

Annex to: Risk assessment of N-nitrosamines in food
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Annex B – BMD analyses

Selection of the BMR

The BMR (benchmark response) used is an extra risk of 10% compared to the controls.

The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

Software Used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-packag PROAST, version 70.0, for the underlying calculations.

Specification of Deviations from Default Assumptions

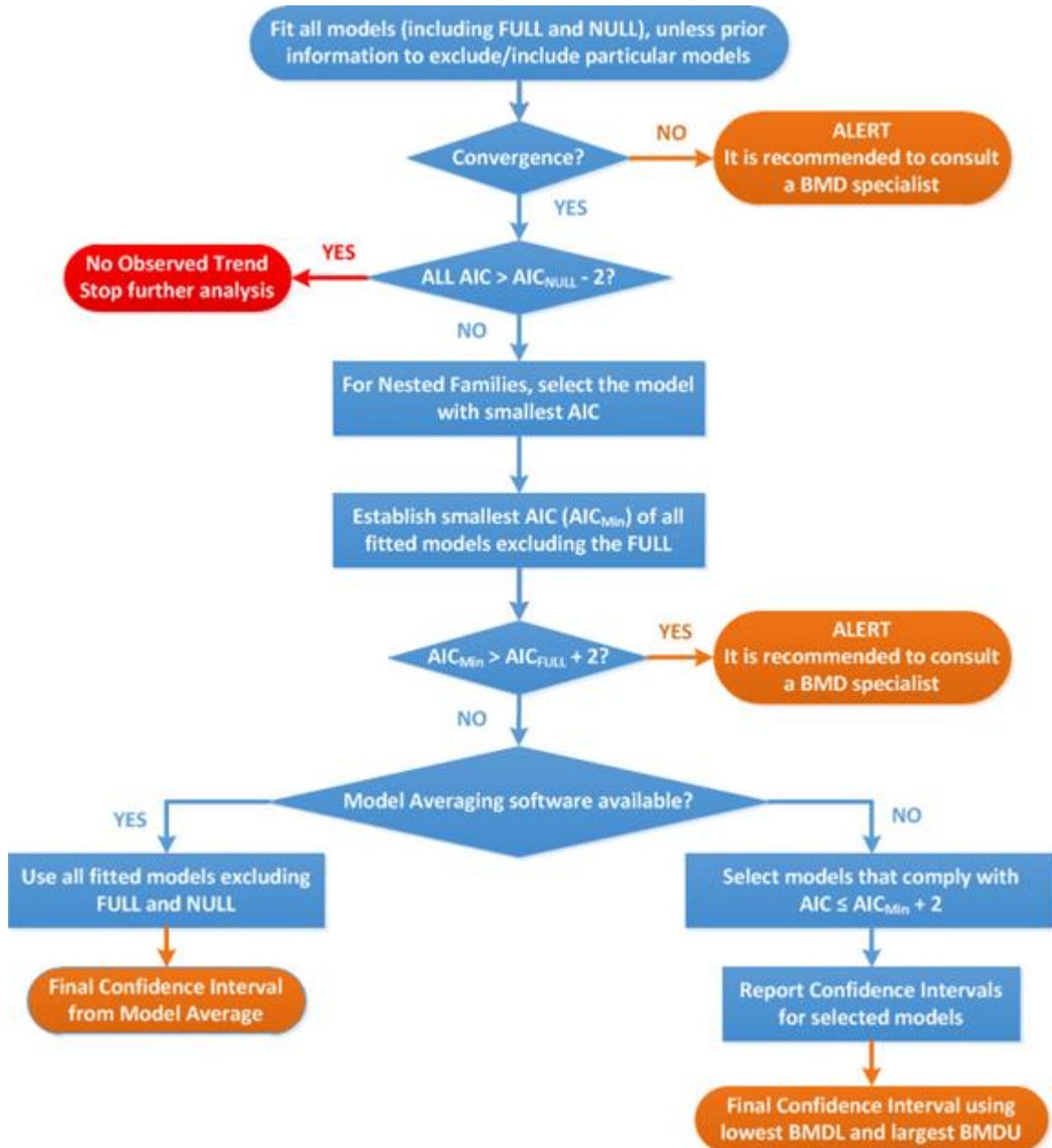
Dose-response models

Default set of fitted models:

Model	Number of parameters	Formula
Null	1	$y = a$
Full	no. of groups	$y = \text{group mean}$
Logistic	2	$y = 1 + \exp(-a - bx)$
Probit	2	$y = \text{pnorm}((x-a) \cdot b)$
Log-logistic	3	$y = a + 1 - a \cdot \exp(c \cdot \log(bx))$
Log-probit	3	$y = a + (1-a) \cdot \text{pnorm}(c \cdot \log(xb))$
Weibull	3	$y = a + (1-a)(1 - \exp(-(xb)^c))$
Gamma	3	$y = \text{pgamma}(bx; c)$
Two-stage	3	$y = a + (1-a)(1 - \exp(-xb - c(xb)^2))$
Exp model 3	3	$y = a \cdot \exp(bxd)$
Exp model 5	4	$y = a \cdot (c - (c-1) \exp(-bxd))$
Hill model 3	3	$y = a \cdot (1 - xdbd + xd)$
Hill model 5	4	$y = a \cdot (1 + (c-1)xdbd + xd)$

For the Exp and Hill family, we fit models with 3 and 4 parameters as listed in the table. The 3-parameter model is selected if the difference in AIC is smaller than 5, otherwise the 4-parameter model is selected.

Procedure for selection of BMDL



Flowchart for selection of BMDL

B.1. NDMA

B.1.1 Data description

The endpoint to be analyzed is animals with a liver tumour (malignant or non-malignant).

Data used for analysis:

NDMA dose mg/kg bw per day	Number of. tumour cases	Animals (n)	Male (1)/female (2)
0.000	16	192	1
0.002	9	48	1
0.004	10	48	1
0.008	6	48	1
0.017	8	48	1
0.030	7	48	1
0.070	12	48	1
0.100	15	48	1
0.140	23	48	1
0.170	33	48	1
0.200	38	48	1
0.270	38	48	1
0.340	44	48	1
0.410	47	48	1
0.540	48	48	1
0.000	28	192	2
0.003	12	48	2
0.006	10	48	2
0.011	9	48	2
0.020	13	48	2
0.050	14	48	2
0.090	21	48	2
0.140	38	48	2
0.180	38	48	2
0.230	46	48	2
0.280	47	48	2
0.360	48	48	2
0.460	46	48	2
0.550	47	48	2
0.730	44	48	2

Final BMD Values

subgroup	BMDL	BMDU
Males	0.05	0.0863
Females	0.035	0.0625

Confidence intervals for the BMD are based on 200 bootstrap data sets.

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	sens.subgr	conv
null	2	-1252.25	2508.50		NA	NA	NA		NA
full	32	-717.39	1498.78		NA	NA	NA		NA
two.stage-a	4	-811.29	1630.58	no	NA	NA	0.02060	--	yes
log.logist-ab	5	-767.20	1544.40	no	NA	NA	0.02950	2	yes
Weibull-ab	5	-796.75	1603.50	no	NA	NA	0.00683	2	yes
log.prob-ab	5	-778.02	1566.04	no	NA	NA	0.02390	2	yes
gamma-a	4	-804.25	1616.50	no	NA	NA	0.00787	--	yes
LVM: Expon. m5-ab	6	-739.98	1491.96	yes	0.0337	0.0571	0.04460	2	yes
LVM: Hill m5-ab	6	-742.34	1496.68	no	NA	NA	0.05660	2	yes

Estimated Model Parameters

two.stage

estimate for a-1 : 0.09998

estimate for a-2 : 0.1834

estimate for BMD- : 0.02059

estimate for c : 1e-06

log.logist

estimate for a-1 : 0.1166

estimate for a-2 : 0.1735

estimate for BMD-1 : 0.04088

estimate for BMD-2 : 0.02947

estimate for c : 1.831

Weibull

estimate for a-1 : 0.07902

estimate for a-2 : 0.1424

estimate for BMD-1 : 0.008488

estimate for BMD-2 : 0.006831

estimate for c : 0.735

log.prob

estimate for a-1 : 0.1139

estimate for a-2 : 0.1727

estimate for BMD-1 : 0.03234

estimate for BMD-2 : 0.02387

estimate for c : 0.9281

gamma

estimate for a-1 : 0.07585

estimate for a-2 : 0.1553

estimate for BMD- : 0.007872

estimate for c : 0.6609

EXP

estimate for a-1 : 1.32

estimate for a-2 : 1.264

estimate for BMD-1 : 0.06431

estimate for BMD-2 : 0.04456

estimate for c- : 0.5146

estimate for d- : 1.825

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

HILL

estimate for a-1 : 1.311

estimate for a-2 : 1.254

estimate for BMD-1 : 0.07869

estimate for BMD-2 : 0.05661

estimate for c- : 0.5153

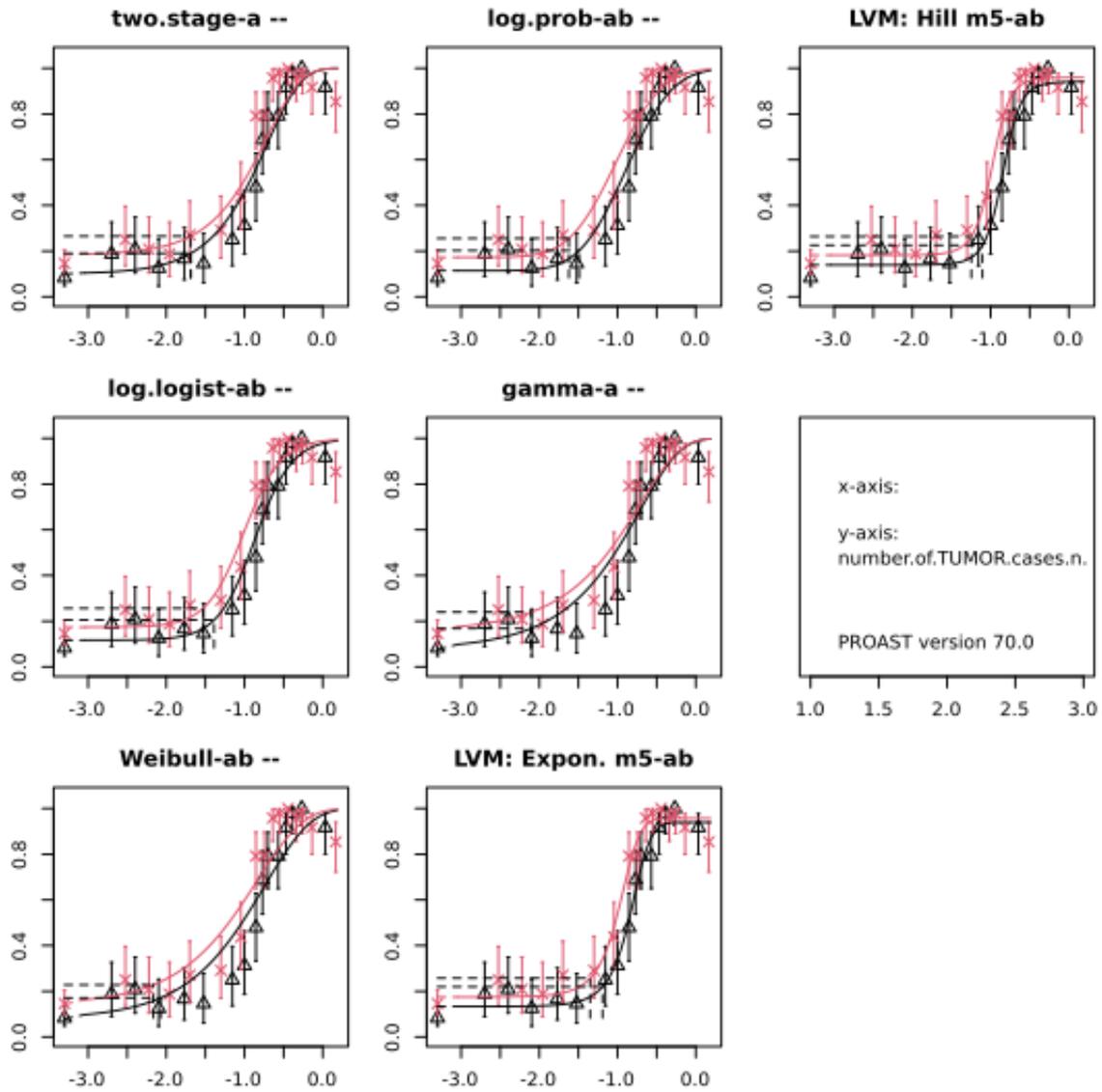
estimate for d- : 2.903

estimate for th(fixed) : 0

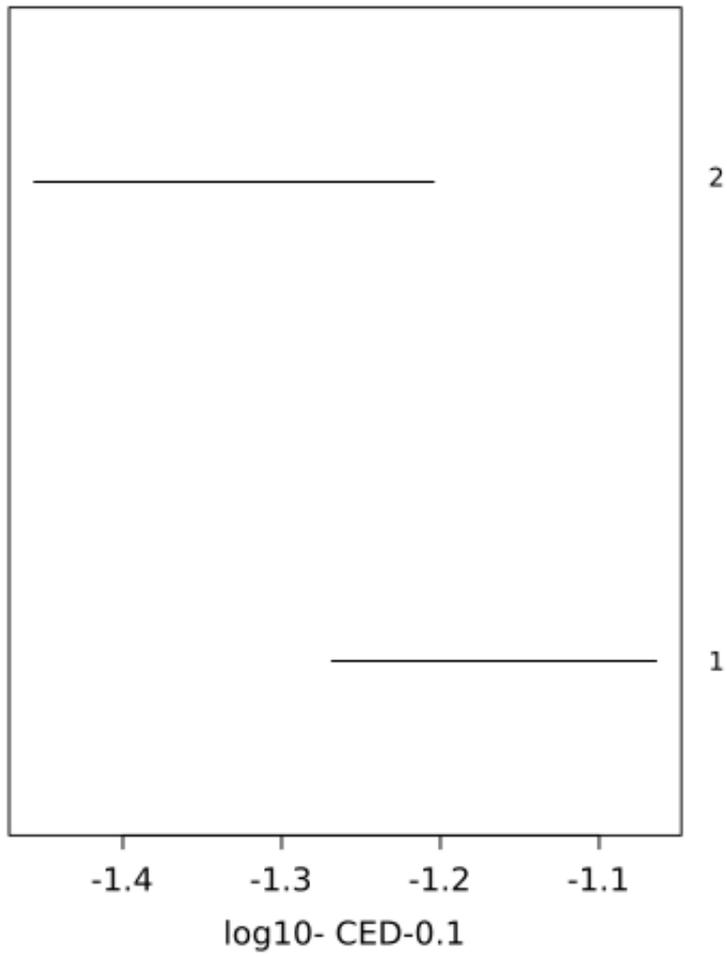
estimate for sigma(fixed) : 0.25

Weights for Model Averaging

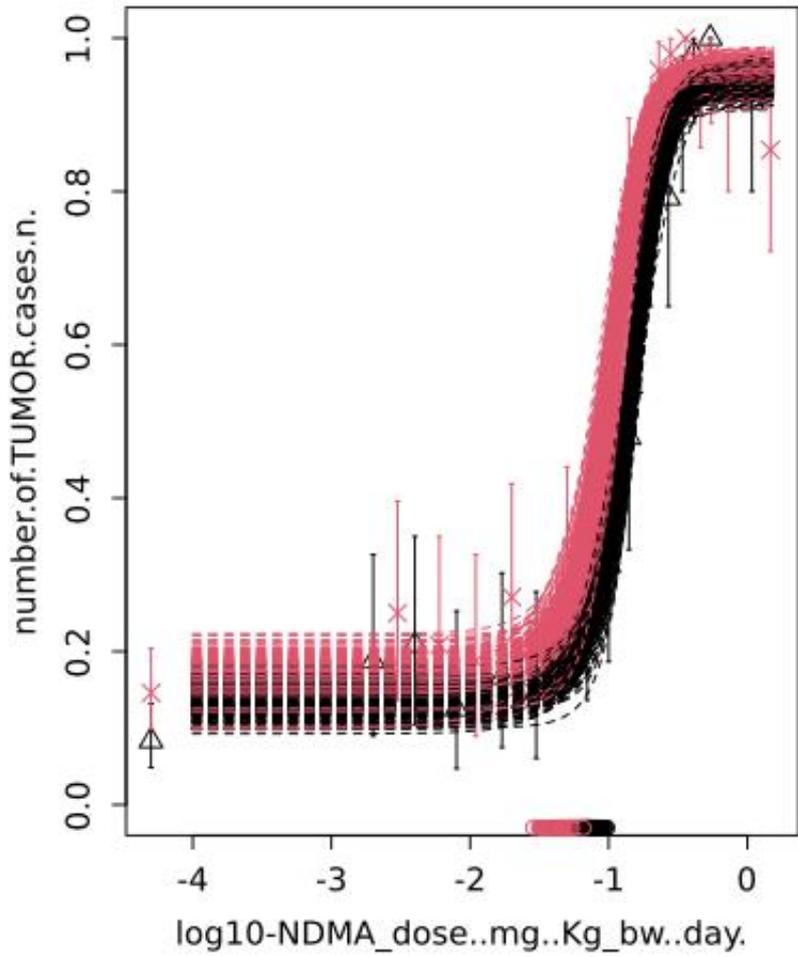
two.stage	log.logist	Weibull	log.prob	gamma	EXP	HILL
0	0	0	0	0	0.91	0.09



BMD confidence intervals based on MA



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
0.054 0.0863
0.035 0.0625

B.2. NDEA

B.2.1 Brantom 1983:

Data Description

The endpoint to be analyzed is animals with a liver tumour (malignant or non-malignant).

Data used for analysis:

NDEA dose Mg/kg bw per day	Number of tumour cases	Animals (n)	Male (1) Female (2)
0.000	15	192	1
0.002	5	48	1
0.004	4	48	1
0.008	10	48	1
0.017	3	48	1
0.030	10	48	1
0.070	19	48	1
0.100	28	48	1
0.140	19	48	1
0.170	29	48	1
0.200	26	48	1
0.270	31	48	1
0.340	28	48	1
0.410	32	48	1
0.540	28	48	1
1.080	39	48	1
0.000	27	192	2
0.003	10	48	2
0.006	9	48	2
0.011	5	48	2
0.020	15	48	2
0.050	19	48	2
0.090	37	48	2
0.140	42	48	2
0.180	42	48	2
0.230	44	48	2
0.280	45	48	2
0.360	46	48	2

0.460	38	48	2
0.550	44	48	2
0.730	46	48	2
1.470	45	48	2

B.2.1.1 Results for all doses

Final BMD Values

subgroup	BMDL	BMDU
1	0.019	0.0376
2	0.0099	0.0211

Confidence intervals for the BMD are based on 200 bootstrap data sets.

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	sens.sub gr	conv
null	2	-1219.32	2442.64		NA	NA	NA		NA
full	32	-812.23	1688.46		NA	NA	NA		NA
two.stage-ab	5	-891.98	1793.96	no	NA	NA	0.01760	2	yes
log.logist-b	4	-846.36	1700.72	no	NA	NA	0.00561	2	yes
Weibull-ab	5	-854.47	1718.94	no	NA	NA	0.00239	2	yes
log.prob-b	4	-847.47	1702.94	no	NA	NA	0.00564	2	yes
gamma-ab	5	-862.07	1734.14	no	NA	NA	0.00188	2	yes
LVM:xpon. m5-a	5	-844.08	1698.16	no	NA	NA	0.01320	2	yes
LVM: Hill m5-a	5	-842.92	1695.84	yes	0.00928	0.026	0.01630	2	yes

Attention: the AIC of the best model (minimum AIC) is more than two units larger than that of the full model. This might indicate a problem in the data, in particular when the difference is much larger than two units (e.g. > 5). You might check the following options:

1. In real-life studies, not all experimental factors are completely randomized over all animals (experimental units), e.g. animals were housed in the same cage within a given dose group, or order of treatments were not randomized over individual animals. Another option is that individual outlying animals distort the mean response of one or more treatment groups. This may lead to fluctuations in the (mean) responses among treatment groups that are larger than expected from random sampling error, resulting in an AIC difference with the full model larger than 2 units.
2. the data consist of subgroups not taken into account in the model (e.g. various studies, or two sexes)
3. the data contain litter effects not taken into account
4. the response in the top dose group deviates substantially from the fitted model (check the CI around the observed (mean) response);

Associated actions for each of these four options are:

1. the greater scatter in (mean) responses will result in a wider BMD CI; normally, no further action is needed, as the BMD approach is relatively robust to such deviations. You might check this by leaving out specific treatment groups (one by one) and check if this has a major impact on the BMD CI.
2. use the factor defining the subgroups as a covariate and re-analyse the data
3. re-analyse the data with litter effects taken into account
4. consider to leave out the top dose; it is not recommended to leave out two high dose groups.

Estimated Model Parameters

two.stage

estimate for a-1 : 0.1203

estimate for a-2 : 0.1737

estimate for BMD-1 : 0.04335

estimate for BMD-2 : 0.01759

estimate for c : 1e-06

log.logist

estimate for a- : 0.1053

estimate for BMD-1 : 0.02498

estimate for BMD-2 : 0.005606

estimate for c : 1.014

Weibull

estimate for a-1 : 0.0722

estimate for a-2 : 0.1162

estimate for BMD-1 : 0.009227

estimate for BMD-2 : 0.00239

estimate for c : 0.593

log.prob

estimate for a- : 0.1068

estimate for BMD-1 : 0.025

estimate for BMD-2 : 0.005638

estimate for c : 0.5905

gamma

estimate for a-1 : 0.06916

estimate for a-2 : 0.1203

estimate for BMD-1 : 0.00644

estimate for BMD-2 : 0.001882

estimate for c : 0.4654

EXP

estimate for a-1 : 1.489

estimate for BMD-1 : 0.02687

estimate for BMD-2 : 0.0132

estimate for c- : 0.588

estimate for d- : 1.087

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

HILL

estimate for a-1 : 1.478

estimate for BMD-1 : 0.02843

estimate for BMD-2 : 0.01633

estimate for c- : 0.5752

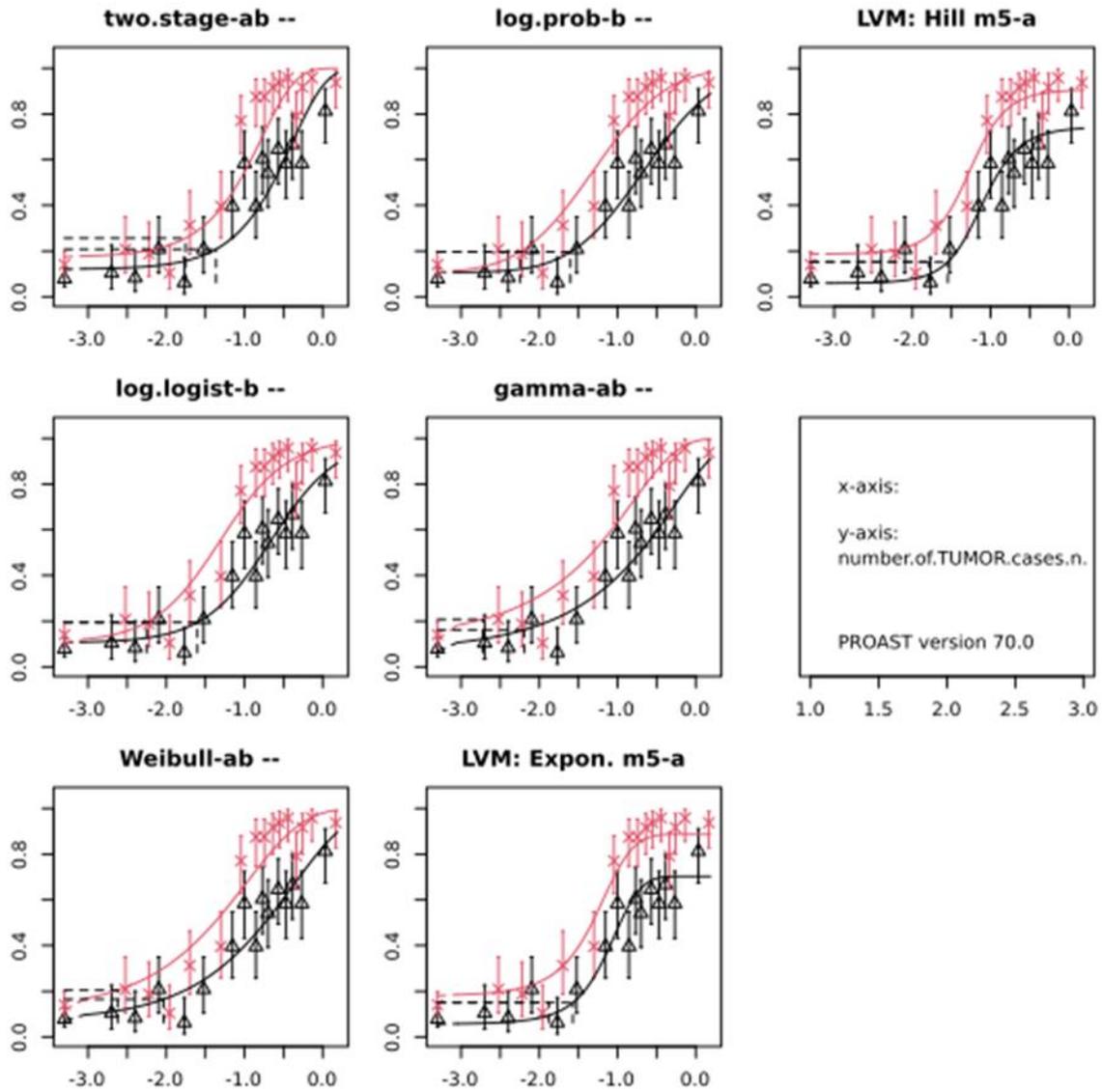
estimate for d- : 1.51

estimate for th(fixed) : 0

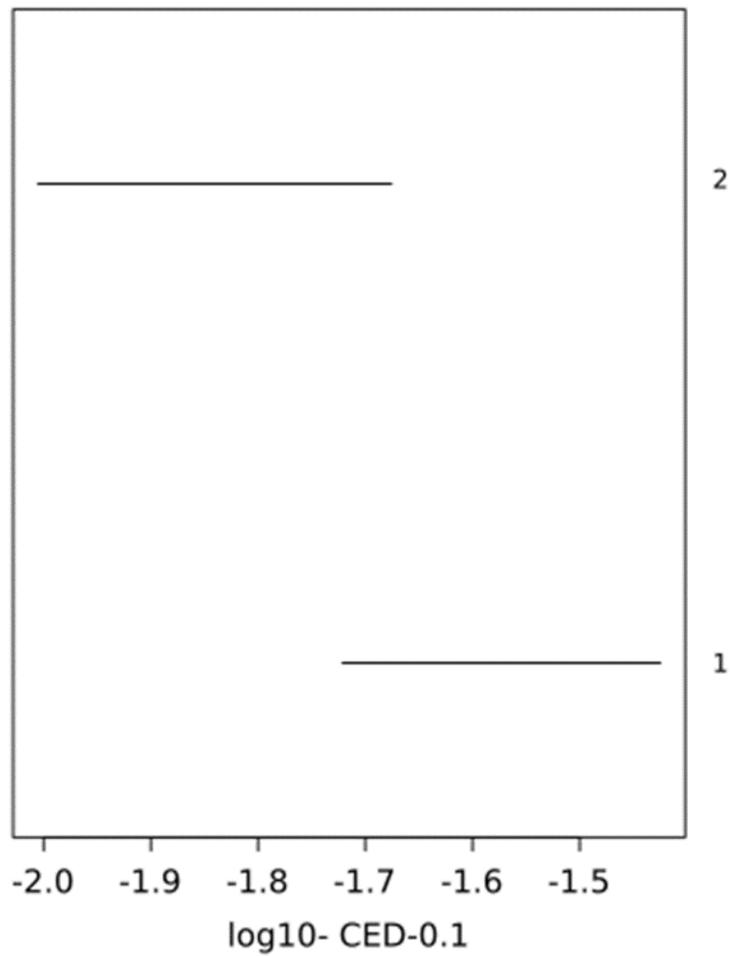
estimate for sigma(fixed) : 0.25

Weights for Model Averaging

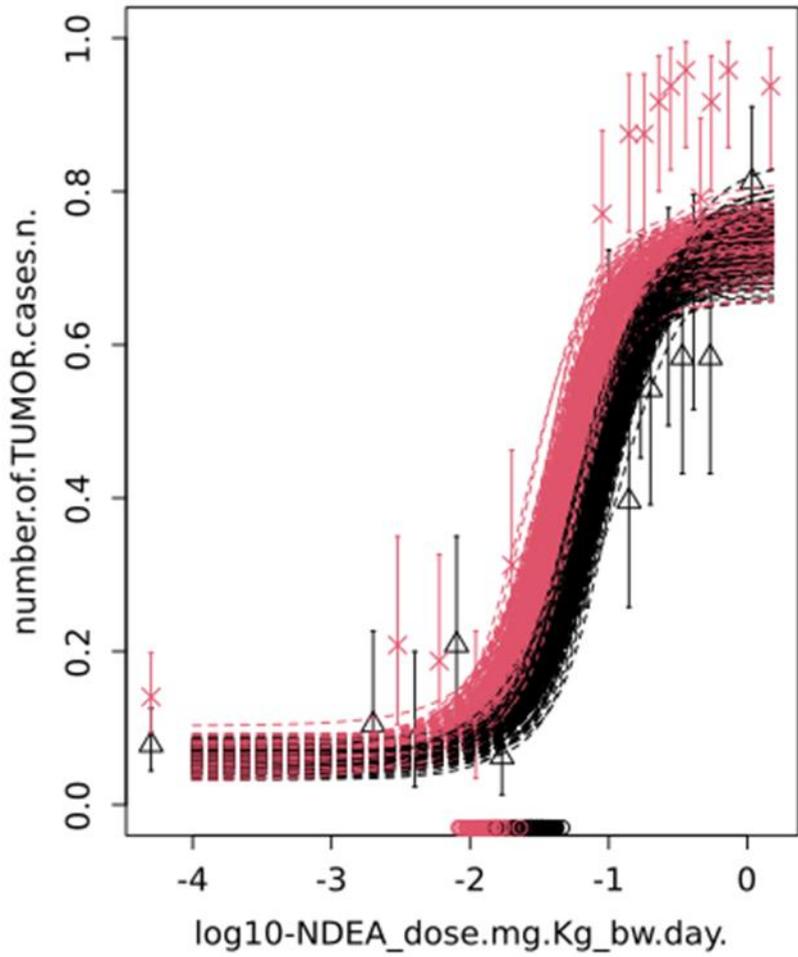
two.stage	log.logist	Weibull	log.prob	gamma	EXP	HILL
0	0.06	0	0.02	0	0.22	0.7



BMD confidence intervals based on MA



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
0.019 0.0376
0.0099 0.0211

B.2.1.2 Results for the first 5 doses

Final BMD Values

subgroup	BMDL	BMDU
1	0.012	0.0459
2	0.0086	0.034

Confidence intervals for the BMD are based on 200 bootstrap data sets.

Response variable: R

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	sens.su bgr	conv
null	2	-362.82	729.64		NA	NA	NA		NA
full	12	-346.57	717.14		NA	NA	NA		NA
two.stage-a	4	-353.12	714.24	yes	0.01290	0.0339	0.0216	--	yes
log.logist-a	4	-353.32	714.64	yes	0.00916	0.0349	0.0205	--	yes
Weibull-a	4	-353.25	714.50	yes	0.00924	0.0348	0.0204	--	yes
log.prob-a	4	-353.56	715.12	yes	0.00858	0.0353	0.0205	--	yes
gamma-a	4	-353.27	714.54	yes	0.00910	0.0344	0.0199	--	yes
LVM: Expon. m3-a	4	-352.72	713.44	yes	0.00511	0.0307	0.0156	2	yes
LVM: Hill m3-a	4	-352.72	713.44	yes	0.00511	0.0307	0.0156	2	yes

Estimated Model Parameters

two.stage

estimate for a-1 : 0.0829

estimate for a-2 : 0.1522

estimate for BMD- : 0.0216

estimate for c : 4.068

log.logist

estimate for a-1 : 0.08305

estimate for a-2 : 0.1529

estimate for BMD- : 0.02053

estimate for c : 1.281

Weibull

estimate for a-1 : 0.08233

estimate for a-2 : 0.1521

estimate for BMD- : 0.02041

estimate for c : 1.178

log.prob

estimate for a-1 : 0.08478

estimate for a-2 : 0.1547

estimate for BMD- : 0.02046

estimate for c : 0.6752

gamma

estimate for a-1 : 0.08152

estimate for a-2 : 0.1515

estimate for BMD- : 0.01987

estimate for c : 1.15

EXP

estimate for a-1 : 1.416

estimate for BMD-1 : 0.02413

estimate for BMD-2 : 0.01557

estimate for d- : 0.7691

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

HILL

estimate for a-1 : 1.416

estimate for BMD-1 : 0.02413

estimate for BMD-2 : 0.01557

estimate for d- : 0.7702

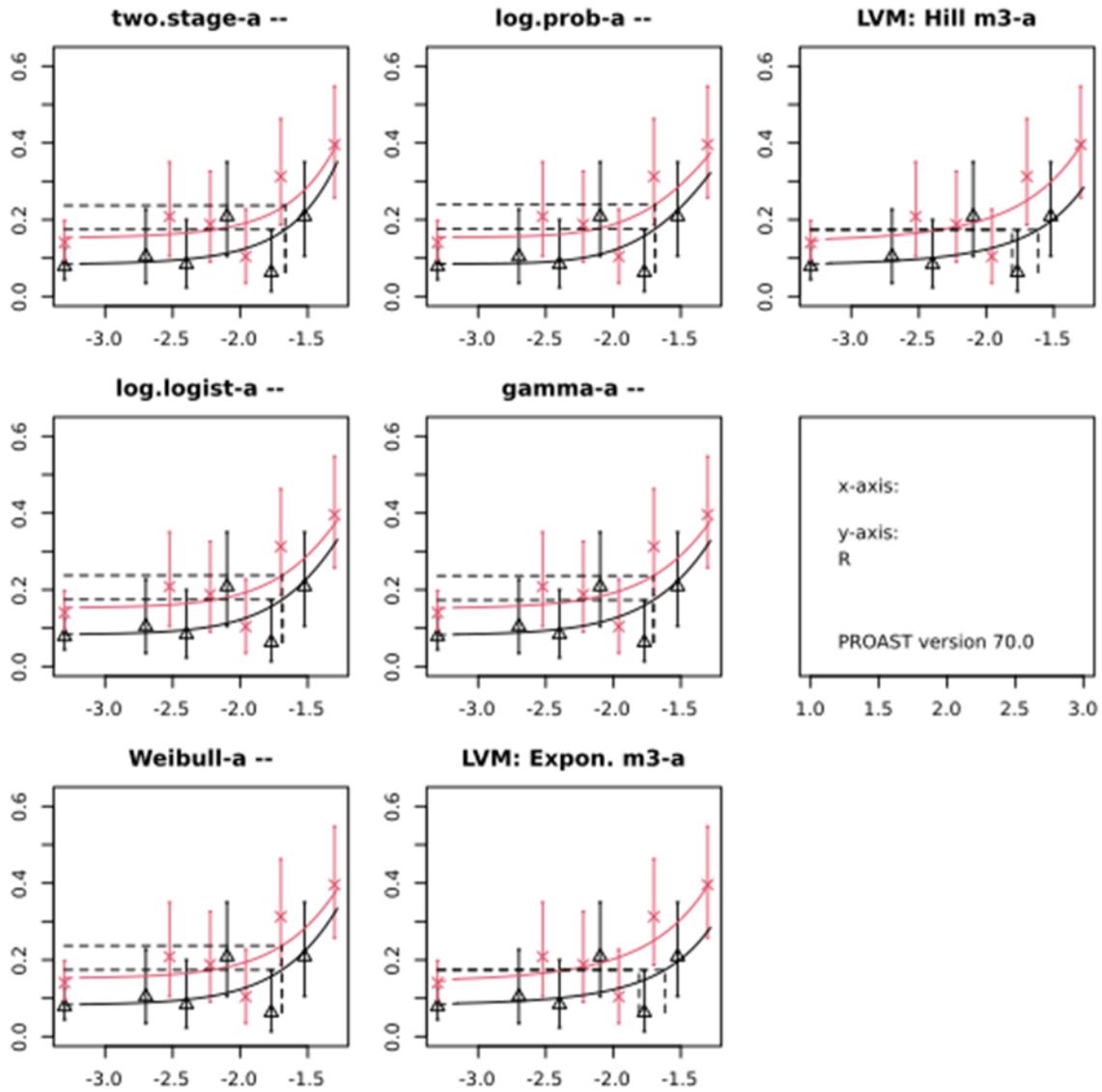
estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

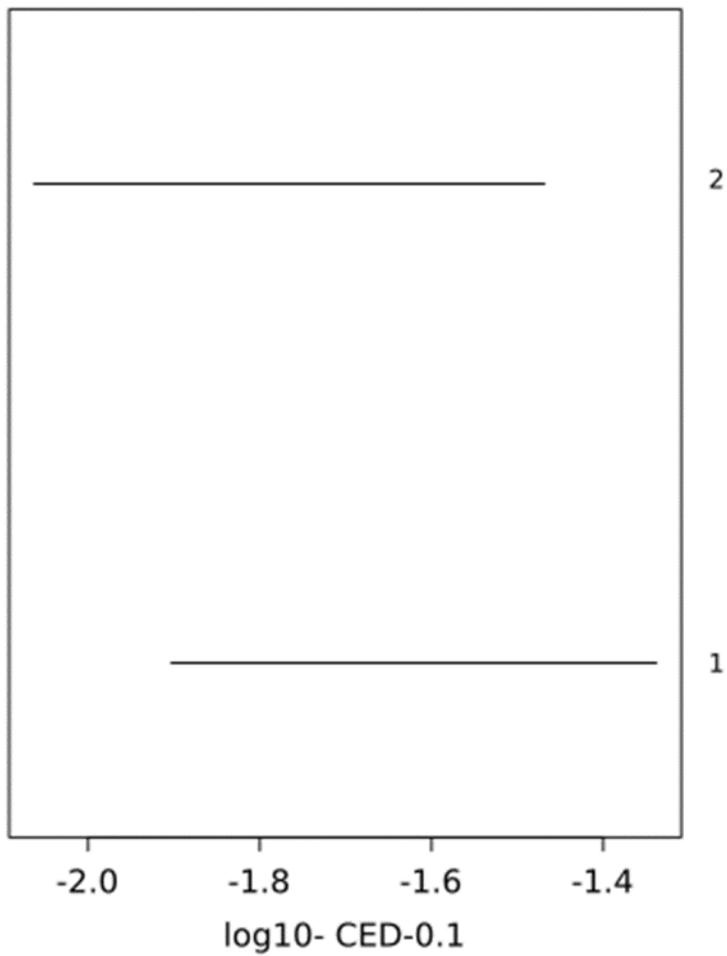
Weights for Model Averaging

two.stage	log.logist	Weibull	log.prob	gamma	EXP	HILL
0.14	0.11	0.12	0.09	0.12	0.21	0.21

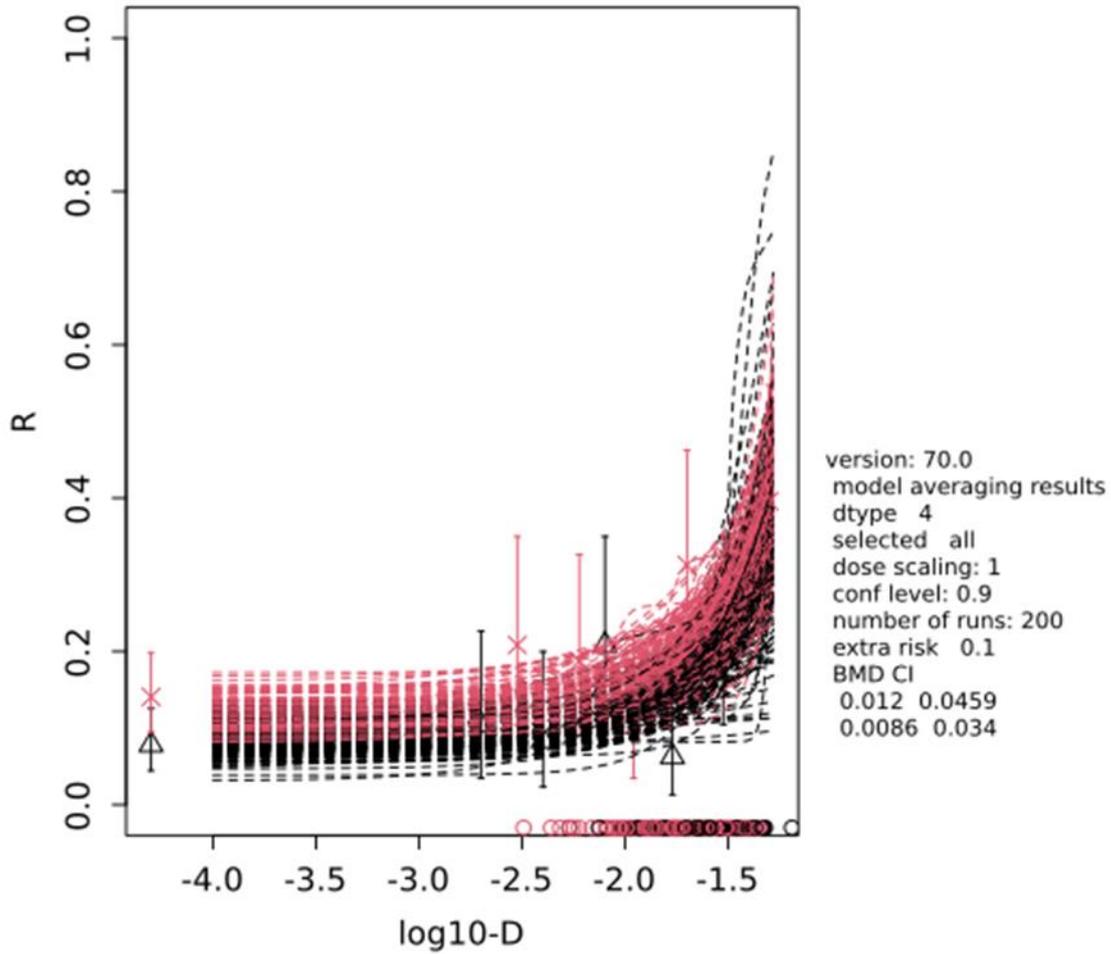
Visualization



BMD confidence intervals based on MA



bootstrap curves based on model averaging



B.2.2 Berger et al, 1987

Data Description

The endpoint to be analyzed is: any liver tumour

Data used for analysis:

NDEA dose mg/kg bw per day	Number of animals with any liver tumour	Total number of animals
0.000	3	500
0.007	2	80
0.023	3	80
0.070	36	80

B.2.2.1 Results

subgroup	BMDL	BMDU
all	0.028	0.0536

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
null	1	-166.85	335.70		NA	NA	NA	NA
full	4	-95.54	199.08		NA	NA	NA	NA
two.stage	3	-96.60	199.20	yes	0.0233	0.0349	0.0303	no
log.logist	3	-96.43	198.86	yes	0.0241	0.0468	0.0343	yes
Weibull	3	-96.37	198.74	yes	0.0241	0.0478	0.0346	yes
log.prob	3	-96.58	199.16	yes	0.0249	0.0437	0.0337	yes
gamma	3	-96.50	199.00	yes	0.0238	0.0451	0.0342	yes
LVM:Expon. m³-	3	-95.98	197.96	yes	0.0254	0.0503	0.0360	yes
LVM:Hill m³-	3	-95.98	197.96	yes	0.0253	0.0503	0.0359	yes

Estimated Model Parameters

two.stage

estimate for a- : 0.006691

estimate for BMD- : 0.03027

estimate for c : 61330000

log.logist

estimate for a- : 0.00797

estimate for BMD- : 0.03428

estimate for c : 2.757

Weibull

estimate for a- : 0.007694

estimate for BMD- : 0.03463

estimate for c : 2.436

log.prob

estimate for a- : 0.008562

estimate for BMD- : 0.03374

estimate for c : 1.566

gamma

estimate for a- : 0.008192

estimate for BMD- : 0.03425

estimate for c : 3.587

EXP

estimate for a- : 1.853

estimate for BMD- : 0.03596

estimate for d- : 0.9808

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

HILL

estimate for a- : 1.853

estimate for BMD- : 0.03594

estimate for d- : 0.9852

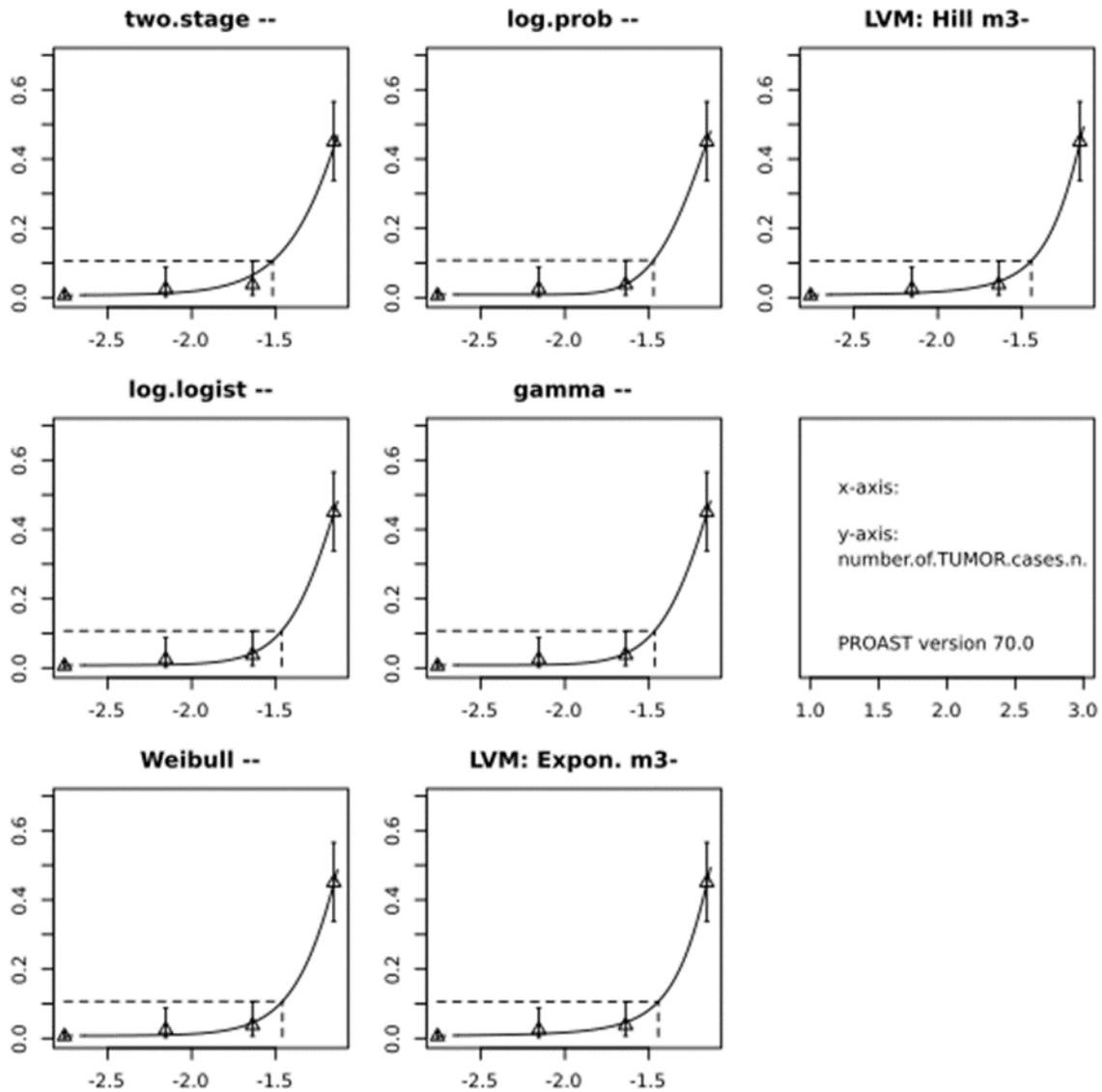
estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

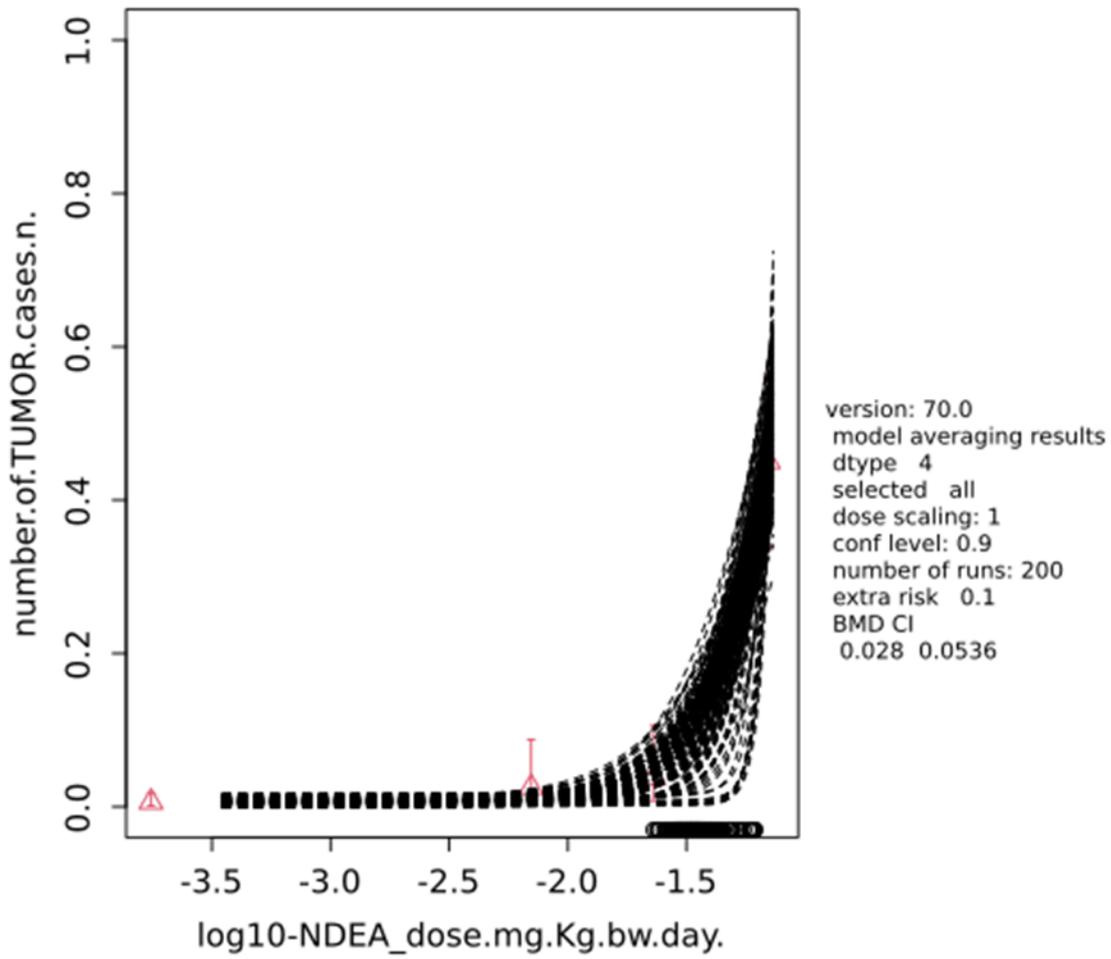
Weights for Model Averaging

two.stage	log.logist	Weibull	log.prob	gamma	EXP	HILL
0.11	0.13	0.14	0.11	0.12	0.2	0.2

Visualisation



bootstrap curves based on model averaging



B.3. NMOR

Data Description

The endpoint to be analyzed is animal with any liver tumour.

Data used for analysis:

NMOR dose mg/kg bw per day	Number of animals with any liver tumour	Total number of animals
0	1	100
0.003	6	100
0.006	5	100
0.016	7	48
0.039	9	48
0.093	22	48
0.229	23	24

Results

Final BMD Values

Subgroup	BMDL	BMDU
all	0.014	0.0376

Confidence intervals for the BMD are based on 200 bootstrap data sets.

Fitted Models

Model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
null	1	-202.62	407.24		NA	NA	NA	NA
full	7	-128.51	271.02		NA	NA	NA	NA
two.stage	3	-130.71	267.42	yes	0.0133	0.0340	0.0207	yes
log.logist	3	-133.80	273.60	no	NA	NA	0.0268	yes
Weibull	3	-131.75	269.50	no	NA	NA	0.0226	yes
log.prob	3	-134.42	274.84	no	NA	NA	0.0278	yes
gamma	3	-132.20	270.40	no	NA	NA	0.0195	yes
LVM: Expon. m3-	3	-130.48	266.96	yes	0.0119	0.0341	0.0206	yes
LVM: Hill m3-	3	-130.50	267.00	yes	0.0119	0.0342	0.0206	yes

Estimated Model Parameters

two.stage

estimate for a- : 0.02566

estimate for BMD- : 0.02067

estimate for c : 1.492

log.logist

estimate for a- : 0.04051

estimate for BMD- : 0.02678

estimate for c : 1.801

Weibull

estimate for a- : 0.0322

estimate for BMD- : 0.02256

estimate for c : 1.333

log.prob

estimate for a- : 0.04491

estimate for BMD- : 0.02778

estimate for c : 1.092

gamma

estimate for a- : 0.02798

estimate for BMD- : 0.01948

estimate for c : 1.307

EXP

estimate for a- : 1.644

estimate for BMD- : 0.02059

estimate for d- : 0.5948

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

HILL

estimate for a- : 1.643

estimate for BMD- : 0.02064

estimate for d- : 0.599

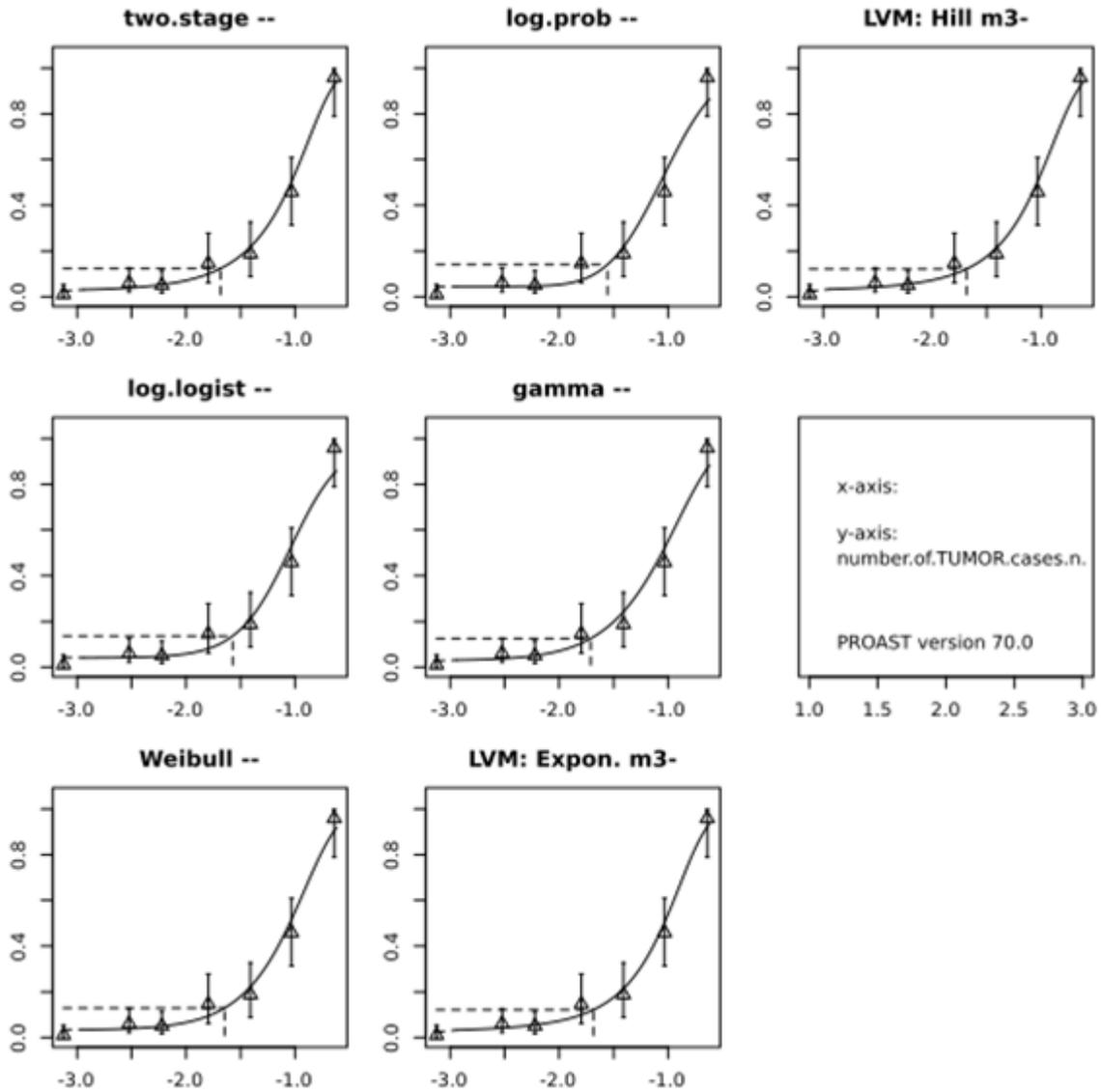
estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

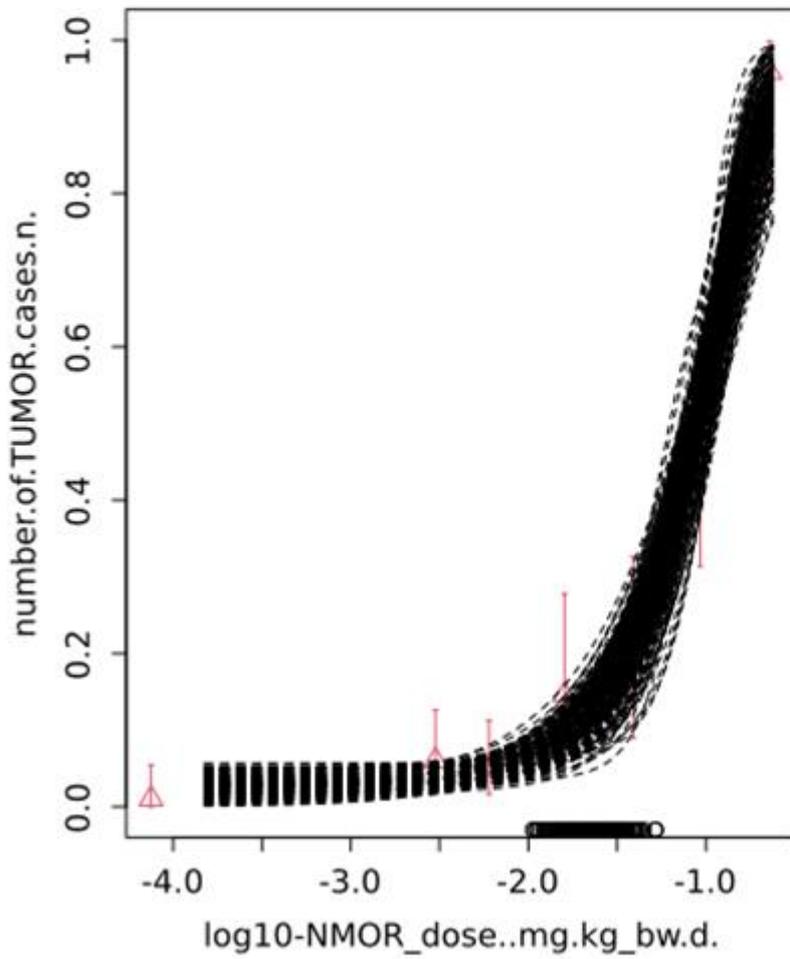
Weights for Model Averaging

two.stage	log.logist	Weibull	log.prob	gamma	EXP	HILL
0.24	0.01	0.09	0.01	0.05	0.3	0.3

Visualisation



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
0.014 0.0376

B.4. NPYR

Data Description

The endpoint to be analyzed is animal with any liver tumour.

Data used for analysis: NPYR dose mg/kg bw per day	Number of animals with any liver tumour	Total number of animals
0.000	3	500
0.029	1	80
0.095	4	80
0.290	17	80

Results

Final BMD Values:

subgroup	BMDL	BMDU
all	0.13	0.264

Confidence intervals for the BMD are based on 200 bootstrap data sets.

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
null	1	-109.27	220.54		NA	NA	NA	NA
full	4	-80.98	169.96		NA	NA	NA	NA
two.stage	3	-81.00	168.00	yes	0.120	0.228	0.177	yes
log.logist	3	-80.98	167.96	yes	0.117	0.223	0.168	yes
Weibull	3	-80.98	167.96	yes	0.120	0.225	0.170	yes
log.prob	3	-80.99	167.98	yes	0.111	0.216	0.159	yes
gamma	3	-80.98	167.96	yes	0.119	0.223	0.168	yes
LVM: Expon. m3-	3	-81.03	168.06	yes	0.125	0.235	0.181	yes
LVM: Hill m3-	3	-81.03	168.06	yes	0.125	0.235	0.181	yes

Estimated Model Parameters

two.stage

estimate for a- : 0.005901

estimate for BMD- : 0.1768

estimate for c : 27.08

log.logist

estimate for a- : 0.005975

estimate for BMD- : 0.1675

estimate for c : 1.568

Weibull

estimate for a- : 0.005957

estimate for BMD- : 0.1702

estimate for c : 1.493

log.prob

estimate for a- : 0.006117

estimate for BMD- : 0.1593

estimate for c : 0.7714

gamma

estimate for a- : 0.005977

estimate for BMD- : 0.1681

estimate for c : 1.608

EXP

estimate for a- : 1.878

estimate for BMD- : 0.1806

estimate for d- : 0.6546

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

HILL

estimate for a- : 1.878

estimate for BMD- : 0.1805

estimate for d- : 0.6568

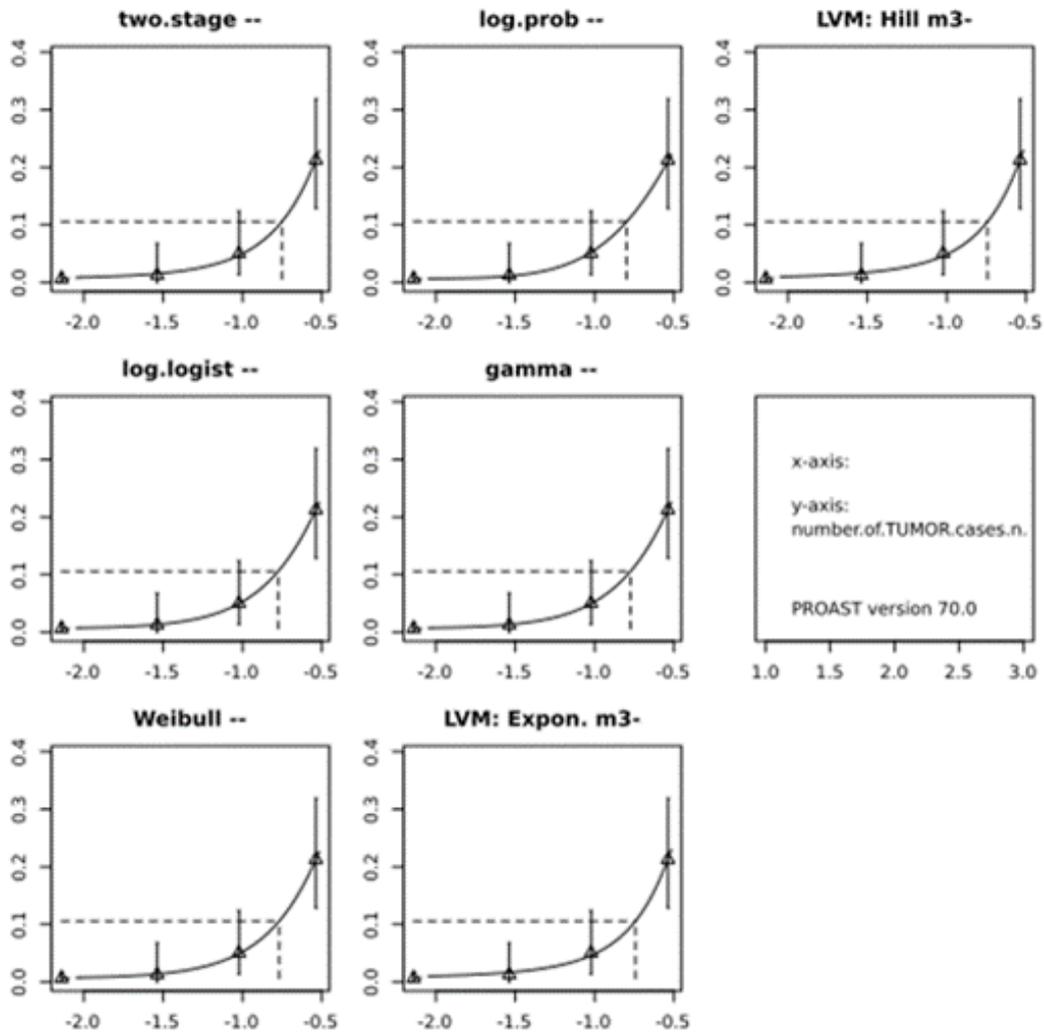
estimate for th(fixed) : 0

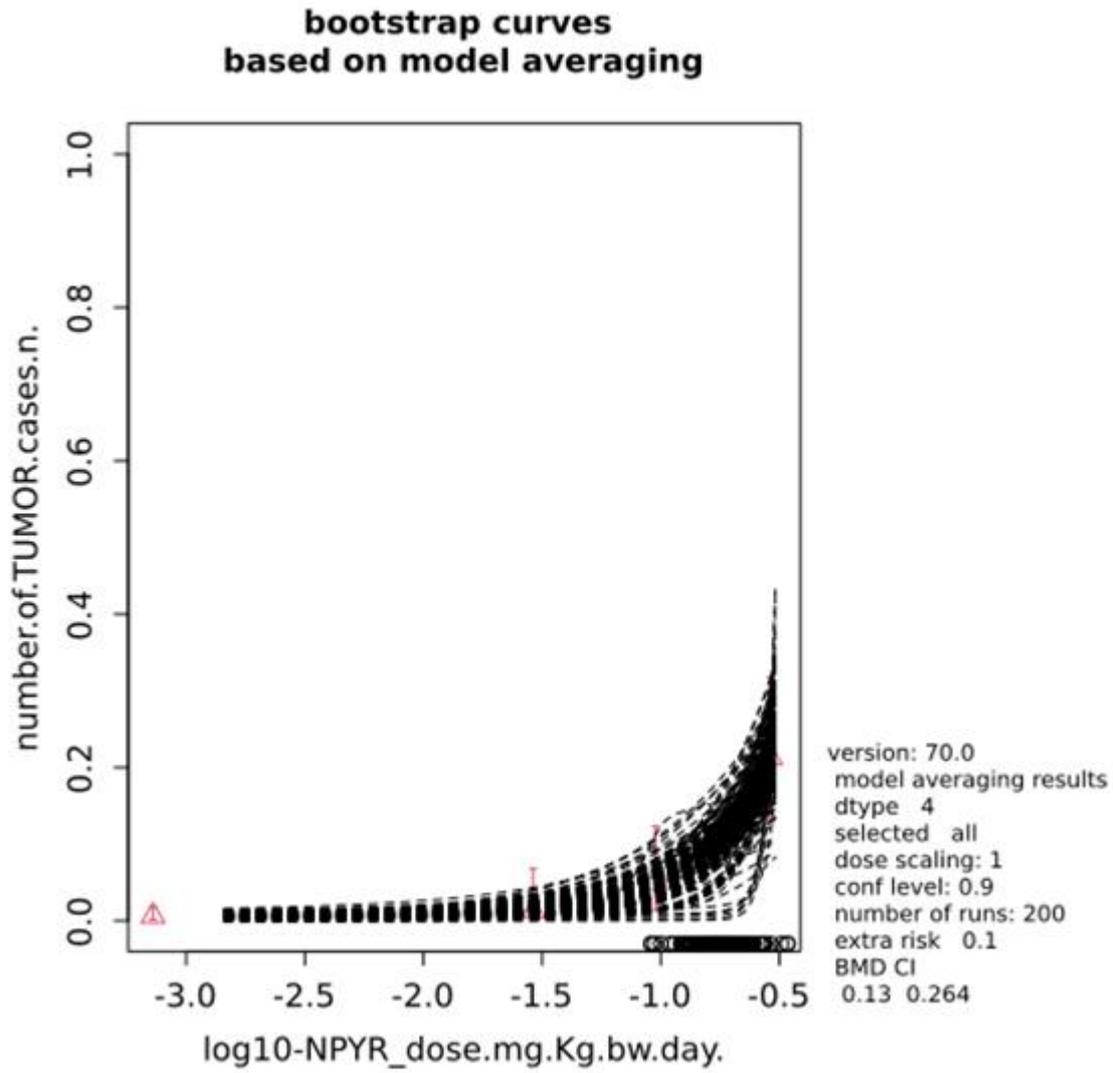
estimate for sigma(fixed) : 0.25

Weights for Model Averaging

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

Visualization





B.5. NPIP

A.1.1 Data Description

The endpoint to be analyzed is animal with any liver tumour.

Data used for analysis:

NPIP dose mg/kg bw per day	Number of animals with any liver tumour	Total number of animals
0	0	40
0.02	3	78
0.09	6	75
0.4	16	34
2.1	11	34

Results

Final BMD Values

subgroup	BMDL	BMDU
all	0.062	0.213

Confidence intervals for the BMD are based on 200 bootstrap data sets.

Response variable: number.of.TUMOR.cases.n.

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
null	1	-104.71	211.42		NA	NA	NA	NA
full	5	-78.53	167.06		NA	NA	NA	NA
two.stage	3	-91.99	189.98	no	NA	NA	0.3280	yes
log.logist	3	-84.70	175.40	no	NA	NA	0.0682	yes
Weibull	3	-85.19	176.38	no	NA	NA	0.0663	yes
log.prob	3	-84.25	174.50	no	NA	NA	0.0689	yes
gamma	3	-85.56	177.12	no	NA	NA	0.0664	yes
LVM: Expon. m5-	4	-80.46	168.92	yes	0.0604	0.206	0.1110	yes
LVM: Hill m5-	4	-80.65	169.30	yes	0.0768	0.172	0.1060	yes

Estimated Model Parameters

two.stage

estimate for a- : 0.04576

estimate for BMD- : 0.328

estimate for c : 1e-06

log.logist

estimate for a- : 1e-06

estimate for BMD- : 0.0682

estimate for c : 0.5809

Weibull

estimate for a- : 1e-06

estimate for BMD- : 0.06632

estimate for c : 0.4927

log.prob

estimate for a- : 1e-06

estimate for BMD- : 0.06886

estimate for c : 0.3358

gamma

estimate for a- : 1e-06

estimate for BMD- : 0.06643

estimate for c : 0.4366

EXP

estimate for a- : 1.661

estimate for BMD- : 0.1112

estimate for c- : 0.6427

estimate for d- : 1.663

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

HILL

estimate for a- : 1.635

estimate for BMD- : 0.1064

estimate for c- : 0.6517

estimate for d- : 3.333

estimate for th(fixed) : 0

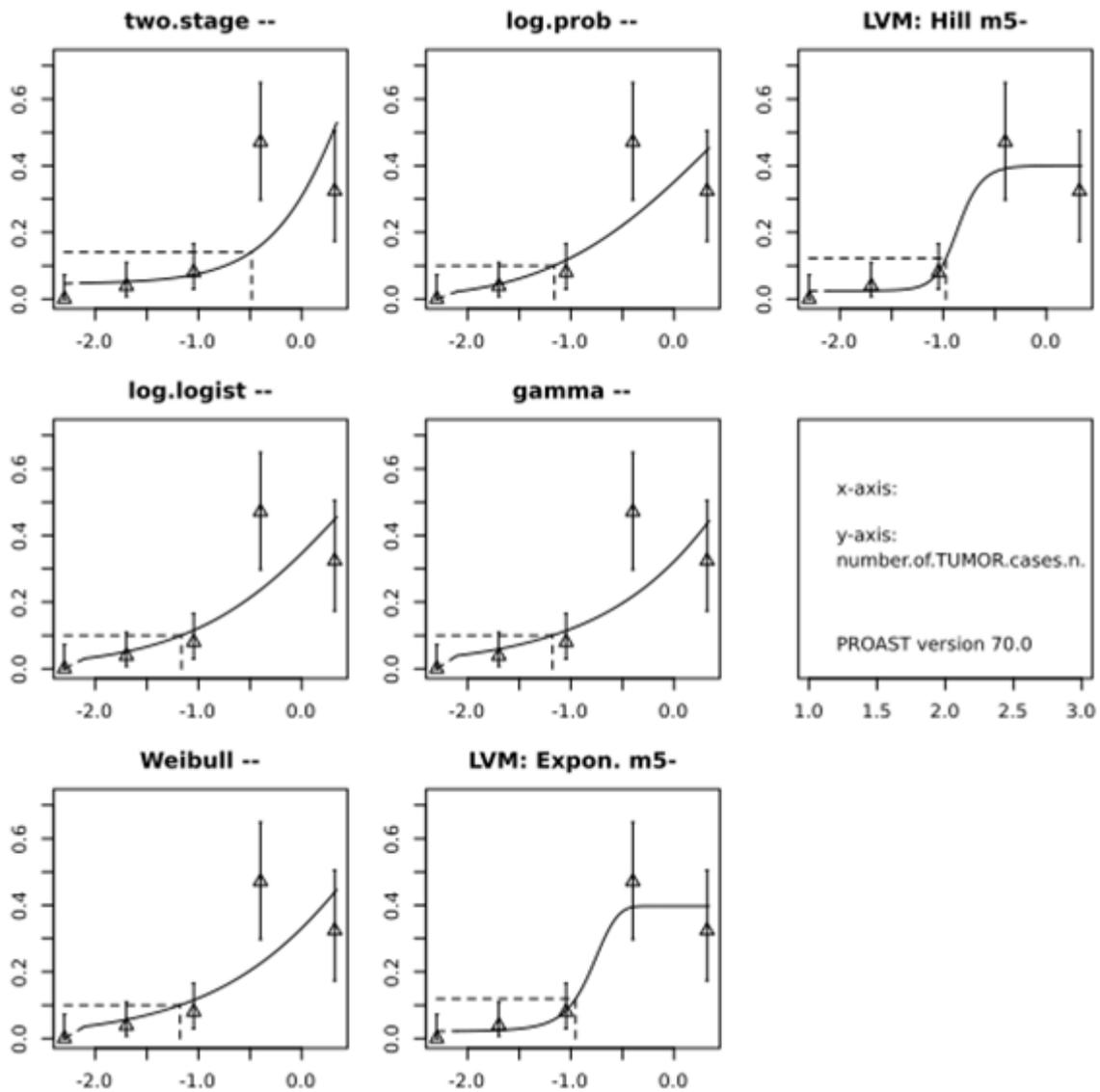
estimate for sigma(fixed) : 0.25

Weights for Model Averaging

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

0 0.02 0.01 0.03 0.01 0.51 0.42

Visualization



bootstrap curves based on model averaging

