

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS
IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT**

Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity

Benchmark dose modelling of riddelliine and lasiocarpine

This Annex provides details of the approach used and results obtained from the benchmark dose modelling using the data from the National Toxicology Program studies on riddelliine and lasiocarpine.

**Secretariat
July 2008**

Approach used in selecting BMD models for PA risk assessment

The US EPA BMD software, version 1.4.1 was used for modelling the dose response for liver hemangiosarcoma incidence in rats and mice in two 2-year studies, one with ridelline and one with lasiocarpine.

In the study with ridelline, multiple doses were only administered to female rats and male mice. The study with lasiocarpine was conducted with male and female rats. The dietary administration levels described in the lasiocarpine report were converted into the equivalent mg/kg b.w./day level using the approximation advised by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in their guidelines for preparing working papers.

The following dose-response models for carcinogenicity data were fitted:

- Gamma multihit model
- Log-logistic model
- Multi-stage model
- Log-probit model
- Quantal linear model
- Weibull model

The BMD10 and BMDL10 values for a 10% increase in incidence of haemangiosarcomas compared with the background incidence in controls were estimated by performing 250 iterations.

The acceptability of a model can be based on several criteria. Some models are 'nested' related models, such that by leaving out a parameter one model reduces to the other. The fit for such models should not be significantly worse than the fit provided by the 'full' model, using the likelihood ratio test. The full model is a model that does not assume any dose-response function (its parameters are simply the frequencies per dose level (Filipsson *et al.*, 2003).

For example, when the full model has 8 parameters a fitted dose-response model with 3 parameters should result in a log-likelihood no more than 5.54 lower than the log-likelihood associated with the full model. The table below shows the critical differences in log-likelihood values for various differences in number of parameters between the models to be compared, (i.e. various numbers of degrees of freedom).

Number of degrees of freedom	Critical difference in log-likelihood
1	1.92
2	2.00
3	3.91
4	4.74
5	5.54
6	6.30
7	7.03
8	7.75

Table showing critical values of log-likelihoods making an increase by a number of parameters (= number of degrees of freedom) to result in a significantly better fit.

While the likelihood ratio test can be applied only to nested models, the Akaike Information Criterion (AIC) has been proposed as an approximate criterion for comparing the fitness of non-nested models.

For the current assessment the approach adopted by JECFA in its recent evaluation of Ochratoxin A (2007) and by EFSA in its assessment of PAHs (2008) was used. This approach involves assessment of statistics on the goodness of fit calculated by the BMD software. The lower the chi-square value the better the fit, and the calculated *P*-value should be greater than 0.1. This *P*-value was therefore applied as a rejection level.

For the multi-stage model the degree of the polynomial was adjusted sequentially in order to determine what degree provided the better fit. The model with the highest *P*-value and lowest AIC value [Akaike information criterion; an alternative measure proposed for comparing the fit of different models] was selected.

References

EFSA (2008). Draft opinion of the Scientific Panel on Contaminants in the Food Chain on polycyclic aromatic hydrocarbons (PAHs) in Food. *In Press*

Filipsson AF, Sand S, Nilsson J & Victorin K. (2003). The benchmark dose method--review of available models, and recommendations for application in health risk assessment. *Critical Reviews in Toxicology* 33: 505-542.

JECFA (2007). Evaluation of Ochratoxin A. *WHO Food Additive Series* 59: 357-429.

http://whqlibdoc.who.int/publications/2008/9789241660594_eng.pdf

JECFA (2001). Guidelines for the preparation of working papers for the Joint FAO/WHO Expert Committee on Food Additives. pp. 18-19.

http://www.who.int/ipcs/food/jecfa/en/contaminant_guidelines.pdf

BMD modelling for lasiocarpine

BMD10 and BMDL10 calculations based on incidence of liver hemangiosarcoma in male rats administered lasiocarpine in the diet (NCI-CG-TR-39)

Model	Log likelihood (parameters)	p-value	AIC	Chi-square	P-value	Accept	BMD ₁₀ (mg/kg bw per day)	BMDL ₁₀ (mg/kg bw per day)
Full model	-43.95 (4)							
Gamma	-44.50 (1)	0.77	91.0	1.14	0.77	Yes	0.158	0.117
Logistic	-48.17 (2)	0.01	100.3	6.30	0.04	No	0.393	0.302
Log - Logistic	-44.25 (2)	0.74	92.5	0.61	0.74	Yes	0.134	0.078
Multistage	-44.50 (1)	0.77	91.0	1.14	0.77	Yes	0.158	0.117
Quantal-linear	-44.50 (1)	0.77	91.0	1.14	0.77	Yes	0.158	0.117
Probit	-47.86 (2)	0.02	99.7	5.97	0.05	No	0.370	0.288
Log-Probit	-45.00 (2)	0.55	92.0	2.21	0.53	Yes	0.260	0.200
Weibull	-44.50 (1)	0.77	91.0	1.14	0.77	Yes	0.158	0.117
Reduced model	-57.71 (1)	<0.001						

Range of BMD₁₀s in accepted models: 0.134-0.260 mg/kg bw/day
Range of BMDL₁₀s in accepted models: 0.078-0.200 mg/kg bw/day

BMD10 and BMDL10 calculations based on incidence of liver hemangiosarcoma in female rats administered lasiocarpine in the diet (NCI-CG-TR-39)

[NB: data entered for highest dose restricted to those animals that survived beyond 52 weeks]

Model	Log likelihood (parameters)	p-value	AIC	Chi-square	P-value	Accept	BMD ₁₀ (mg/kg bw per day)	BMDL ₁₀ (mg/kg bw per day)
Full model	-33.68 (4)							
Gamma	-38.12 (1)	0.03	78.24	10.04	0.02	No	0.206	0.142
Logistic	-40.00 (2)	0.002	84.00	9.82	0.01	No	0.658	0.393
Log - Logistic	-37.07(1)	0.08	76.15	7.24	0.07	No	0.162	0.100
Multistage	-38.12 (1)	0.03	78.24	10.04	0.02	No	0.206	0.142
Quantal-linear	-38.12(1)	0.03	78.24	10.04	0.02	No	0.206	0.142
Probit	-39.91 (2)	0.002	83.82	9.83	0.01	No	0.607	0.367
Log-Probit	-40.70 (2)	<0.001	85.40	12.40	0.002	No	0.523	0.220
Weibull	-38.12 (1)	0.03	78.24	10.04	0.02	No	0.206	0.142
Reduced model	-41.14 (1)	0.002						

None of the models are accepted.

BMD modelling for ridelliine

BMD10 and BMDL10 calculations based on incidence of liver hemangiosarcoma in female rats administered ridelliine by oral gavage (NTP TR 508)

Model	Log likelihood (parameters)	p-value	AIC	Chi-square	P-value	Accept	BMD ₁₀ (mg/kg bw per day)	BMDL ₁₀ (mg/kg bw per day)
Full model	-38.90 (6)							
Gamma	-38.92 (2)	0.99	81.8	0.02	0.99	Yes	0.388	0.301
Logistic	-40.32 (2)	0.59	84.6	2.11	0.72	Yes	0.508	0.418
Log - Logistic	-38.95 (2)	0.99	81.9	0.05	0.99	Yes	0.389	0.303
Multistage	-39.02 (2)	0.99	82.0	0.15	0.99	Yes	0.414	0.307
Quantal-linear	-54.78 (1)	N/A	111.6	23.1	0.0003	No	0.133	0.103
Probit	-39.64 (2)	0.83	83.3	1.02	0.91	Yes	0.458	0.378
Log-Probit	-38.90 (2)	1	81.8	0.00	1.0	Yes	0.377	0.301
Weibull	-39.00 (2)	0.99	82.0	0.11	0.99	Yes	0.406	0.305
Reduced model	-119.66 (1)	<0.001						

Range of BMD₁₀s in accepted models: 0.377-0.508 mg/kg bw/day

Range of BMDL₁₀s in accepted models: 0.301-0.418 mg/kg bw/day

BMD10 and BMDL10 calculations based on incidence of liver hemangiosarcoma in male mice administered ridelliine by oral gavage (NTP TR 508)

Model	Log likelihood (parameters)	p-value	AIC	Chi-square	P-value	Accept	BMD ₁₀ (mg/kg bw per day)	BMDL ₁₀ (mg/kg bw per day)
Full model	-54.90 (5)							
Gamma	-56.31 (3)	0.24	118.6	2.04	0.36	Yes	1.498	1.090
Logistic	-57.13 (2)	0.22	118.3	4.44	0.22	Yes	1.419	1.175
Log - Logistic	-56.32 (3)	0.24	118.6	2.04	0.36	Yes	1.539	1.093
Multistage	-56.44 (2)	0.38	116.9	2.40	0.49	Yes	1.450	1.116
Quantal-linear	-67.36 (2)	<0.001	138.72	19.4	0.0002	No	0.541	0.407
Probit	-57.73 (2)	0.13	119.5	6.05	0.11	Yes	1.252	1.040
Log-Probit	-56.31 (3)	0.25	118.6	2.04	0.36	Yes	1.434	1.062
Weibull	-56.32 (3)	0.24	118.6	2.04	0.36	Yes	1.615	1.122
Reduced model	-103.04 (1)	<0.001						

Range of BMD₁₀s in accepted models: 1.252-1.615 mg/kg bw/day

Range of BMDL₁₀s in accepted models: 1.040-1.175 mg/kg bw/day