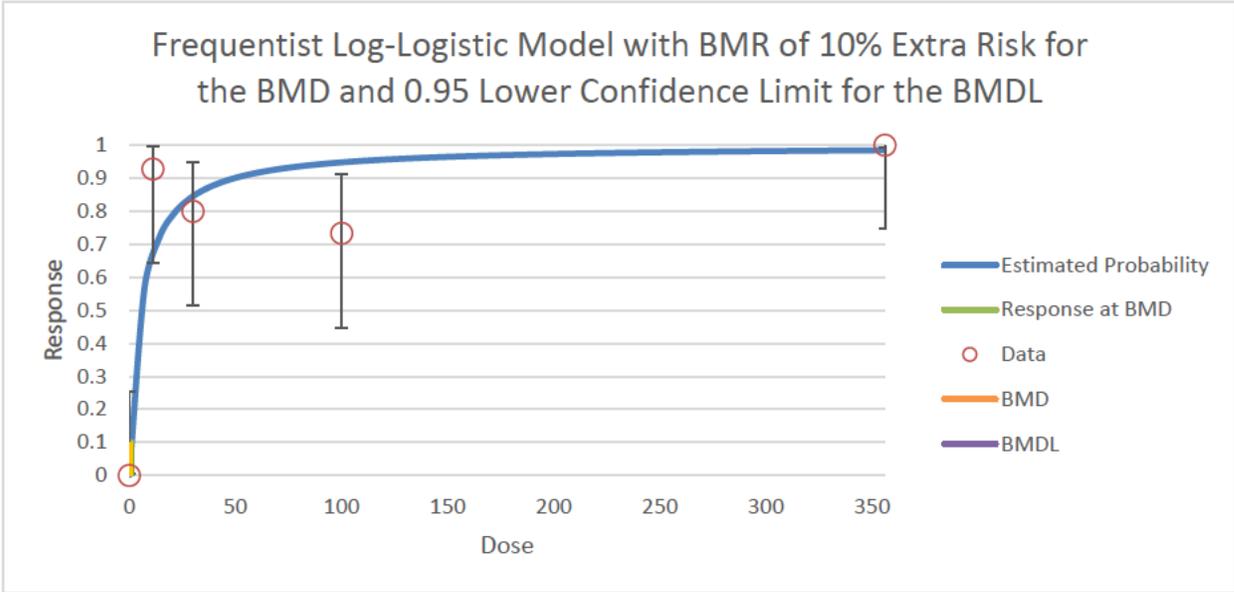
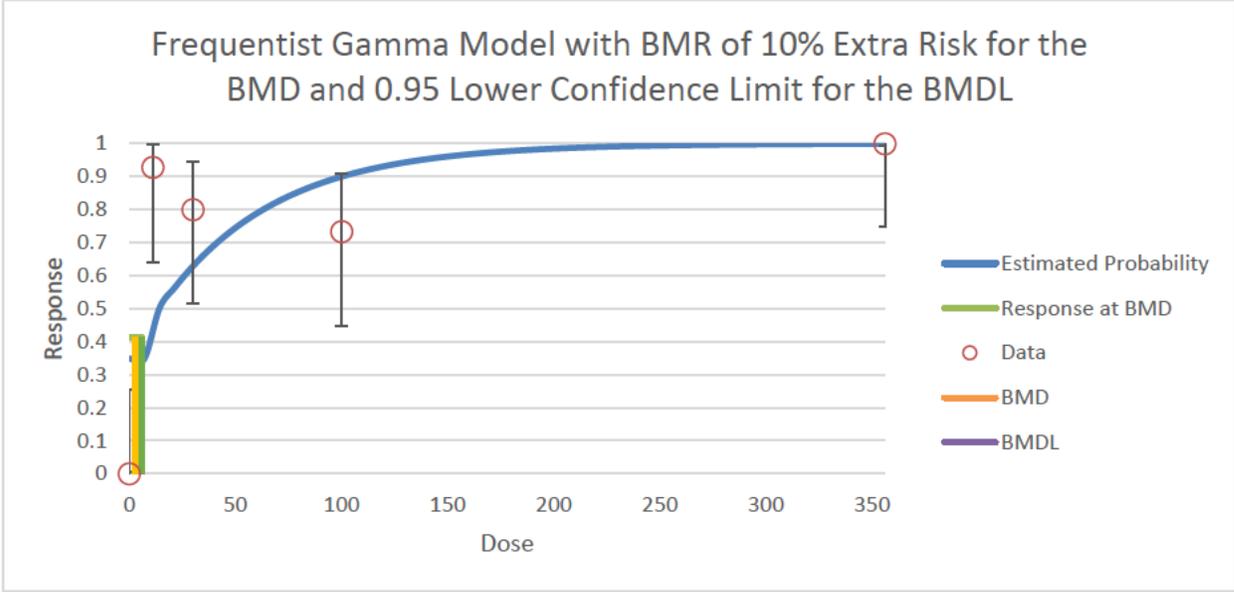




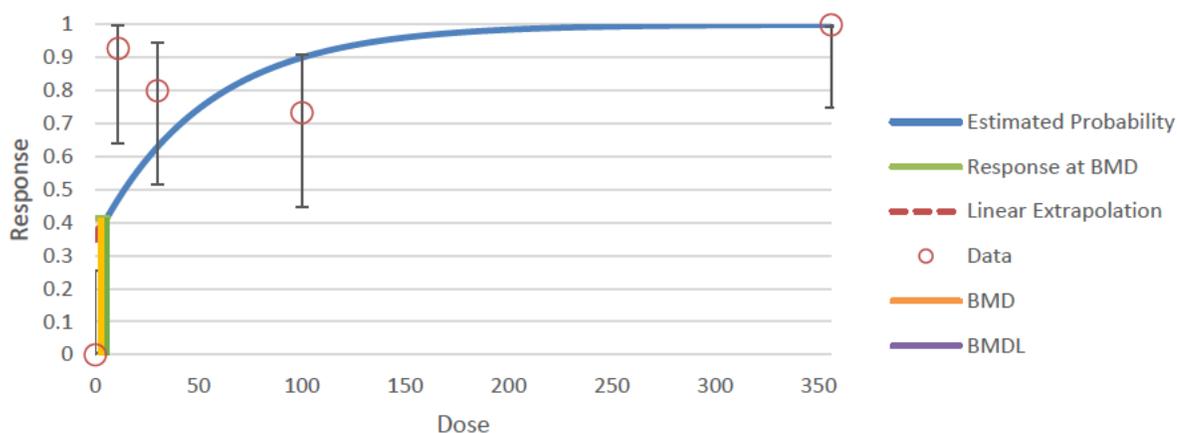
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Mucosa-female

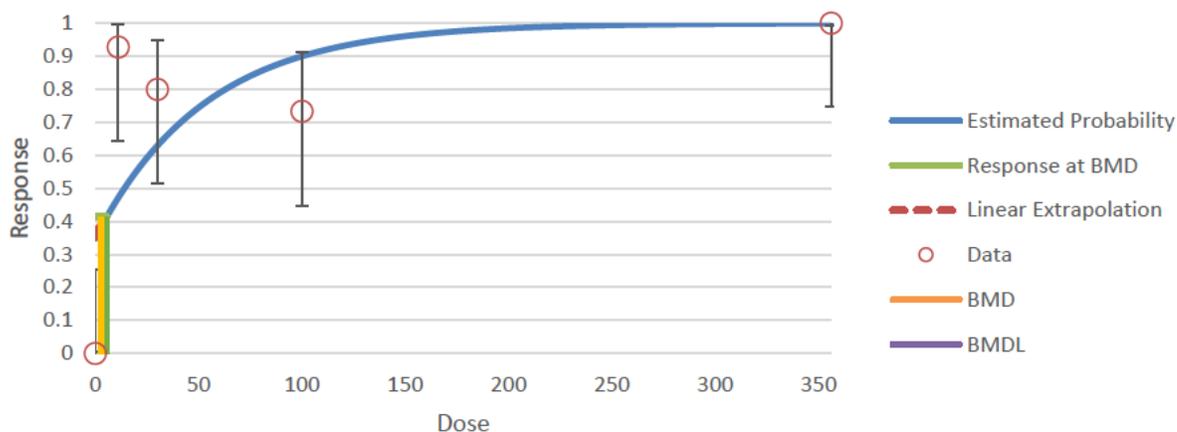




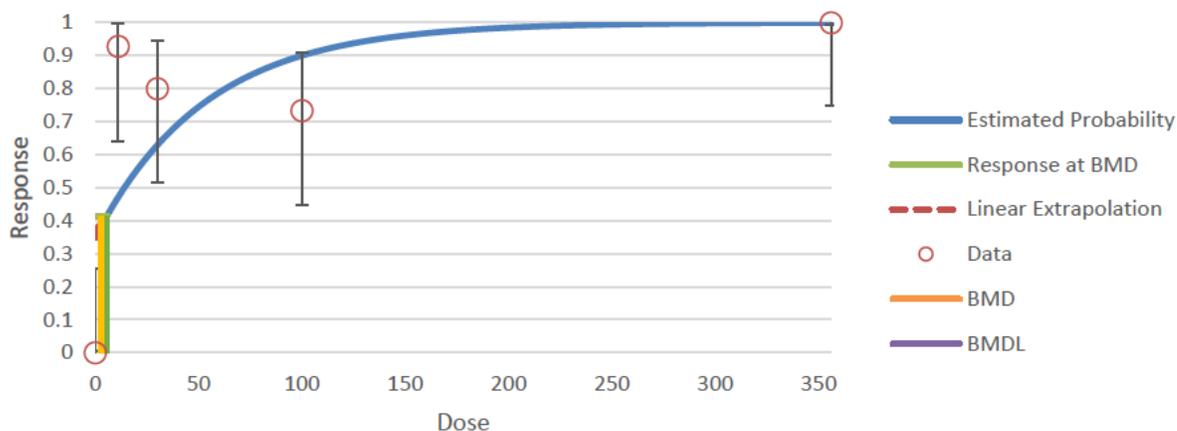
Frequentist Multistage Degree 4 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



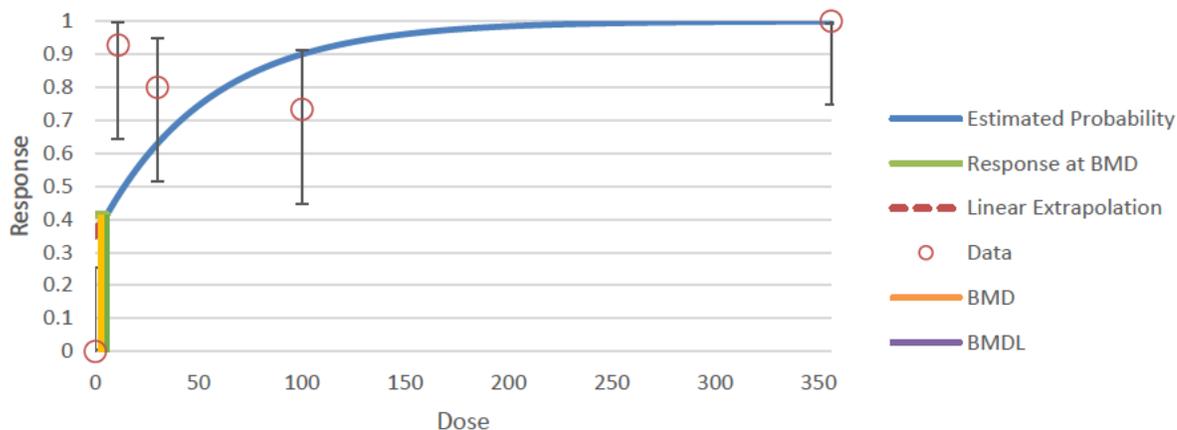
Frequentist Multistage Degree 3 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

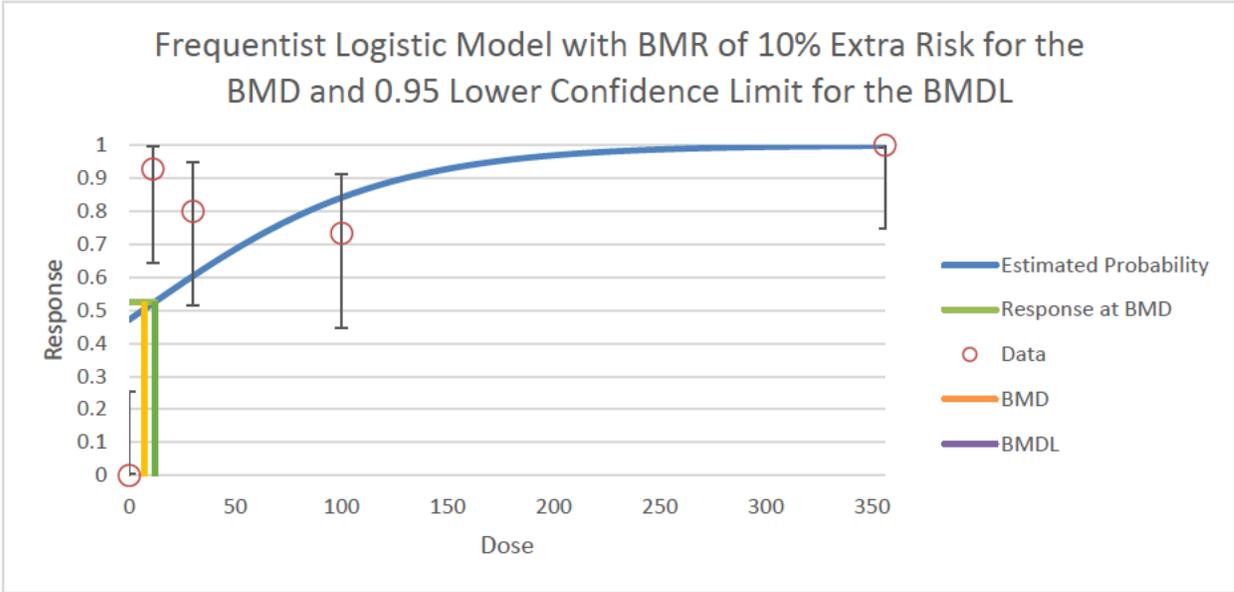
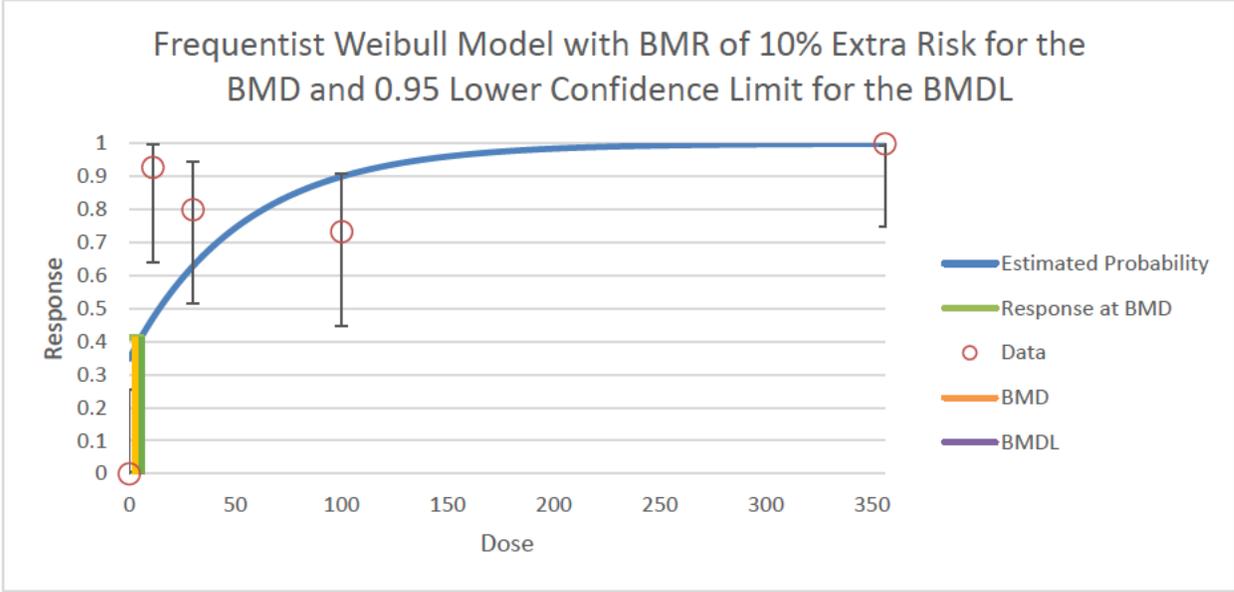


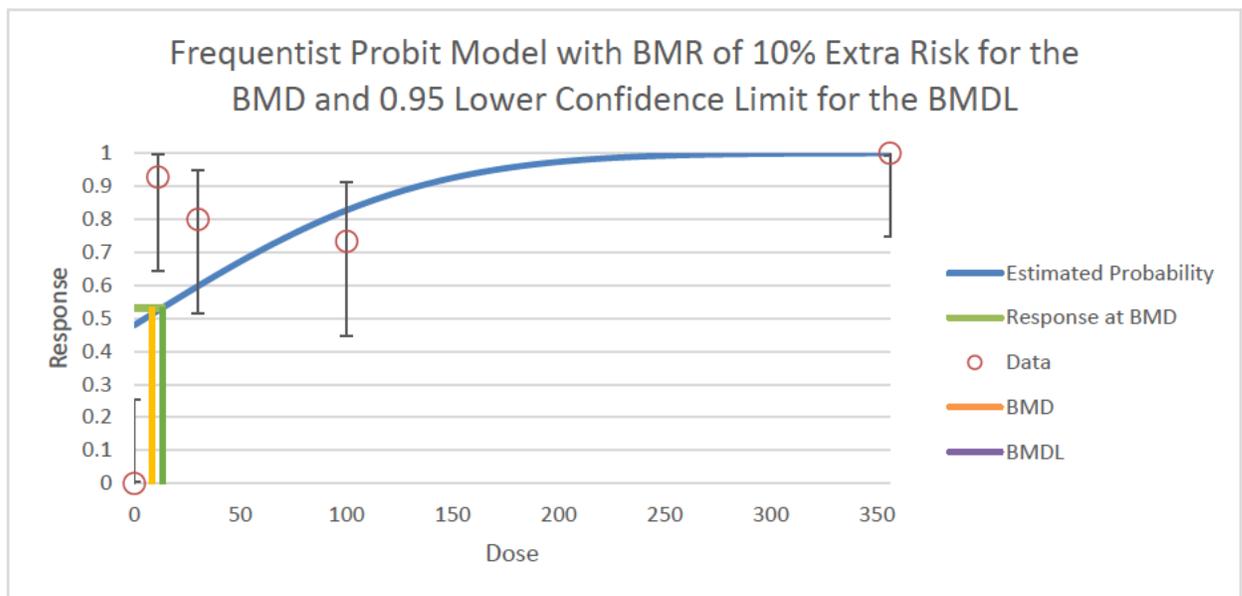
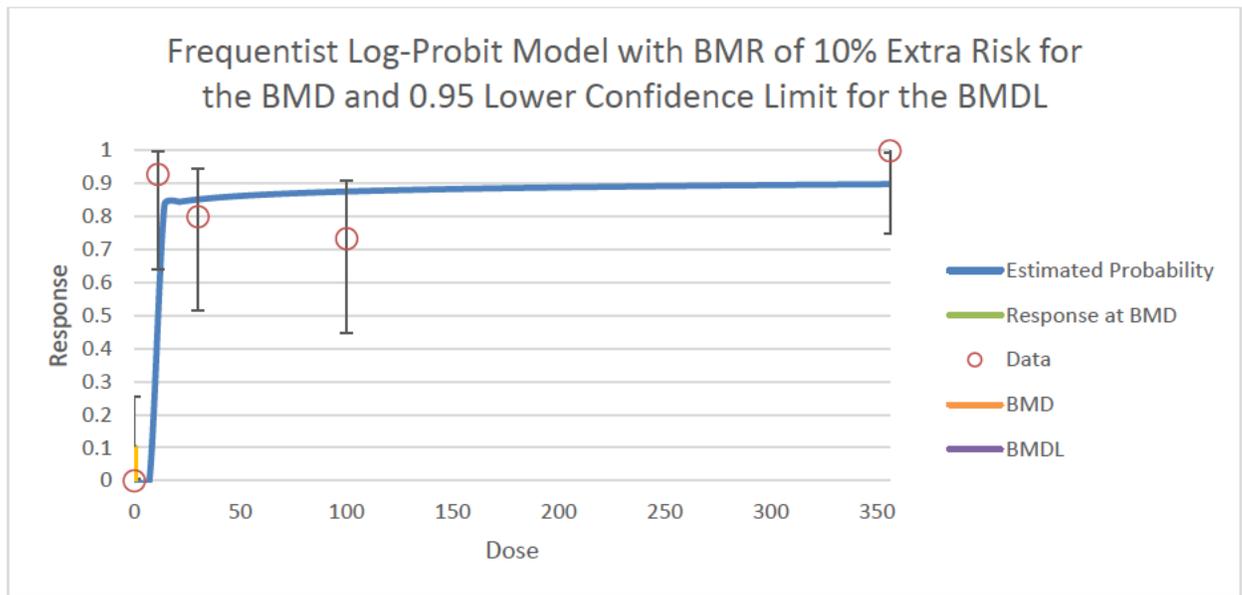
Frequentist Multistage Degree 2 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



Frequentist Multistage Degree 1 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

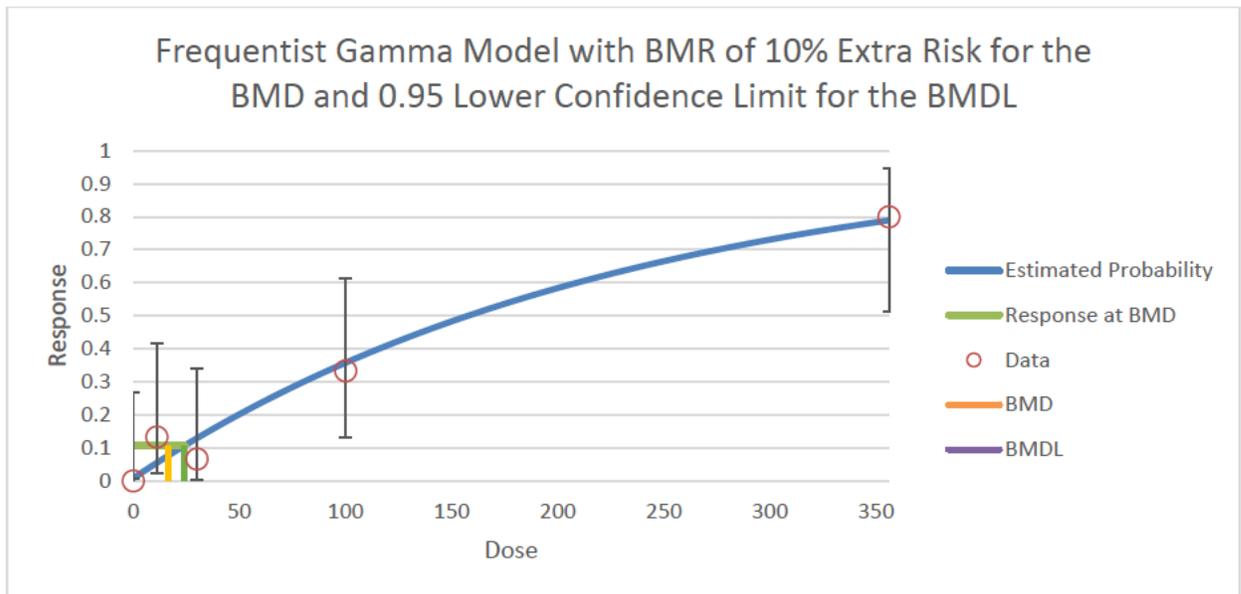
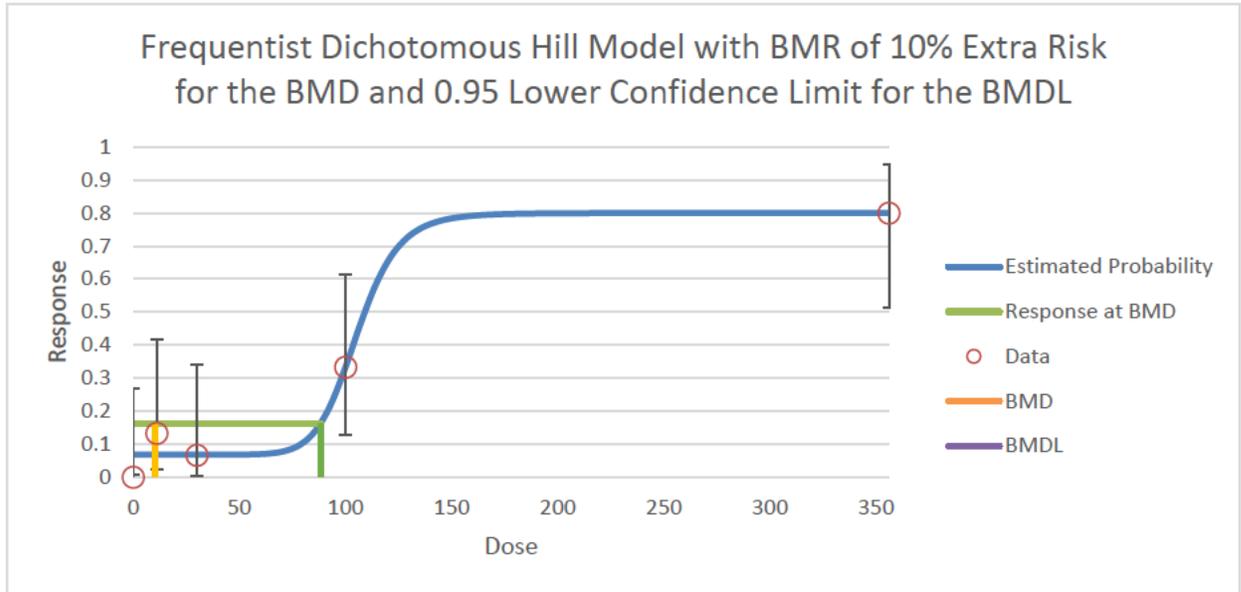




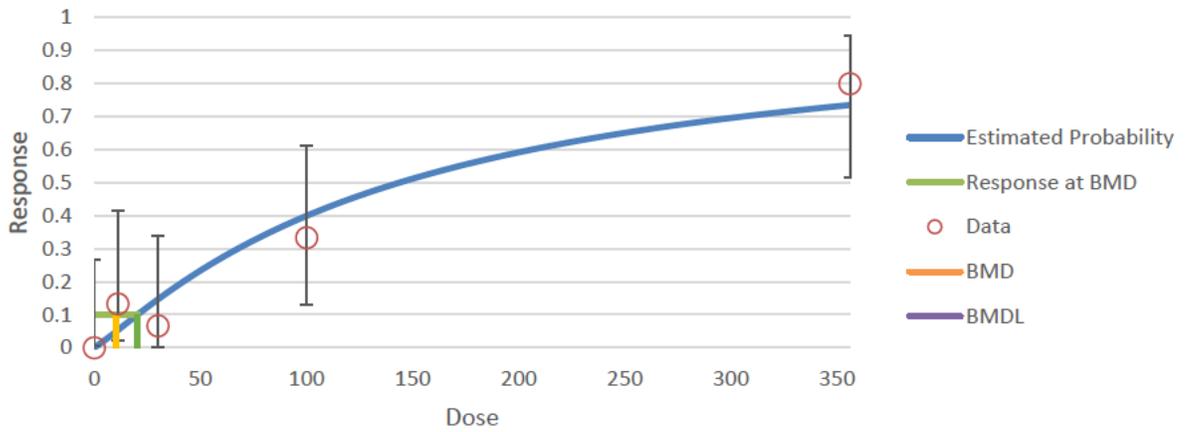


Plot of model average is not available from BMDS Version 3.2.0.1.

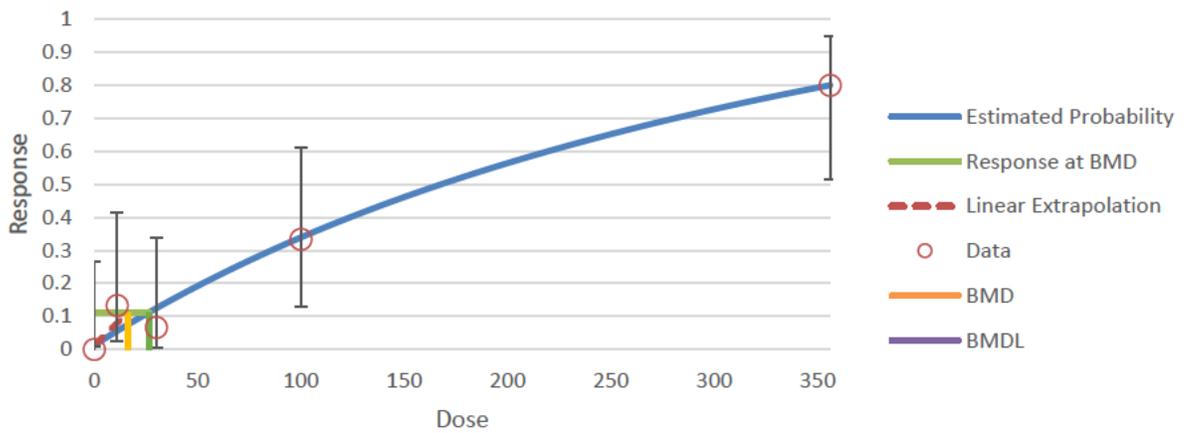
Ventral-male



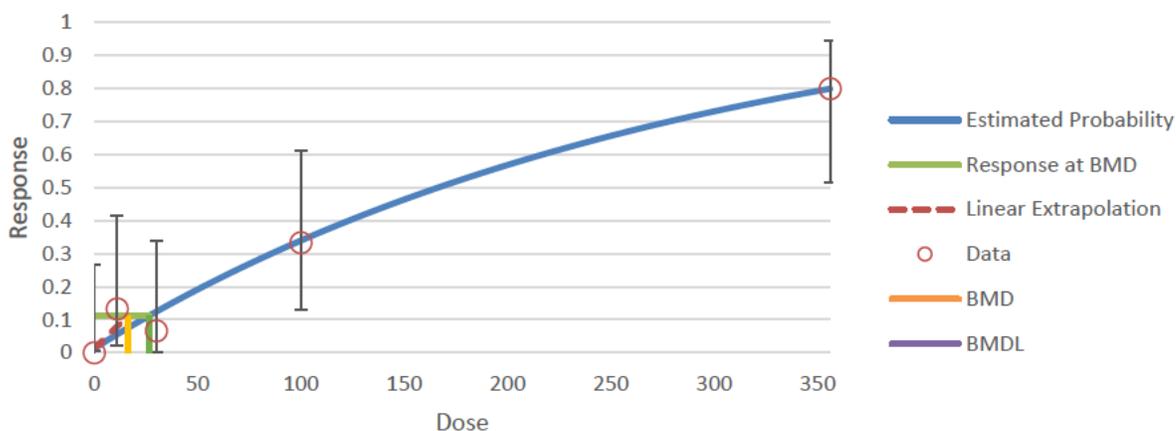
Frequentist Log-Logistic Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



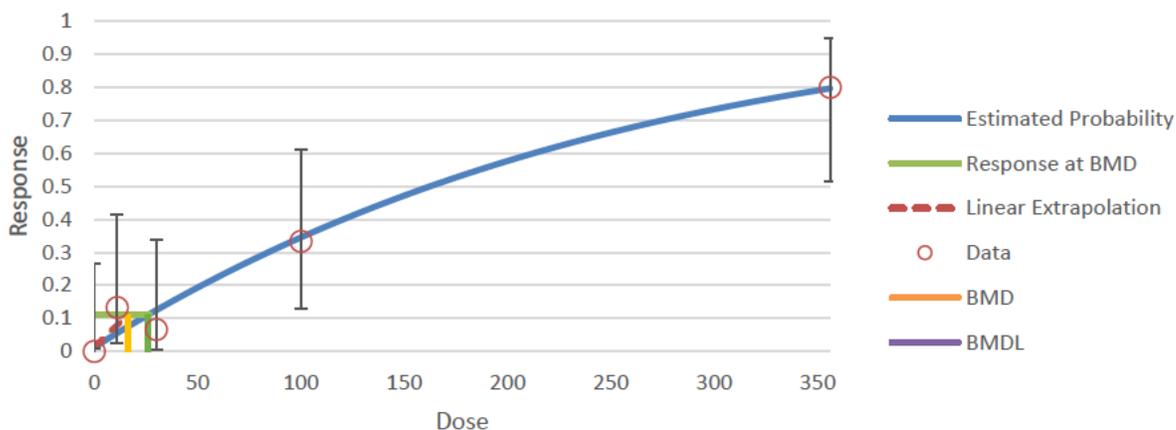
Frequentist Multistage Degree 4 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



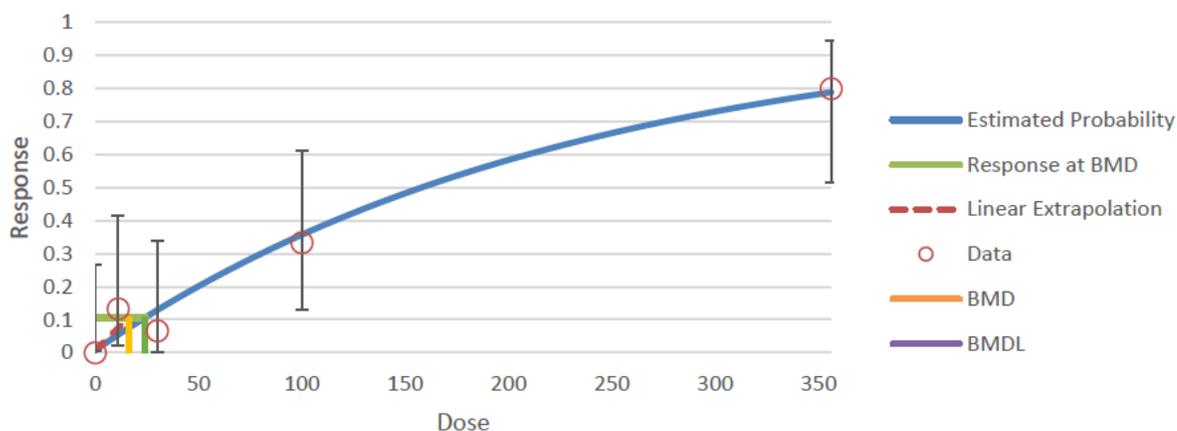
Frequentist Multistage Degree 3 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



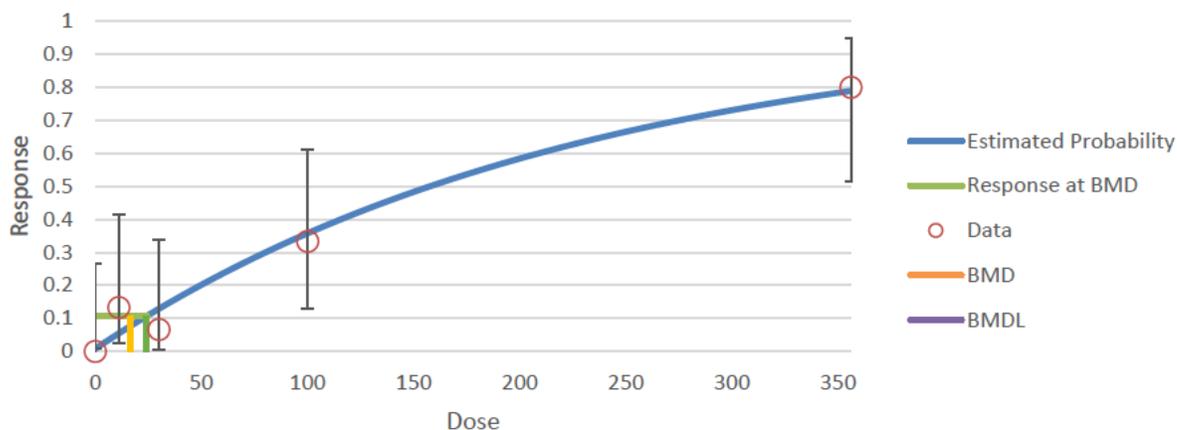
Frequentist Multistage Degree 2 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



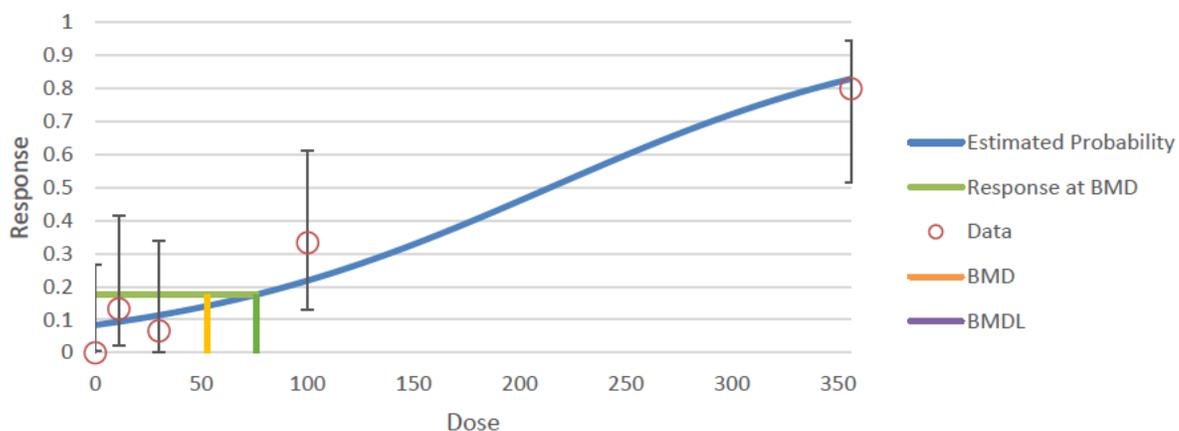
Frequentist Multistage Degree 1 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



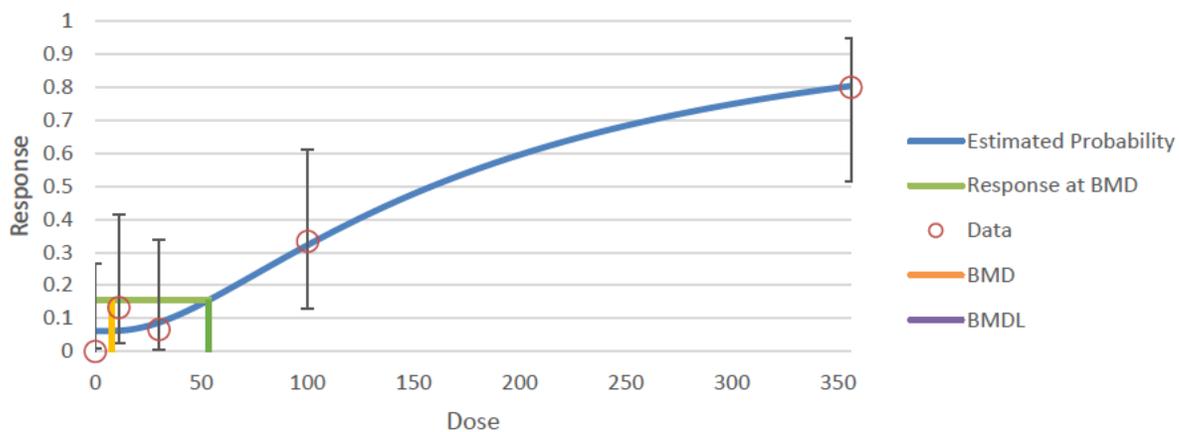
Frequentist Weibull Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

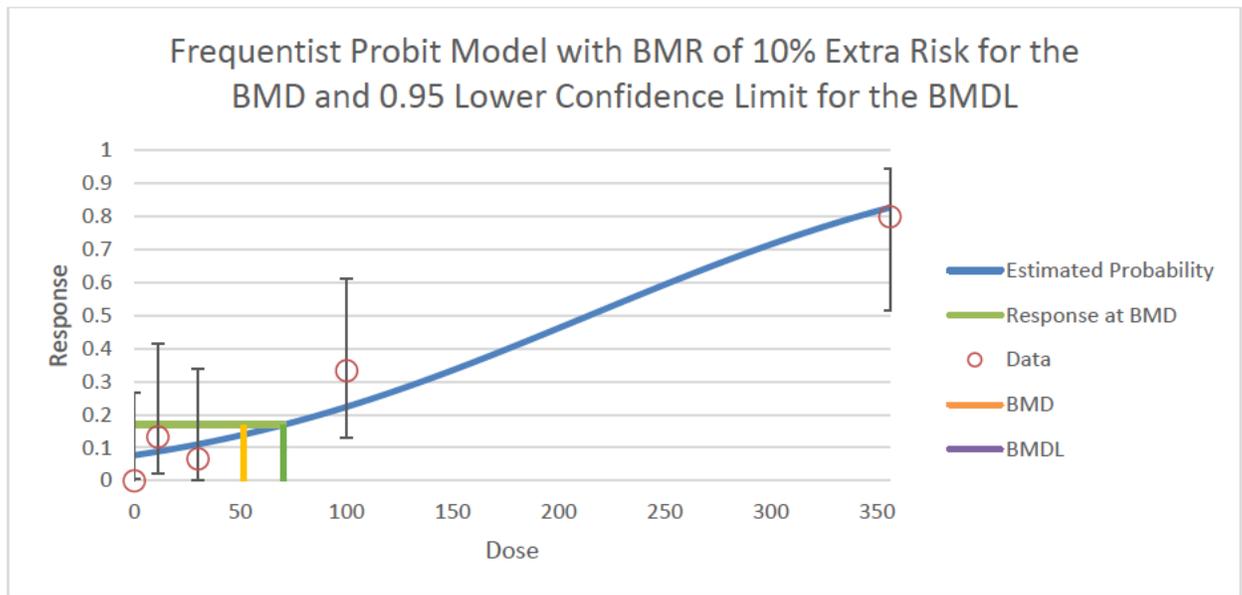


Frequentist Logistic Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



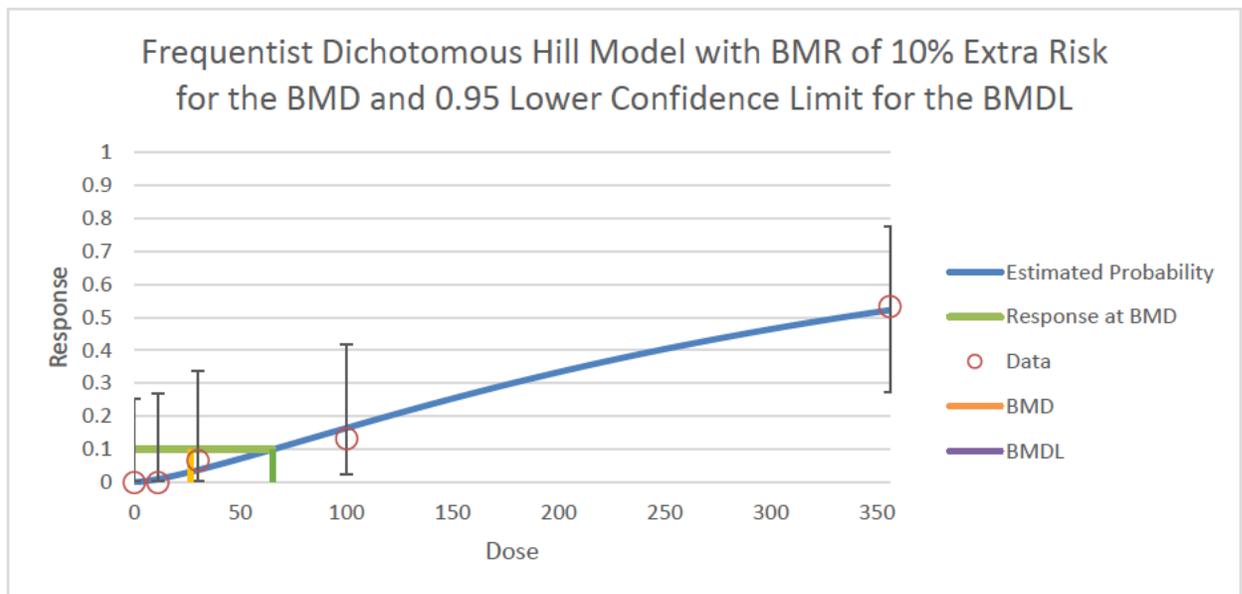
Frequentist Log-Probit Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



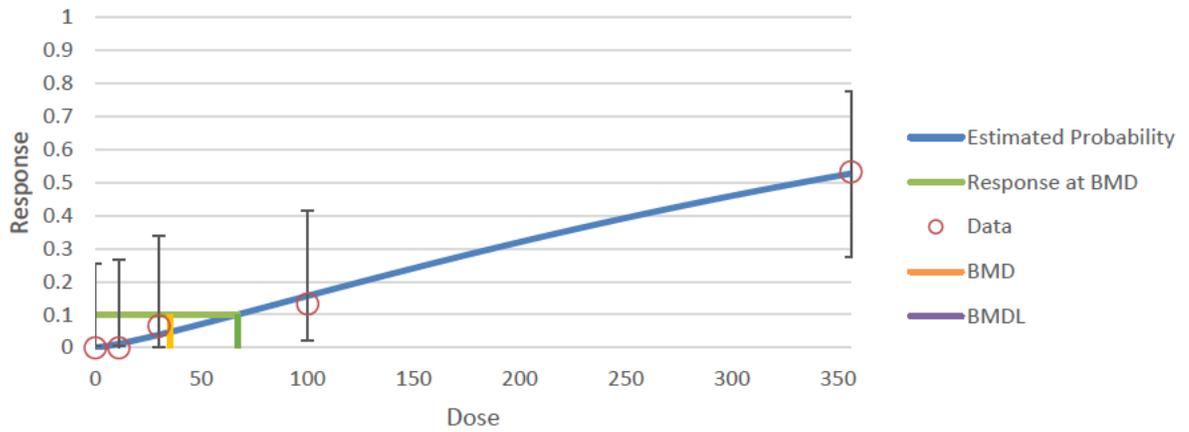


Plot of model average is not available from BMDS Version 3.2.0.1.

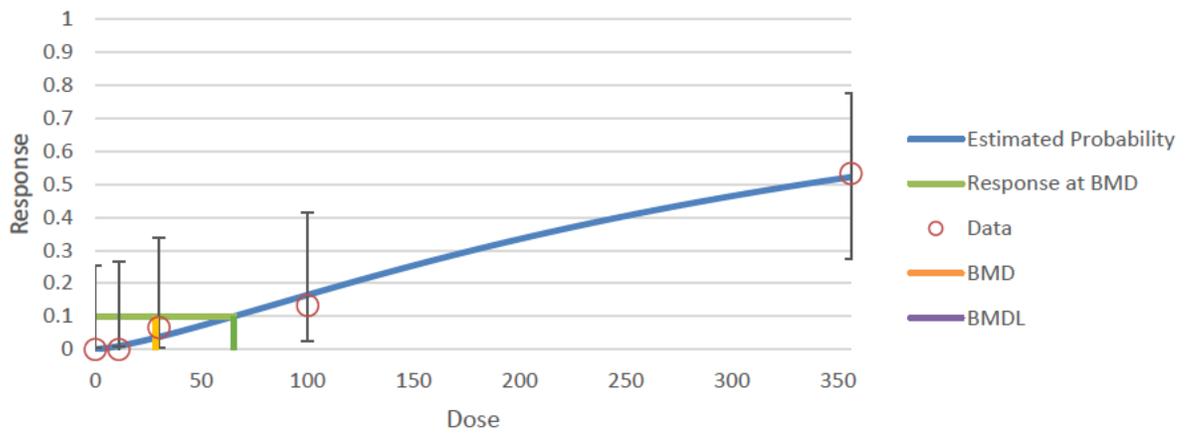
Ventral-female



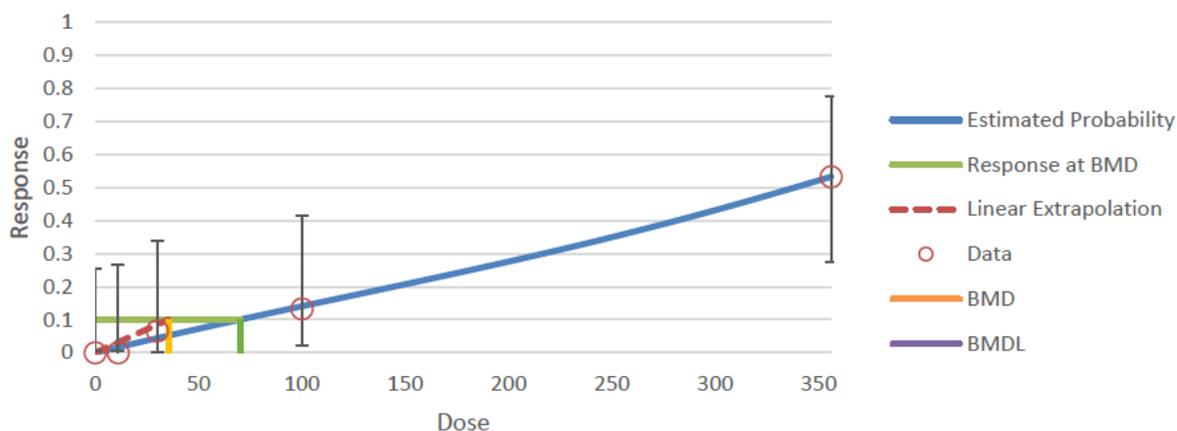
Frequentist Gamma Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



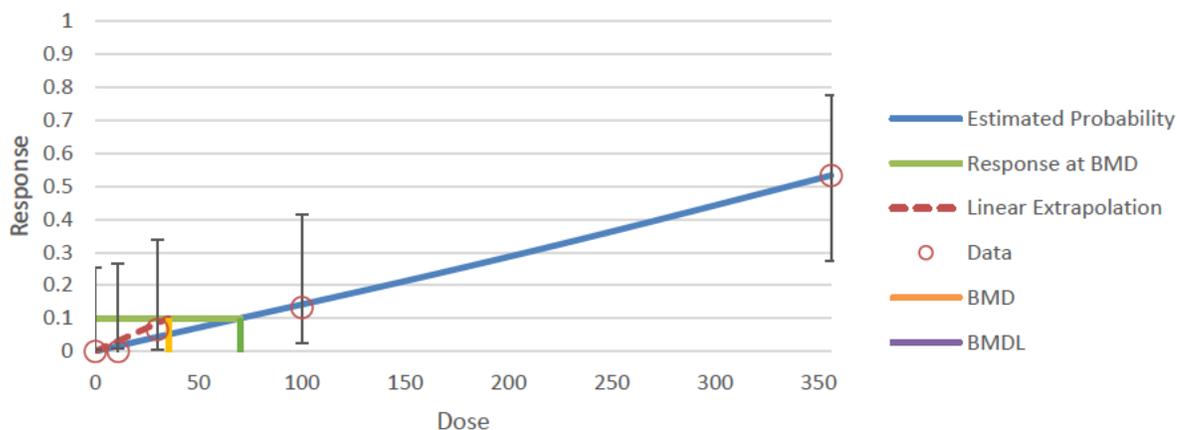
Frequentist Log-Logistic Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



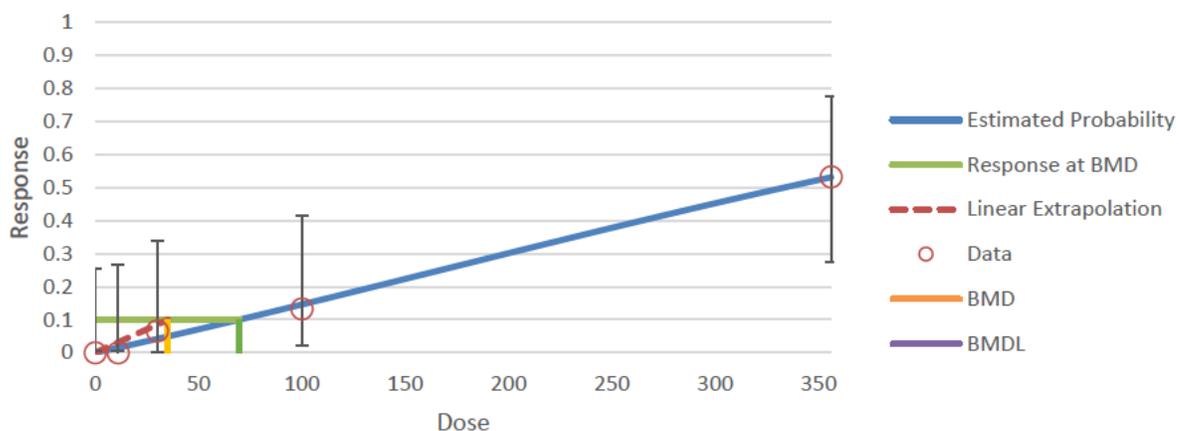
Frequentist Multistage Degree 4 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



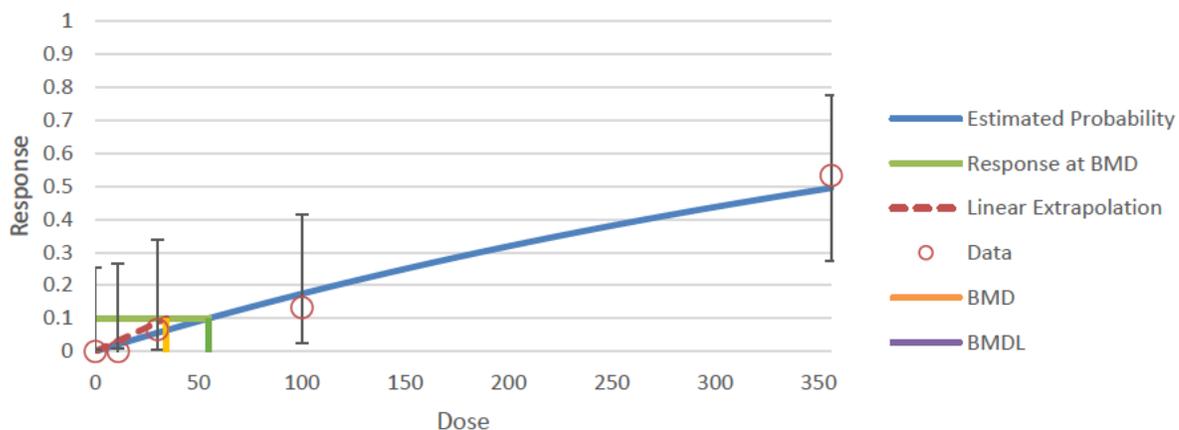
Frequentist Multistage Degree 3 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



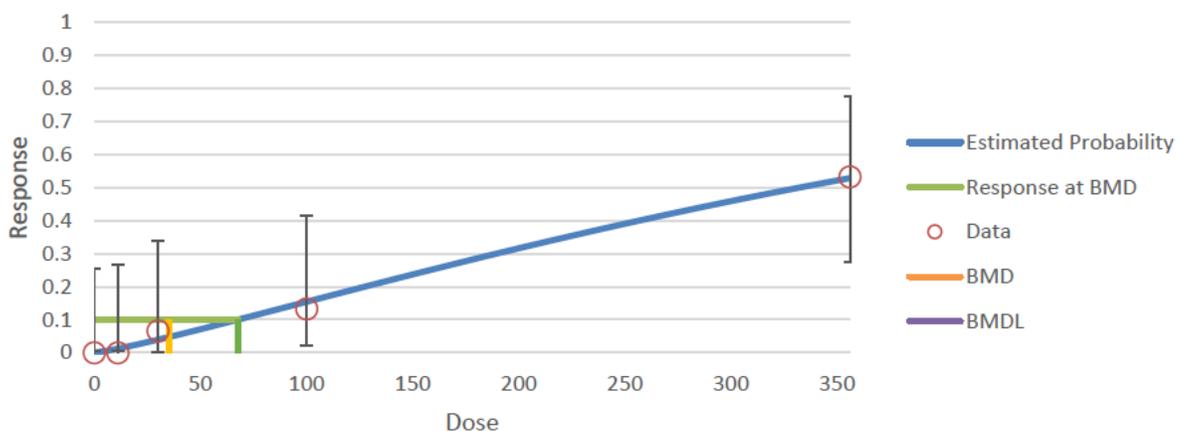
Frequentist Multistage Degree 2 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



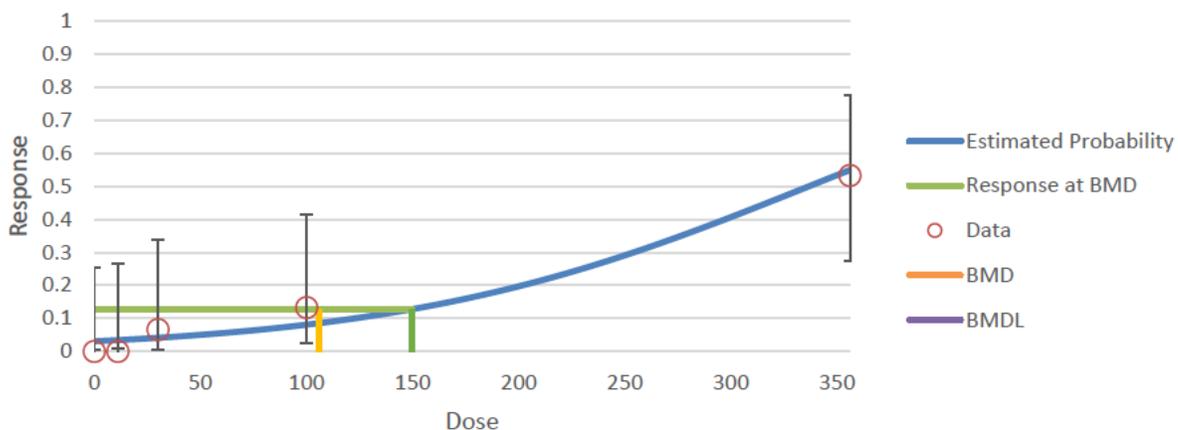
Frequentist Multistage Degree 1 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

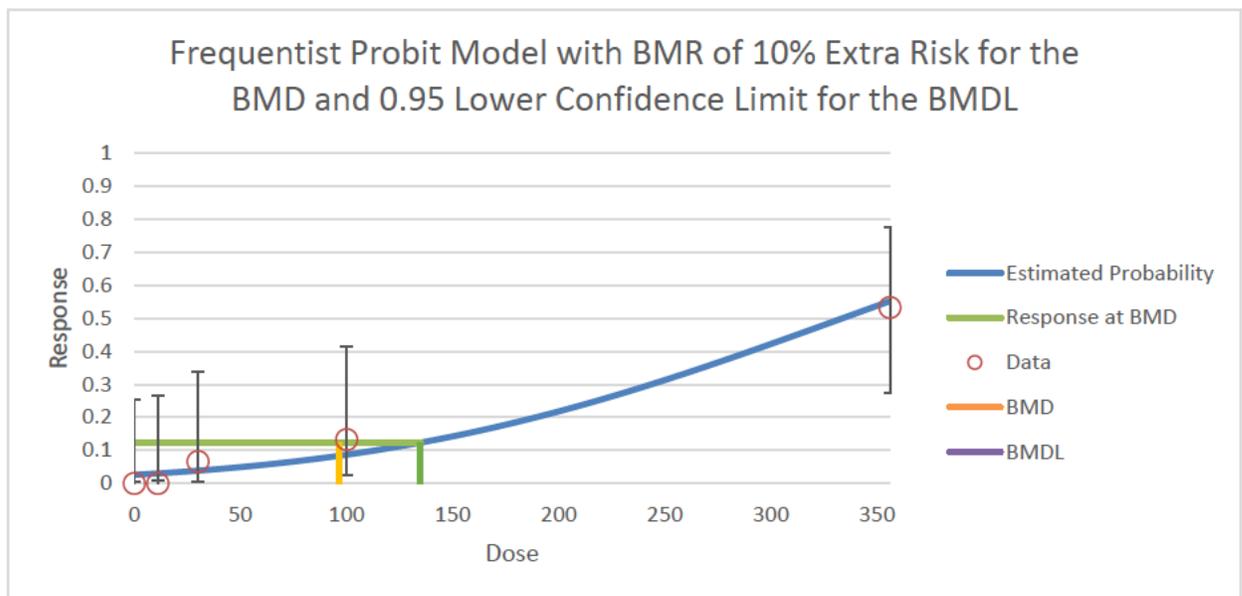
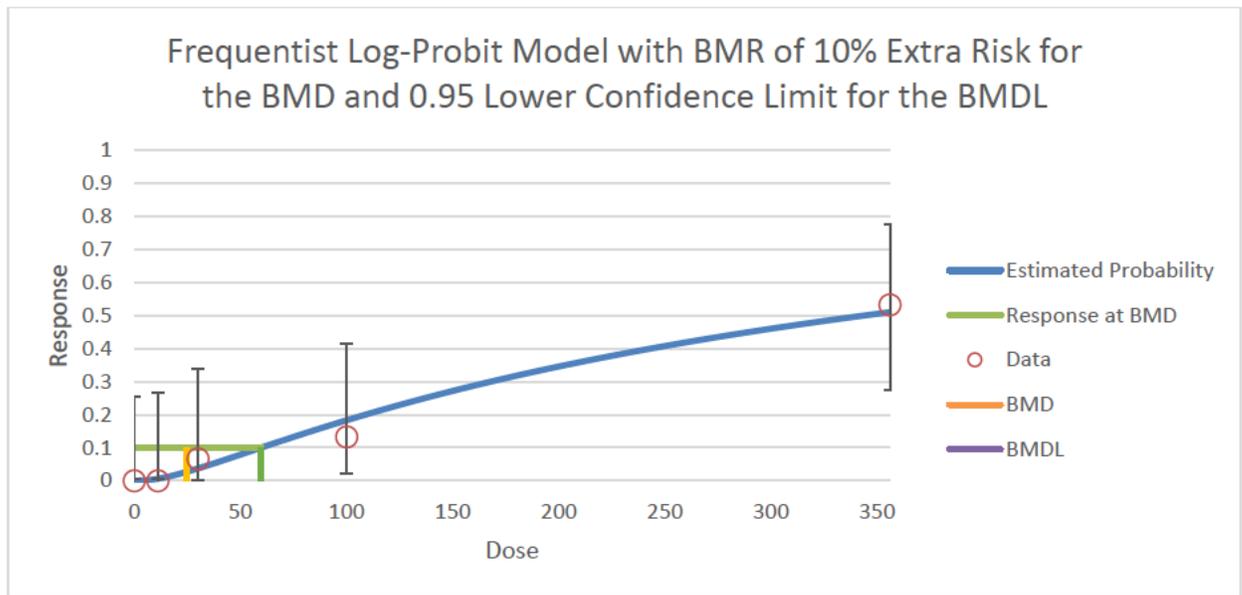


Frequentist Weibull Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



Frequentist Logistic Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL





Plot of model average is not available from BMDS Version 3.2.0.1.

Conclusions

A BMD analysis was conducted for the squamous metaplasia incidence data in accordance with the EFSA (2022) BMD guidance. An evaluation of the data usability to estimate BMD using dose-response modeling found that both the Mucosa-male and the Mucosa-female datasets lack sufficient information between the control dose and first treatment dose to provide reliable BMD estimates. The data usability evaluation found both Ventral-male and Ventral datasets can provide reliable BMD estimates.

Dose-response modeling using EFSA BMD app resulted in model average estimates for Mucosa-male and Mucosa-female that failed EFSA (2022) diagnostic ratio criteria for reliable BMD estimate. EFSA BMD app estimated reliable BMD estimates for the Ventral-male and Ventral-female datasets. For Ventral-male, EFSA BMD app estimated a BMDL of 8.57 mg/m³ and for the Ventral-female a BMDL of 33.8 mg/m³.

A preliminary analysis of the Mucosa-male dataset was conducted using the newly released EPA BMDS Version 3.3. However, the Bayesian model average estimates failed the EFSA (2022) diagnostic ratio criteria.

Additional dose-response modeling was conducted using EPA BMDS Version 3.2 given the unreliable estimates for Mucosa-male and Mucosa-female. For the Mucosa-male dataset, the BMDS Version 3.2 Bayesian model average estimate of BMDL = 1.15 mg/m³, and it fulfills EFSA (2022) diagnostic ratio criteria. For the Mucosa-female dataset, BMDS Version 3.2 analysis failed to obtain adequate model fit (p-value>0.1 criterion) for all models so that the results are not considered usable. For the Ventral-male dataset, the BMDS Version 3.2 Bayesian model average estimate for BMDL = 20.11 mg/m³, and it fulfills EFSA (2022) diagnostic ratio criteria. For the Ventral-female dataset, the BMDS Version 3.2 Bayesian model average estimate for BMDL = 41.85 mg/m³, and it fulfills EFSA (2022) diagnostic ratio criteria.

The results of the data usability and dose-response analyses led to the conclusion that reliable BMD estimates could not be obtained for the Mucosa-male and Mucosa-female datasets.

References

European Food Safety Authority Scientific Committee (EFSA). 2022. Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 20(10):7584, <https://doi.org/10.2903/j.efsa.2022.7584>

European Food Safety Agency Benchmark Dose Modelling app. Available from: <https://r4eu.efsa.europa.eu/app/bmd> (Accessed October 24, 2022).

██████████ 1992. A subchronic (3-month) inhalation toxicity study of Pyrethrum extract in the rat via whole-body exposures. Final report. ██████████. September 14, 1992.

U.S. Environmental Protection Agency. Benchmark Dose Software (BMDS) Version v3.2.0.1. National Center for Environmental Assessment. United States Environmental Protection Agency. Available from: <https://www.epa.gov/bmds/download-bmds> (Accessed October 24, 2022).

U.S. Environmental Protection Agency. Benchmark Dose Software (BMDS) Version v3.3. National Center for Environmental Assessment. United States Environmental Protection Agency. Available from: <https://www.epa.gov/bmds/download-bmds> (PC-version), <https://www.epa.gov/bmds/bmds-online> (Web-version) (Accessed October 26, 2022).

Appendix A

Results from EFSA BMD App Analysis

Mucosa-male

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
null	1	-42.15	86.30		NA	NA	NA	NA
full	5	-21.18	52.36		NA	NA	NA	NA
two.stage	3	-21.37	48.74	yes	0.967000	2.50	1.490	yes
log.logist	3	-21.53	49.06	yes	0.128000	6.65	2.500	yes
Weibull	3	-21.28	48.56	yes	0.004630	4.87	0.810	yes
log.prob	3	-21.40	48.80	yes	0.088900	6.69	2.260	yes
gamma	3	-21.25	48.50	yes	0.000000	5.41	0.511	yes
LVM: Expon. m3-	3	-21.25	48.50	yes	0.001120	3.91	0.373	yes
LVM: Hill m3-	3	-21.25	48.50	yes	0.000586	3.92	0.378	yes

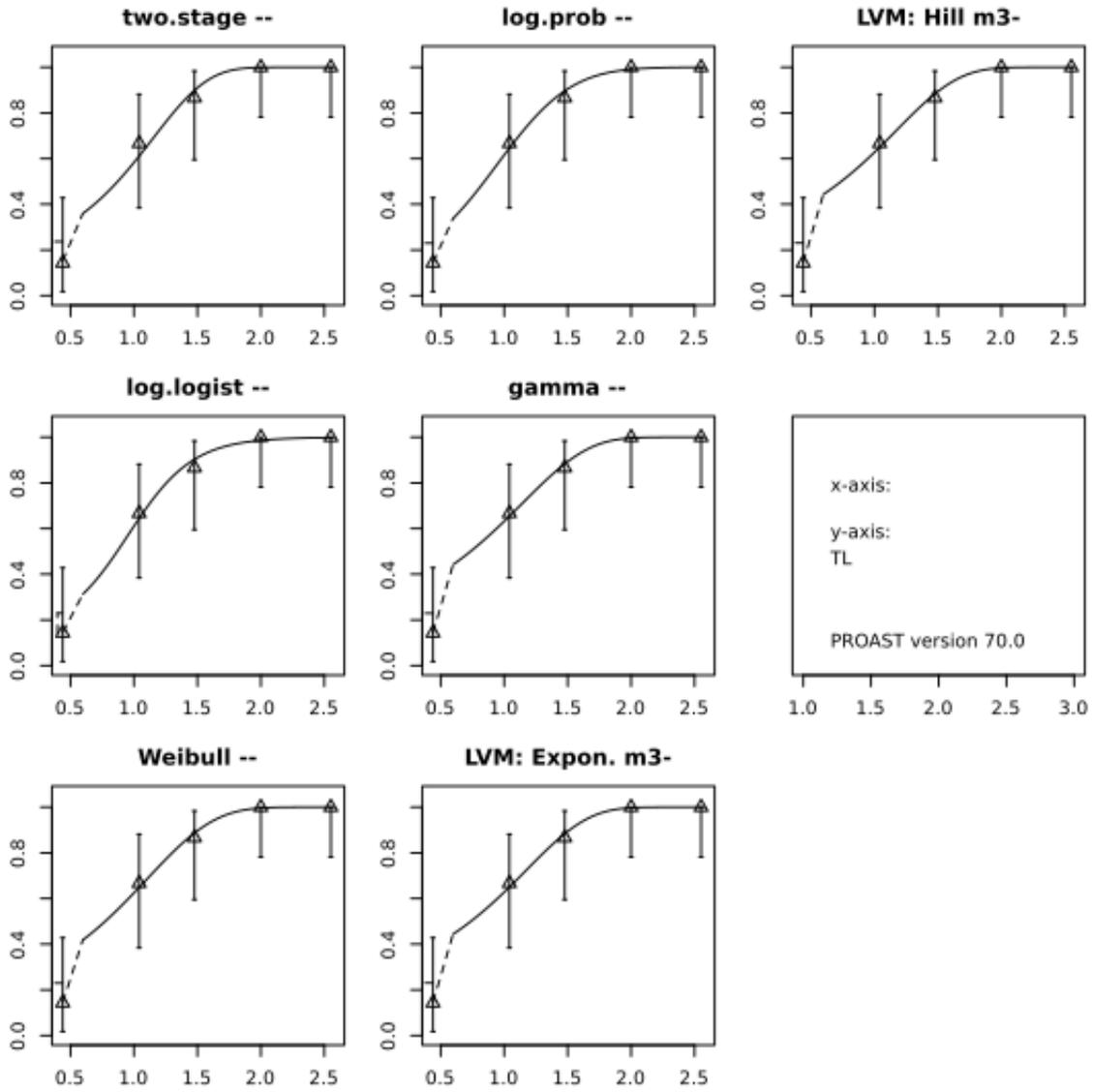
Weights for Model Averaging

two.stage	log.logist	Weibull	log.prob	gamma	EXP	HILL
0.14	0.12	0.15	0.13	0.15	0.15	0.15

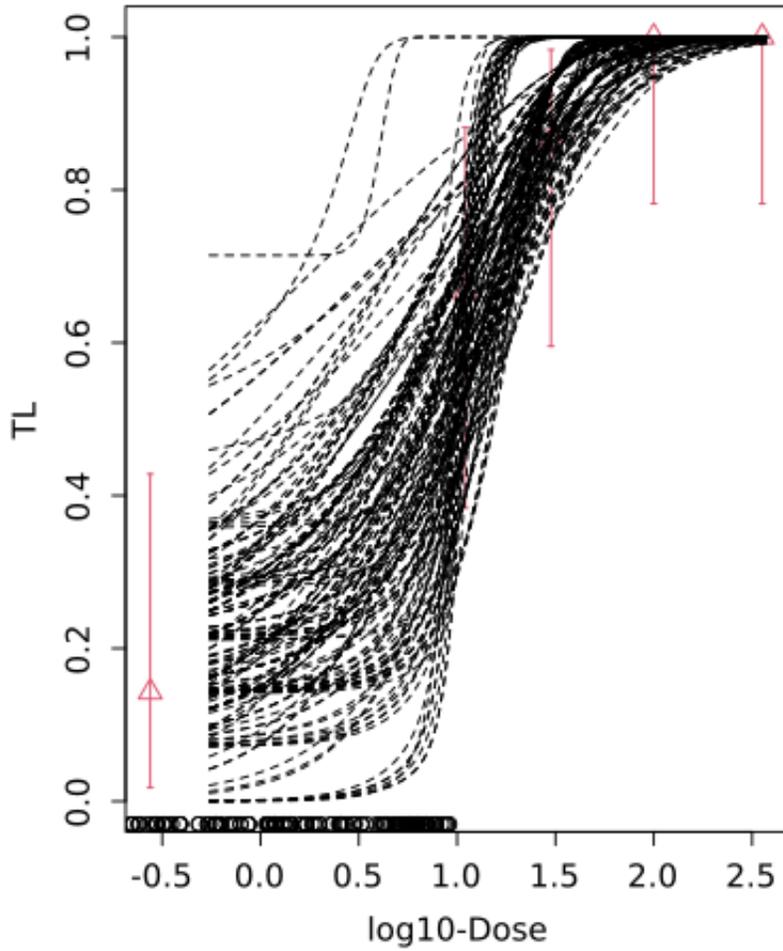
Final BMD Values

subgroup	BMDL	BMDU
all	0.02	7.38

Visualization



**bootstrap curves
based on model averaging**



version: 70.0
model averaging results
dtype 4
selected all
dose scaling: 1
conf level: 0.9
number of runs: 200
extra risk 0.1
BMD CI
0.022 7.38

Mucosa-female

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
null	1	-45.86	93.72		NA	NA	NA	NA
full	5	-19.81	49.62		NA	NA	NA	NA
two.stage	3	-35.80	77.60	no	NA	NA	5.630000	yes
log.logist	3	-23.31	52.62	yes	0	0.113000	0.000001	yes
Weibull	3	-23.40	52.80	yes	0	0.010300	0.000001	yes
log.prob	3	-23.30	52.60	yes	0	0.099200	0.000001	yes
gamma	3	-23.85	53.70	yes	0	0.000999	0.000001	yes
LVM: Expon. m3-	3	-26.47	58.94	no	NA	NA	0.014100	yes
LVM: Hill m3-	3	-24.85	55.70	no	NA	NA	0.001310	yes

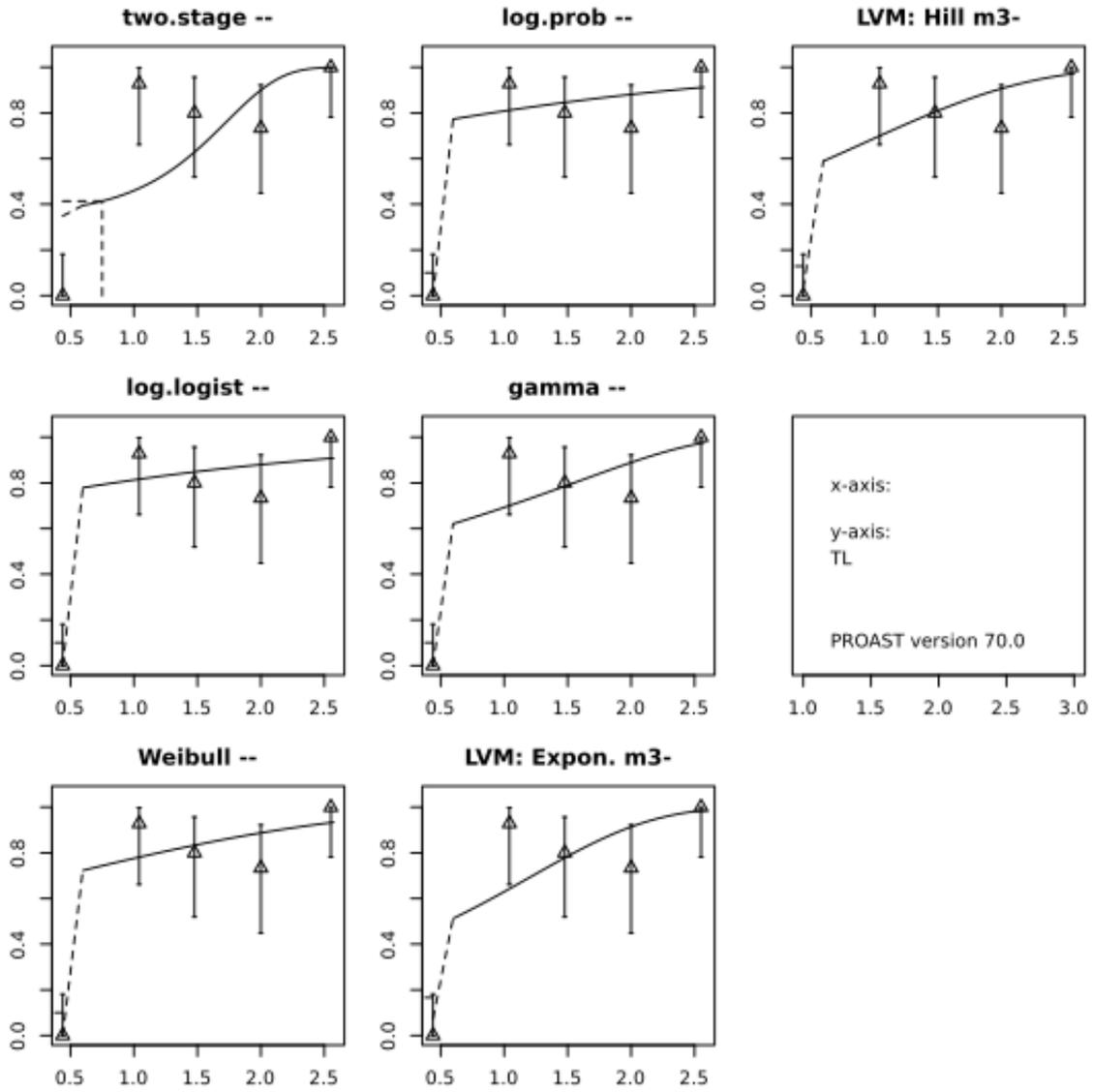
Weights for Model Averaging

two.stage	log.logist	Weibull	log.prob	gamma	EXP	HILL
0	0.27	0.24	0.27	0.15	0.01	0.06

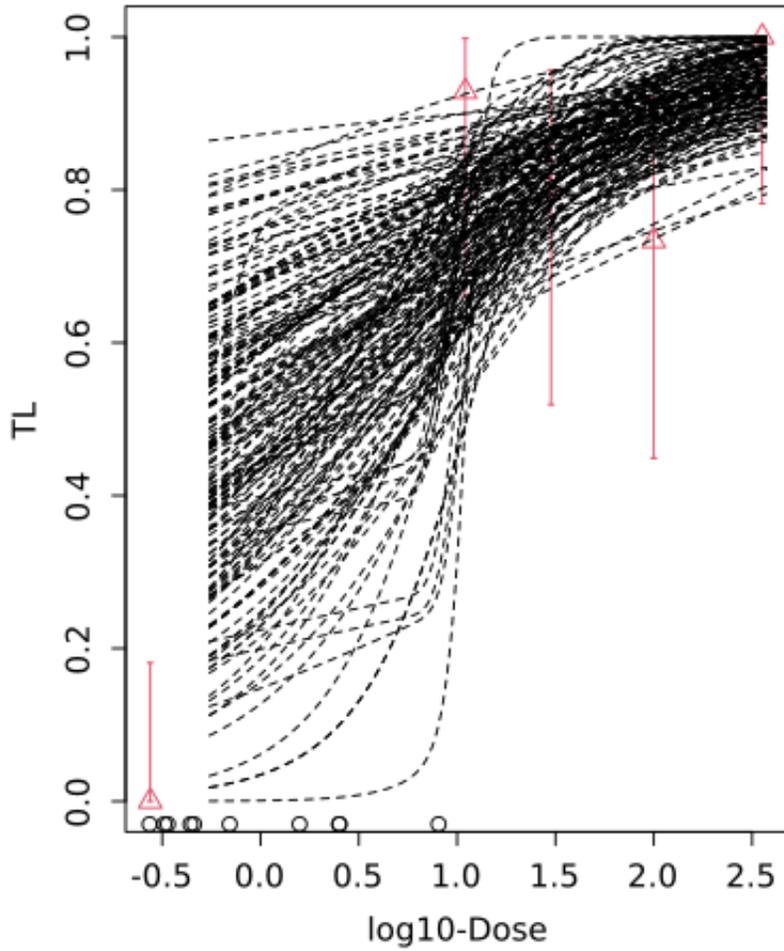
Final BMD Values

subgroup	BMDL	BMDU
all	0	0.16

Visualization



**bootstrap curves
based on model averaging**



version: 70.0
model averaging results
dtype 4
selected all
dose scaling: 1
conf level: 0.9
number of runs: 200
extra risk 0.1
BMD CI
2e-11 0.161

Ventral-male

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
null	1	-43.18	88.36		NA	NA	NA	NA
full	5	-26.62	63.24		NA	NA	NA	NA
two.stage	3	-27.72	61.44	yes	16.30	95.6	26.3	yes
log.logist	3	-28.10	62.20	yes	6.69	106.0	20.4	yes
Weibull	3	-27.67	61.34	yes	5.57	94.3	19.8	yes
log.prob	3	-28.05	62.10	yes	7.70	107.0	53.5	yes
gamma	3	-27.64	61.28	yes	4.39	95.0	18.8	yes
LVM: Expon. m3-	3	-27.53	61.06	yes	5.77	85.7	22.9	yes
LVM: Hill m3-	3	-27.53	61.06	yes	5.78	85.9	23.0	yes

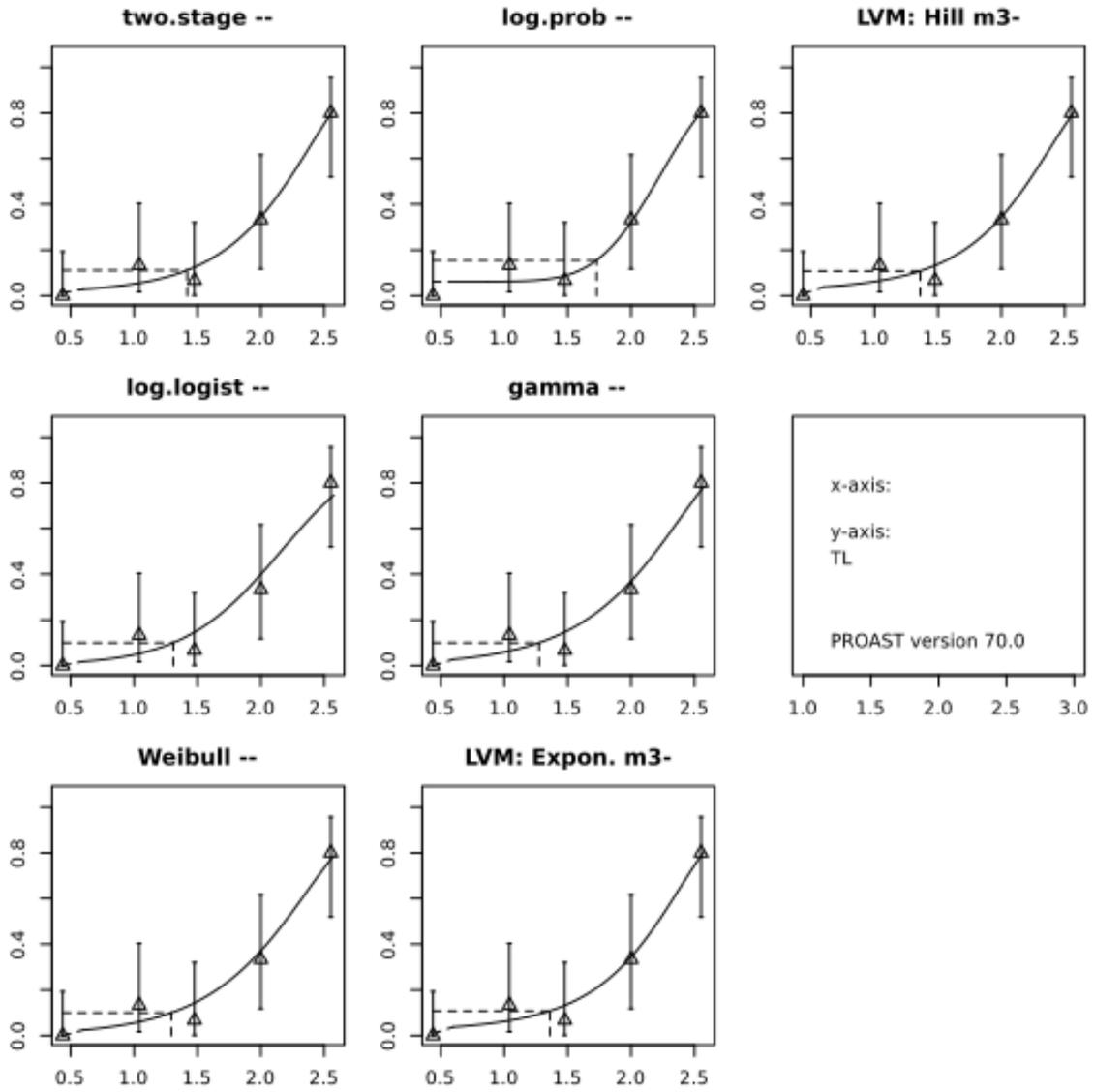
Weights for Model Averaging

two.stage	log.logist	Weibull	log.prob	gamma	EXP	HILL
0.14	0.1	0.15	0.1	0.16	0.17	0.17

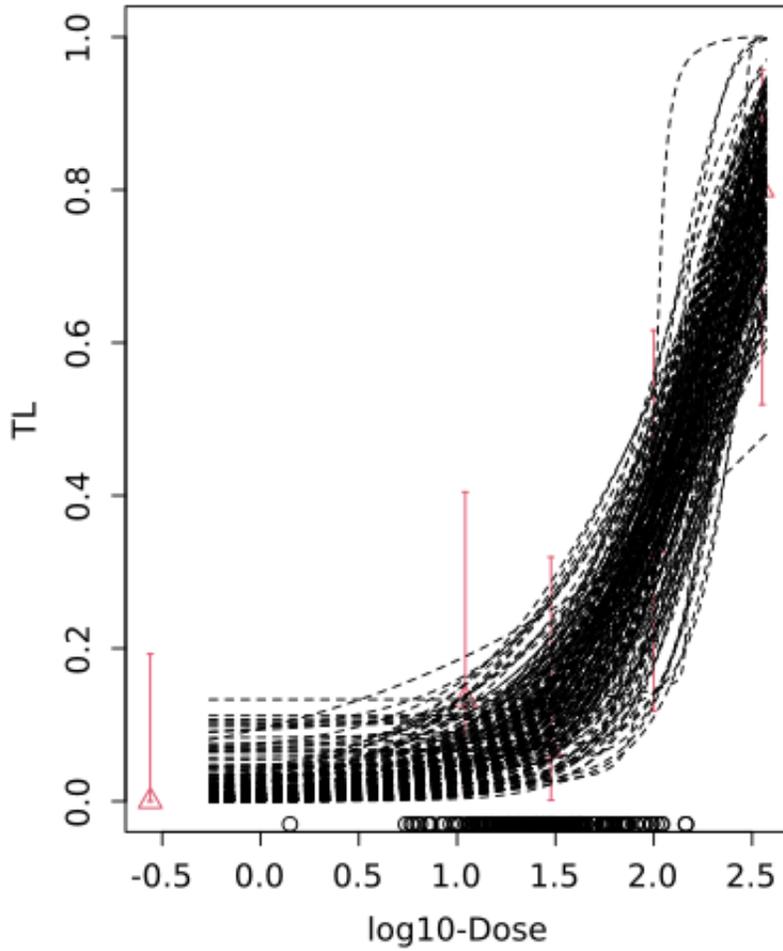
Final BMD Values

subgroup	BMDL	BMDU
all	8.57	81.1

Visualization



**bootstrap curves
based on model averaging**



version: 70.0
model averaging results
dtype 4
selected all
dose scaling: 1
conf level: 0.9
number of runs: 200
extra risk 0.1
BMD CI
8.6 81.1

Ventral-female

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
null	1	-31.11	64.22		NA	NA	NA	NA
full	5	-19.93	49.86		NA	NA	NA	NA
two.stage	3	-20.25	46.50	yes	35.2	150	69.6	yes
log.logist	3	-20.27	46.54	yes	25.0	162	65.3	yes
Weibull	3	-20.25	46.50	yes	24.3	166	67.6	yes
log.prob	3	-20.31	46.62	yes	24.8	153	59.7	yes
gamma	3	-20.25	46.50	yes	24.0	158	66.8	yes
LVM: Expon. m3-	3	-20.26	46.52	yes	24.9	185	66.3	yes
LVM: Hill m3-	3	-20.26	46.52	yes	24.6	185	66.3	yes

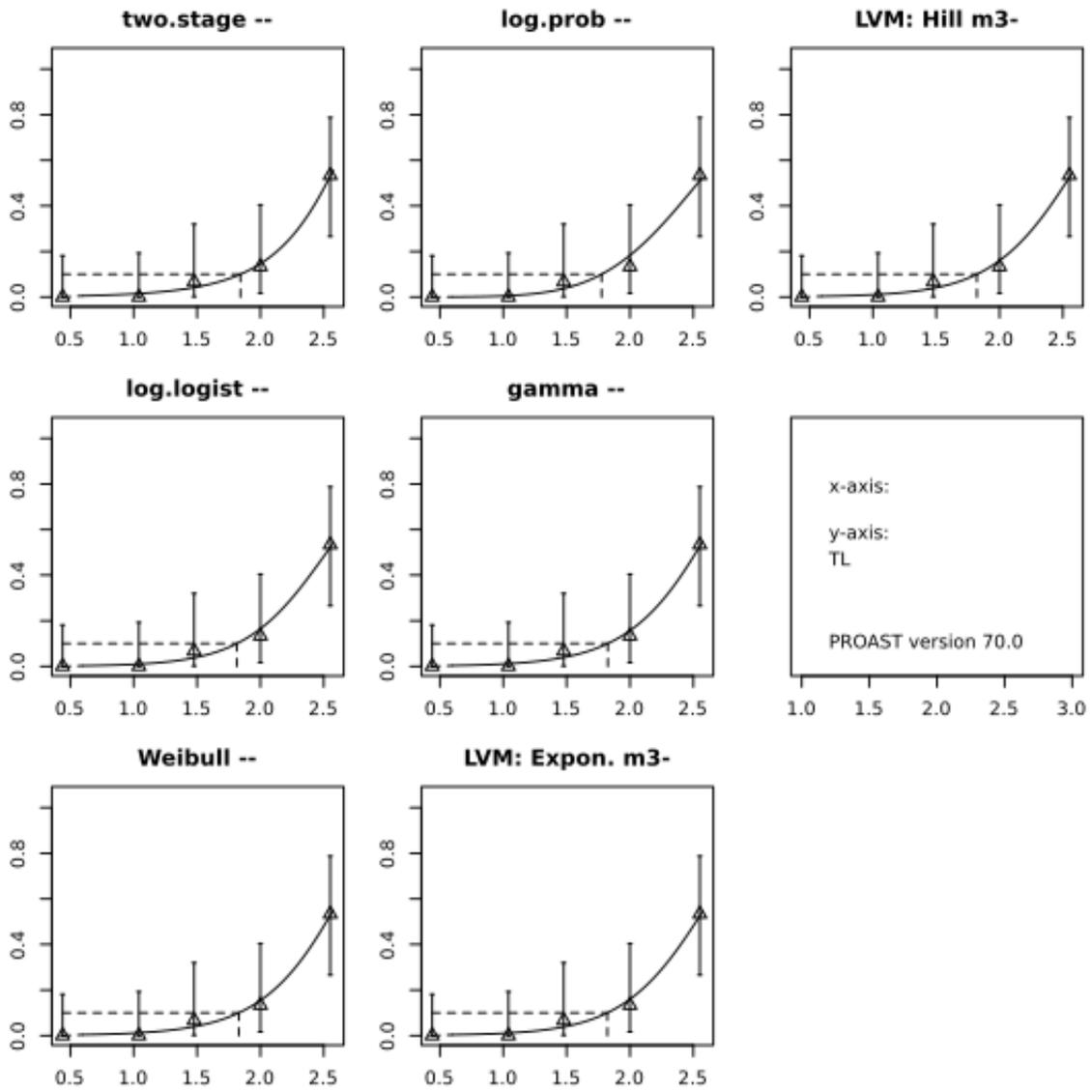
Weights for Model Averaging

two.stage	log.logist	Weibull	log.prob	gamma	EXP	HILL
0.14	0.14	0.14	0.14	0.14	0.14	0.14

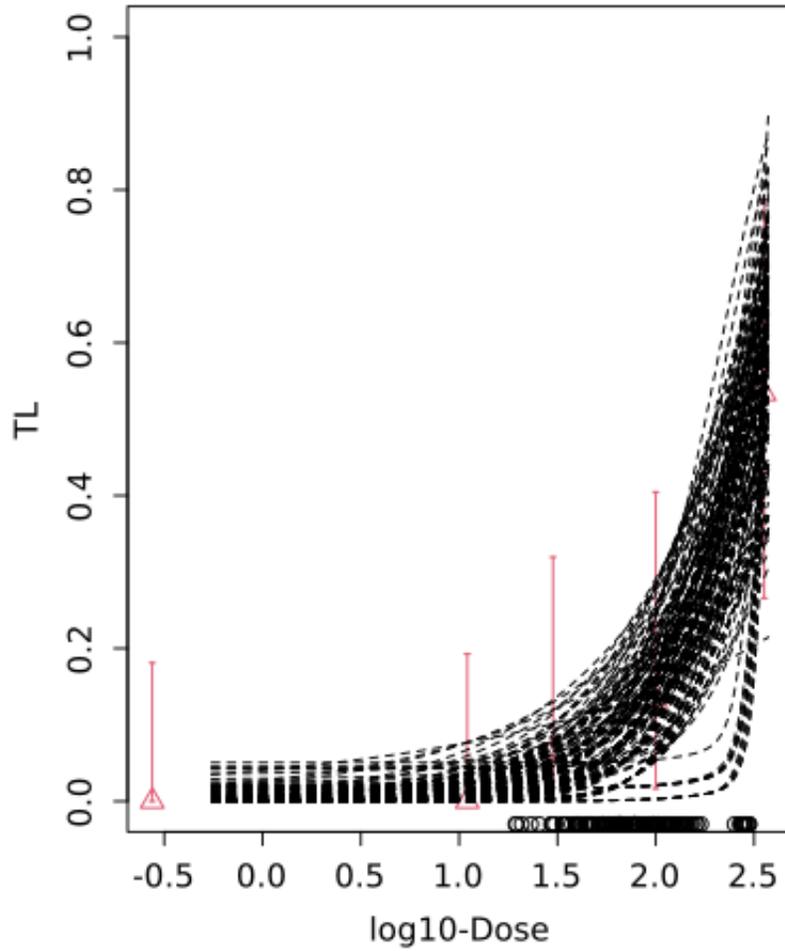
Final BMD Values

subgroup	BMDL	BMDU
all	33.8	268

Visualization



bootstrap curves based on model averaging



version: 70.0
model averaging results
dtype 4
selected all
dose scaling: 1
conf level: 0.9
number of runs: 200
extra risk 0.1
BMD CI
34 268

Appendix B

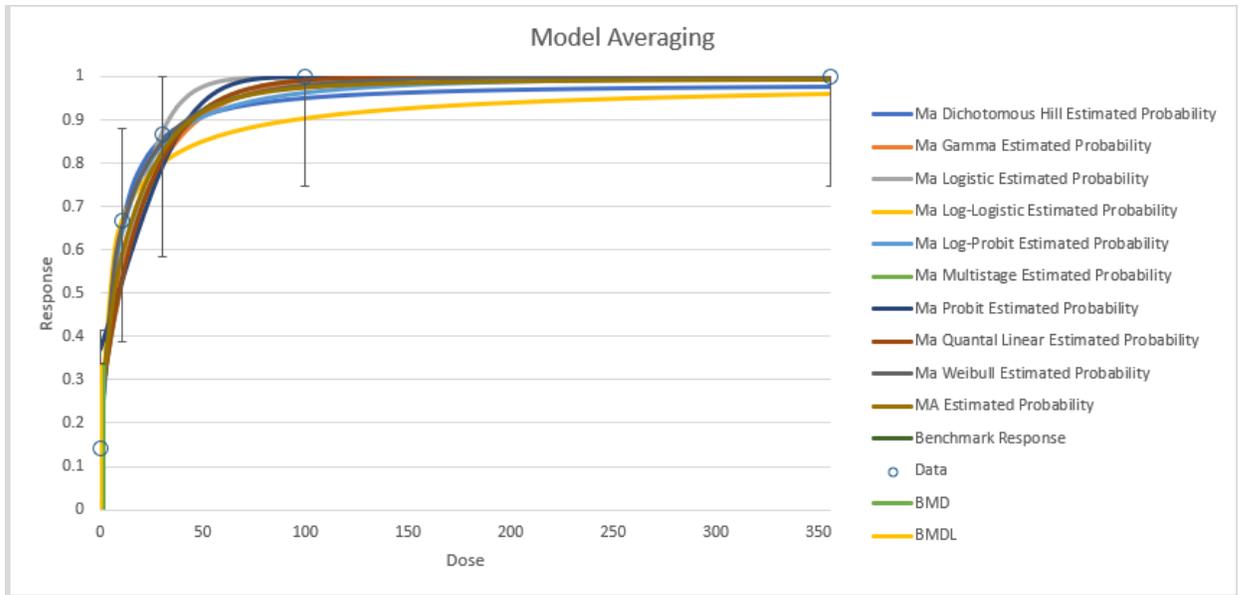
Results from BMDS Version 3.3 Analysis of Mucosa-male Dataset

BMDS Version 3.3, PC-version

Model	Analysis Type	Restriction	BMD	BMDL	BMDU	P Value	AIC
Dichotomous Hill	frequentist	Restricted	2.4963	0.3276	6.6502	0.9818	49.0592
Gamma	frequentist	Restricted	1.4869	0.9672	5.7133	0.9917	46.7450
Log-Logistic	frequentist	Restricted	2.4963	0.3276	6.6502	0.9818	49.0592
Multistage Degree 3	frequentist	Restricted	1.4869	0.9672	4.5205	0.9917	46.7450
Multistage Degree 2	frequentist	Restricted	1.4869	0.9672	4.5205	0.9917	46.7450
Multistage Degree 1	frequentist	Restricted	1.4869	0.9672	2.4990	0.9917	46.7450
Weibull	frequentist	Restricted	1.4869	0.9672	5.0205	0.9917	46.7450
Logistic	frequentist	Unrestricted	3.2558	2.1792	4.8519	0.7883	48.6251
Log-Probit	frequentist	Unrestricted	2.2634	0.0896	6.6727	0.9870	48.7934
Probit	frequentist	Unrestricted	3.6977	2.7847	5.0712	0.6038	49.7671
Quantal Linear	frequentist	Unrestricted	1.4869	0.9672	2.4990	0.9917	46.7450
Model Average	Bayesian MA	-	1.7187	0.0621	5.2529	-	-

MA - Individual Models

Model	Posterior Probability	BMD	BMDL	BMDU
Dichotomous Hill	0.0504	1.2535	0.1157	4.1376
Gamma	0.0290	1.4515	0.1915	5.2829
Logistic	0.0000	3.4576	2.6719	5.6652
Log-Logistic	0.0025	0.3790	0.0131	2.5489
Log-Probit	0.0171	1.3537	0.1831	4.5053
Multistage	0.4005	2.3155	1.3644	4.9428
Probit	0.1026	4.2868	2.7042	8.7141
Quantal Linear	0.0000	2.3155	2.0560	4.9428
Weibull	0.3979	0.4455	0.0245	2.2982



BMDS Version 3.3, Web-version

Model	Analysis Type	Restriction	BMD	BMDL	BMDU	P Value	AIC
Hill	frequentist	Restricted	2.496	0.328	6.65	0.982	49.059
Gamma	frequentist	Restricted	1.487	0.967	5.713	0.992	46.745
LogLogistic	frequentist	Restricted	2.496	0.328	6.65	0.982	49.059
Multistage 1	frequentist	Restricted	1.487	0.967	2.499	0.992	46.745
Weibull	frequentist	Restricted	1.487	0.967	5.024	0.992	46.745
Logistic	frequentist	Unrestricted	3.256	2.179	4.852	0.788	48.625
LogProbit	frequentist	Unrestricted	2.263	0.09	6.673	0.987	48.793
Probit	frequentist	Unrestricted	3.698	2.785	5.071	0.604	49.767
Quantal Linear	frequentist	Unrestricted	1.487	0.967	2.499	0.992	46.745
Model Average	Bayesian MA	-	1.014	0.041	5.406	-	-

MA - Individual Models

Model	Posterior Probability	BMD	BMDL	BMDU
Hill	0.0721	1.2535	0.1157	4.1376
Gamma	0.0415	1.4515	0.1915	5.2829
Logistic	0.0000	3.4576	2.6719	5.6652
LogLogistic	0.0036	0.3790	0.0131	2.5489
LogProbit	0.0245	1.3537	0.1831	4.5053
Multistage 2°	0.1417	2.3240	1.3666	5.0094
Probit	0.1468	4.2868	2.7042	8.7141
Quantal Linear	0.0000	2.3155	2.0560	4.9428
Weibull	0.5698	0.4455	0.0245	2.2982

