CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name: N,N-Dimethylacetamide

EC Number: 204-826-4

CAS Number: 127-19-5

Index Number: 616-011-00-4

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***N,N-Dimethylacetamide***
EC number:	***204-826-4***
CAS number:	***127-19-5***
Annex VI Index number:	***616-011-00-4***
Degree of purity:	***99 – 100 % (according to the information received in the registration dossiers)***
Impurities:	***None reported***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Repr. 1B H360D*** C≥ 5% Acute Tox.4* H332 Acute Tox.4* H312
Current proposal for consideration by RAC	Repr. 1B; H360D; (removal of the SCL)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 1B H360D*** Acute Tox.4* H332 Acute Tox.4* H312

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation (only relevant part shown)

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification
3.7.	Reproductive toxicity	Repr. 1B; H360D	none	Repr. 1B; H360D; SCL: ≥ 5.0%

Labelling:

Signal word: Danger

Hazard statements: H360D: May damage the unborn child
H312: Harmful in contact with skin
H332: Harmful if inhaled

Pictograms: GHS07 and GHS08

Precautionary statements: Not relevant

Proposed notes assigned to an entry:

: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

DMAC is currently classified for developmental toxicity as Repr. 1B; H360D with an SCL of 5%. The justification of this classification for developmental toxicity and setting of the current SCL for DMAC can be found in the Annexes 1 and 2 of this report.

2.2 Short summary of the scientific justification for the CLH proposal

DMAC is a CLP 1B reproductive toxicant for which the Member State Committee has agreed on identification as Substance of Very High Concern. The agreement and the support document of the Member State Committee is available here: http://echa.europa.eu/identification-of-svhc/-/substance/768/search/127-19-5/term. ECHA has prepared a 'Background document for N,N-Dimethylacetamide (DMAC)' in the context of ECHA's fourth Recommendation for the inclusion of substances in Annex XIV (authorisation). In view of authorisation it is warranted to explore whether the currently allocated 5% according to CLP is appropriate.

According to the criteria in the 'Guidance on the Application of the CLP Criteria' (as described in tables 3.7.2.5.4 and 3.7.2.5.5 of this guidance) the current SCL of 5% for developmental toxicity of DMAC should be withdrawn. According to the CLP Regulation the GCL for a Category 1B reproductive toxicant is 0.3 %.

The removal of the current SCL is warranted because of the potency of DMAC as a reproductive toxicant, as demonstrated by its ED_{10} for the relevant reproductive effects. Analysis of the oral reproductive studies showed multiple ED_{10} levels for effects fulfilling the classification criteria for developmental toxicity with values between 4 and 400 mg/kg bw/day. These values correspond to a medium potency group (i.e. boundaries: 4 mg/kg bw/day < ED_{10} value < 400 mg/kg bw/day) for DMAC (no modifying factors affecting the preliminary potency). In combination with the already established category 1 classification for reproductive toxicity (Repr. 1B; H360D), this provides a basis for removing the SCL of 5% for DMAC. According to the CLP Regulation the GCL for DMAC as a Category 1B reproductive toxicant is 0.3 %.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Acute Tox. 4 * H312

Acute Tox 4* H332

Repr. 1B; H360D: C ≥ 5%

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The table below provides an overview of the self-classification of the registrants concerning reproductive toxicity (ECHA C&L inventory as accessed on February 6th, 2013).

Table 4: Overview of the self-classification for reproductive toxicity of DMAC by the registrants.

Classification category for reproductive toxicity	**SCL**	**Total number of notifiers**	**% of notifiers**
Repr. 1B	C ≥5%*	673	78.3
Repr. 1B	-	156	18.1
Repr. 1A	-	30	3.6

* According to the C&L inventory of ECHA, this SCL of this self-classification concerns classification for Repr. 1B.

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Not needed.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

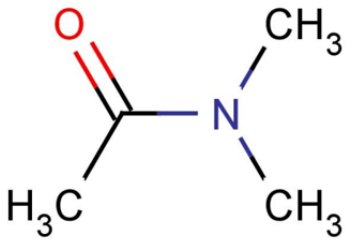
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	204-826-4
EC name:	N,N-Dimethylacetamide (DMAC)
CAS number (EC inventory):	127-19-5
CAS number:	127-19-5
CAS name:	Acetamide, N,N-dimethyl-
IUPAC name:	N,N-Dimethylacetamide
CLP Annex VI Index number:	616-011-00-4
Molecular formula:	C_4 H_9 N O
Molecular weight range:	87.1 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
N,N-Dimethylacetamide		99 – 100 %	According to the information received in the registration dossiers

Current Annex VI entry: not applicable

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Information not relevant			Data from the registration dossiers are provided as confidential information in the IUCLID file.

Current Annex VI entry: not applicable

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
none				According to the information received in the registration dossiers

Current Annex VI entry: not applicable

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	liquid	ECHA REACH Registration dossier
Melting/freezing point	-20 °C	ECHA REACH Registration dossier
Boiling point	166 °C at 1013.25 hPa	ECHA REACH Registration dossier
Relative density	0.94 at 20 °C	ECHA REACH Registration dossier
Vapour pressure	100 Pa at 28 °C	Ref: Lide (2007-2008)
Surface tension	Not needed (data waiving)	ECHA REACH Registration dossier
Water solubility	> 474.7 g/l at 25 °C	ECHA REACH Registration dossier
Partition coefficient n-octanol/water (logP)	-0.77 at 25 °C	Lide (2007-2008)
Flash point	64 °C at 1013.25 hPa (closed cup)	ECHA REACH Registration dossier
Flammability	study scientifically unjustified (data waiving)	ECHA REACH Registration dossier
Explosive properties	Not needed (data waiving)	ECHA REACH Registration dossier
Self-ignition temperature	345 °C at 999 1011 hPa	ECHA REACH Registration dossier
Oxidising properties	no oxidising properties (expert judgement)	ECHA REACH Registration dossier
Granulometry	Not relevant	ECHA REACH Registration dossier
Stability in organic solvents and identity of relevant degradation products	Not relevant (data waiving)	ECHA REACH Registration dossier
Dissociation constant	pKa -0.19 at 25 °C	ECHA REACH Registration dossier
Viscosity	0.92 mPa s (dynamic) at 25 °C	ECHA REACH Registration dossier

2 MANUFACTURE AND USES

2.1 Manufacture

10,000 - 100,000 tonnes per annum (ECHA public registration data, May 2013)

2.2 Identified uses

Summary of available data:

DMAC is used (summary of information provided by the registrants and further data received during the public consultations):

- As a combined solvent and reaction catalyst in the production of agrochemicals, pharmaceuticals and fine chemicals (65-70% of the tonnage);
- As a solvent in the production of man-made fibres (20-25%)
- As a solvent in coatings for industrial use (3-5%)
- As a solvent for polyimide resins used in film production, idem for production of filters and membranes in the medical device industry (dialysis treatment) (<2%)
- In the formulation of paint stripper products by producers of cleaning products for the industrial sector (<1%)
- Other applications (<2.5%)

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not applicable

3.1 *[Insert hazard class when relevant and repeat section if needed]*

3.1.1 Summary and discussion of

3.1.2 Comparison with criteria

3.1.3 Conclusions on classification and labelling

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

4.1.2 Human information

4.1.3 Summary and discussion on toxicokinetics

Kennedy (2012) provides a review of the available data on toxicokinetics. No human data are available. Thus based on available data no differences in toxicokinetics in humans versus animals can be identified.

4.2 Acute toxicity

Not applicable

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

4.2.1.2 Acute toxicity: inhalation

4.2.1.3 Acute toxicity: dermal

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

4.2.3 Summary and discussion of acute toxicity

4.2.4 Comparison with criteria

4.2.5 Conclusions on classification and labelling

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not applicable

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

4.3.2 Comparison with criteria

4.3.3 Conclusions on classification and labelling

4.4 Irritation

Not applicable

4.4.1 Skin irritation

4.4.1.1 Non-human information

4.4.1.2 Human information

4.4.1.3 Summary and discussion of skin irritation

4.4.1.4 Comparison with criteria

4.4.1.5 Conclusions on classification and labelling

4.4.2 Eye irritation

4.4.2.1 Non-human information

4.4.2.2 Human information

4.4.2.3 Summary and discussion of eye irritation

4.4.2.4 Comparison with criteria

4.4.2.5 Conclusions on classification and labelling

4.4.3 Respiratory tract irritation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.4.3.1 Non-human information

4.4.3.2 Human information

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

4.5 Corrosivity

Not applicable

4.5.1 Non-human information

4.5.2 Human information

4.5.3 Summary and discussion of corrosivity

4.5.4 Comparison with criteria

4.5.5 Conclusions on classification and labelling

4.6 Sensitisation

Not applicable

4.6.1 Skin sensititsation

4.6.1.1 Non-human information

4.6.1.2 Human information

4.6.1.3 Summary and discussion of skin sensitisation

4.6.1.4 Comparison with criteria

4.6.1.5 Conclusions on classification and labelling

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

4.6.2.2 Human information

4.6.2.3 Summary and discussion of respiratory sensitisation

4.6.2.4 Comparison with criteria

4.6.2.5 Conclusions on classification and labelling

4.7 Repeated dose toxicity

Not applicable

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

4.7.1.2 Repeated dose toxicity: inhalation

4.7.1.3 Repeated dose toxicity: dermal

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not applicable

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

4.9 Germ cell mutagenicity (Mutagenicity)

Not applicable

4.9.1 Non-human information

4.9.1.1 In vitro data

4.9.1.2 In vivo data

4.9.2 Human information

4.9.3 Other relevant information

4.9.4 Summary and discussion of mutagenicity

4.9.5 Comparison with criteria

4.9.6 Conclusions on classification and labelling

4.10 Carcinogenicity

Not applicable

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

4.10.1.2 Carcinogenicity: inhalation

4.10.1.3 Carcinogenicity: dermal

4.10.2 Human information

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

4.10.5 Comparison with criteria

4.10.6 Conclusions on classification and labelling

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Inhalation studies

Rat (one generation study, equivalent to OECD GL 415)
Groups of 10 male and 20 female Sprague-Dawley rats were exposed to DMAC vapour at concentrations of 0, 31, 101 or 291 ppm for 6 hours/day, 5 days per week over a period of 10 weeks (corresponding to 28, 90 or 259 mg/kg bw/day).[1] At that point mating was done and subsequently the treatment continued 7 days/week for 7-8 weeks up to weaning of the offspring. No clinical signs were seen. No effect on body weights. No adverse effects occurred on mating, fertility, gestation, parturition, litter size, number of pups and survival of pups. The only effect was reduced pup weight and increased liver weight in pups at the highest concentration. In parent animals liver weights were increased at 101 and 291 ppm (Ferenz and Kennedy 1986).

[1] Calculated route-to-route based on the defaults as given in the REACH guidance R.8 (Table R.8-17). Mean body weight rat 0.43 kg, inhalation volume 18.1 litres/hour. Calculation based on equal absorption of DMAC via inhalation and orally.

Rat, male fertility study (one generation study, equivalent to OECD GL 415)
Groups of 12 male Sprague-Dawley rats were exposed to a vapour of DMAC (purity 99.8%) for 6 hours/day, 5 days per week for 69 days. After 43 exposure days males were mated to untreated virgin females. Mean analytical exposure concentrations were 40, 116, and 386 ppm, respectively. (corresponding to 34, 99 and 330 mg/kg bw/day).[2] A control group was exposed to air containing no DMAC. Dams were sacrified on gestation day 20 and necropsied. Fetuses were examined externally. In the treated animals increased liver weights and liver/body weight ratios were seen in the high- and medium-exposure groups. Reproductive data indicated no treatment-related effects on copulation efficiency or efficiency in effecting pregnancy. No treatment-related effects were found on pre-implantation loss, postimplantation loss, embryotoxicity, or fetotoxicity. No malformed fetuses were seen (Wang et al. 1989).

Dermal studies

A study report was supplied on a dermal one-generation study in rats. This study was carried out during the 1970's by Industrial Bio-Test Laboratories, a lab known to have provided fraudulent reports to sponsors during this period. In absence of independent verification of the study report in question, these data are not considered further here.

4.11.1.2 Human information

Not available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Oral studies

Mouse (prenatal development study)
In a very large but incompletely reported developmental study (BASF 1975), groups of pregnant Albino SPF NMRI mice (number not reported) were given single oral doses of DMAC (technical grade) of 3200 or 1280 µl/kg bw on gestation days 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. Based on the specific gravity of DMAC these dose levels equal about 3000 and 1200 mg/kg bw. For some treatment days one or two additional dose levels were applied (indicated below under results). Each treatment group had its own separate control group. On day 18 of gestation all dams were killed and fetuses were examined for external abnormalities. The numbers of implantation sites, resorptions (early, late) were recorded, as were the numbers of live and dead fetuses, fetal weights and fetal sex. One third of the fetuses were examined for visceral abnormalities. The remaining fetuses were examined skeletally. Visceral and skeletal malformations were not reported separately (incidences summed). No tables with results were provided in the report submitted.
In none of the groups signs of maternal toxicity were observed. After treatment on gestation day 6 total number of live fetuses was decreased (at 3000 mg/kg, no effect at 1200 mg/kg), post implantation loss was increased (at 3000 mg/kg, no effect at 1200 mg/kg), fetal weights were decreased (both dose levels), the incidence of visceral abnormalities was increased (at 3000 mg/kg exencephalia, micrognatia, anophthalmia, hydrocephalus, cleft palate, fused ribs in 18/209 fetuses

[2] Calculated route-to-route based on the defaults as given in the REACH guidance R.8 (Table R.8-17). Mean body weight male rat 0.50 kg, inhalation volume 20.5 litres/hour. Calculation based on equal absorption of DMAC via inhalation and orally.

versus 0/203 in controls; at 1200 mg/kg cleft palate in 6/243 fetuses versus 0/261 in controls). Conclusion by authors: at 3000 mg/kg embryolethality, fetotoxicity and teratogenicity, at 1200 mg/kg no embryolethality, fetotoxicity or teratogenicity.
After treatment on day 7: incidence of externally detected abnormalities increased (at 3000 mg/kg exencephalia in 6/168 fetuses, brachygnathia inferior in 3/168 fetuses, head malformation in 2/168 fetuses, macroglossia in 2/168 fetuses, anophthalmia in 2/168 fetuses, exophthalmia in 2/168 fetuses versus 0/126 fetuses in controls; at 1200 mg/kg kinked tail in 3/186 fetuses, exencephalia in 3/186 fetuses, in controls 1/216 fetuses with exencephalia). Visceral and skeletal findings: summed incidences of malformations: 22/168 at 3000 mg/kg versus 4/126 in controls; 5/186 at 1200 mg/kg versus 4/216 in controls. Conclusion by authors: at 3000 mg/kg fetotoxicity and teratogenicity but no embryolethality, at 1200 mg/kg no embryolethality or teratogenicity, slight fetotoxicity.
After treatment on day 8: decreased number of live fetuses (at 3000 mg/kg, no effect at 1200 or 600 mg/kg), post-implantation loss increased (at 3000 mg/kg, no effect at 1200 or 600 mg/kg), fetal weight decreased (at 3000 mg/kg, no effect at 1200 or 600 mg/kg), external examination of fetuses showed malformations (at 3000 mg/kg exencephalia, spina bifida, micrognathia, kyphosis, oligodactylia, cleft lip, short tail, split jaws, number of fetuses with these abnormalities not reported, no malformations among control fetuses; at 1200 mg/kg exencephalia in 30/157 fetuses versus 0/142 in controls; at 600 mg/kg exencephalia in 1/157 fetuses versus 0/142 control fetuses). Visceral and skeletal examinations: at 3000 mg/kg malformations in all fetuses versus 0/244 in controls; at 1200 mg/kg malformations in 36/210 fetuses versus 0/216 in controls; at 600 mg/kg malformations in 4/157 fetuses versus 2/142 in controls). Conclusion by authors: at 3000 mg/kg fetotoxicity, teratogenicity and embryolethality, at 1200 mg/kg no embryolethality or fetotoxicity but strong teratogenicity; at 600 mg/kg no embryolethality, fetotoxicity or teratogenicity.
After treatment on day 9: decreased number of live fetuses (at 3000 mg/kg only), post implantation loss increased (at 3000 mg/kg only), fetal weight decreased (3000, 1200 and 600 mg/kg, no effect at 400 mg/kg), placental weight decreased (at 3000 mg/kg only), external examination of fetuses showed malformations (at 3000 mg/kg exencephalia, syndactylia, oligodactylia, polydactylia, accessory toes, number of fetuses with these abnormalities not reported, no malformations among control fetuses; at 1200 mg/kg exencephalia in 12/187 fetuses, scoliosis in 1/157 fetuses, 2 kinked tails among 200 control fetuses; at 600 mg/kg exencephalia in 4/181 fetuses versus 0/142 in controls; at 400 mg/kg exencephalia in 3/300 fetuses, 1 fetus with multiple malformations among 248 control fetuses). Sum-incidences of skeletal and visceral malformations were increased (at 3000 mg/kg 75/115 versus 2/248 in controls, at 1200 mg/kg 115/187 versus 5/200 in controls, at 600 mg/kg 22/181 versus 2/142 in controls, at 400 mg/kg 4/300 versus 6/248 in controls). Conclusion by study-authors: at 3000 mg/kg fetotoxicity, teratogenicity and embryolethality, at 1200 mg/kg no embryolethality but fetotoxicity and teratogenicity; at 600 mg/kg no embryolethality but fetotoxicity and teratogenicity, at 400 mg/kg no embryolethality, fetotoxicity or teratogenicity.
After treatment on days 10, 11, 12, 13 and 14 similar effects were found. Conclusions as drawn by study-authors:
After treatment on day 10: at 3000 mg/kg fetotoxicity, teratogenicity and embryolethality, at 1200 mg/kg no embryolethality but fetotoxicity and teratogenicity; at 600 mg/kg no embryolethality or teratogenicity but weak fetotoxicity.
After treatment on day 11: at 3000 mg/kg no embryolethality but fetotoxicity and teratogenicity, at 1200 mg/kg no embryolethality or teratogenicity but fetotoxicity; at 600 mg/kg no embryolethality, fetotoxicity or teratogenicity.
After treatment on day 12: at 3000 mg/kg no embryolethality but fetotoxicity and teratogenicity, at 1200 mg/kg no embryolethality but doubtful teratogenicity and fetotoxicity, at 600 mg/kg kg no embryolethality, fetotoxicity or teratogenicity.
After treatment on day 13: at 3000 mg/kg embryolethality plus weak fetotoxicity with no teratogenicity, at 1200 mg/kg no embryolethality, teratogenicity or fetotoxicity.

After treatment on day 14: at 3000 mg/kg doubtful embryolethality and fetotoxicity with no teratogenicity, at 1200 mg/kg no embryolethality, teratogenicity or fetotoxicity.
After treatment on day 15: at 3000 mg/kg no embryolethality or teratogenicity but weak fetotoxicity.
Overall in this study the NOAEL (single dose) for maternal toxicity was 3000 mg/kg bw and for developmental toxicity 400 mg/kg bw.

Determination of the ED_{10}-value:
Dose response modeling was done using the Benchmark dose software PROAST. This is described in Annex 3 to the present report. For this mouse study the modeling was carried out for the endpoint 'sum of malformations' as found after dosing on day 9 of gestation (for this treatment day 4 dose levels were tested and the results in question thus are the most complete). For details see Annex 3.

Table 12: Malformations (visceral + skeletal) after single oral dose (day 9) in mouse study (BASF 1975)

	Dose (mg/kg body weight)				
	0	400	600	1200	3000
Malformed fetuses/total no. of fetuses	15/838 (1.8%)	4/300 (1.3%)	22/181 12%	115/187 (61%)	75/115 (65%)
ED_{10}	597 mg/kg bw (572-630)[a]				

[a] lower 5% and upper 95% confidence bounds

An ED_{10}-value was also estimated by interpolation of the malformation incidences. For details see Annex 4. The estimated ED_{10} is 596 mg/kg bw/day.

Mouse (prenatal development study, equivalent to OECD GL 414)
In an incompletely reported developmental study (BASF 1976a, 1976b), groups of pregnant Albino SPF NMRI mice (number not reported) were given oral doses of DMAC (technical grade) of 1280, 427 or 256 µl/kg bw/day from gestation day 6 through 15. Based on the reported density of DMAC these dose levels correspond to about 1200, 400 and 240 mg/kg bw/day. Each dose level had its own control group. On day 18 all dams were killed and fetuses were examined externally. The numbers of implantation sites, resorptions (early, late) were recorded, as were the numbers of live and dead fetuses, fetal weights and fetal sex. One third of the fetuses were examined for visceral abnormalities. The remaining fetuses were examined skeletally. Visceral and skeletal malformations were not reported separately (incidences summed).
At 1200 mg/kg maternal bodyweights were decreased by about 10% compared to concurrent controls (no effect at lower dose levels). The number of live fetuses was decreased at 1200 mg/kg bw (no. of live fetuses 83/141 versus 185/229 in controls; no effect at lower dose levels). Post implantation loss was increased at 1200 mg/kg (41% versus 19% in controls; no effect at lower dose levels). Fetal weights were decreased (at 1200 mg/kg -35% compared to controls, at 400 mg/kg -7% compared to controls, no effect at 240 mg/kg). Placental weights were slightly decreased at all dose levels. At external examinations increased incidences of external malformations were found at 1200 mg/kg (oligodactylia, sydactylia, exencephalia, no eye lid closure, brachygnatia, macroglossia) and 400 mg/kg (exencephalia, no eye lid closure). Visceral examinations showed increased incidences of cleft palate (incidence at 1200 mg/kg 24/45 fetuses versus 0/75 in controls, at 400 mg/kg 4/83 versus 1/71 in controls and at 240 mg/kg 2/80 versus 15/93 in controls). Skeletal examination showed increased incidences of fused ribs at 1200 mg/kg (79/96 versus 0/154 in controls) and at 400 mg/kg (7/169 versus 0/139 in controls) with no increase at 240 mg/kg (1/160 versus 1/183 in controls), Skeletal examinations also showed increased incidences of synostosis of the processus spinalis (at 1200 mg/kg in 18/154 fetuses versus 0/169 in controls) (BASF 1976a, 1976b).
The NOAEL for maternal toxicity in this study was 400 mg/kg bw/day. The NOAEL for developmental effects was 240 mg/kg bw/day.

Determination of the ED_{10}-value:
Dose response modeling was done using the Benchmark dose software PROAST. This is described in Annex 3 to the present report. For this study the modeling was carried out for the effects of cleft palate and fused ribs. For details see Annex 3.

Table 13: Selected malformations after oral dosing to mice on days 6-15 of gestation (BASF 1976a)

	Dose (mg/kg body weight)			
	0	240	400	1200
Cleft palate (no. of fetuses/total no. of fetuses)	16/239 (6.7%)	2/80 (2.5%)	4/83 (4.8%)	24/45 (53%)
ED_{10}	844 mg/kg bw (581-912)[a]			
Fused ribs (no. of fetuses/total no. of fetuses)	1/476 (0.2%)	1/160 (0.6%)	7/169 (4.1%)	79/96 (82%)
ED_{10}	484 mg/kg bw (435-539)[a]			

[a] lower 5% and upper 95% confidence bounds

ED_{10}-values were also estimated by interpolation of the data on cleft palate and fused ribs. For details see Annex 4. The estimated ED_{10}-values are 597 mg/kg bw/day (cleft palate) and 463 mg/kg bw/day (fused ribs).

Rat (prenatal development study, equivalent to OECD GL 414)
In an incompletely reported developmental study (BASF 1976c, 1976d), groups of 18-24 pregnant Sprague-Dawley rats were given oral doses of DMAC (technical grade) of 1020, 340 or 113 µl/kg bw/day from gestation day 6 through 15. Based on the reported density of DMAC these dose levels correspond to about 960, 323 and 106 mg/kg bw/day respectively. Each dose level had its own control group. On day 20 all dams were killed and fetuses were examined externally. The numbers of implantation sites, resorptions (early, mid-late, late) were recorded, as were the numbers of live and dead fetuses, fetal weights and fetal sex. One third of the fetuses were examined for visceral abnormalities (according to Wilson). The remaining fetuses were examined skeletally. Visceral and skeletal malformations were not reported separately (incidences summed).
Maternal growth was decreased severely at 960 mg/kg and moderately at 323 mg/kg (no effect at 106 mg/kg). Vaginal bleeding occurred in 5 and 6 dams at 323 and 960 mg/kg respectively (zero incidence in controls). At 960 mg/kg all embryos died, mostly in the mid-late period (no live fetuses in this group). At 323 mg/kg placental weights were decreased and the number of live fetuses was also slightly decreased (mean 11.00 per dam versus 12.68 per dam in controls; the embryo's died mainly in the mid and late period). Fetal weights were slightly decreased at this level. At external examination of fetuses increased numbers of malformations were found at 323 mg/kg only (6 fetuses with anasarca, 2 with aplasia of the tale, 1 with atresia; control incidence zero). Visceral examination showed increased incidence of malformations at 323 mg/kg (incidence 18/91 versus 2/94 in controls; the malformations were split/aplastic vertebrae, hydroureter). At 106 mg/kg 1/70 fetuses was malformed (aplasia of the tail, atresia ani; incidence in controls 0/73). Conclusion by study-authors: at 960 mg/kg maternal toxicity and complete embryolethality, at 323 mg/kg maternal toxicity, weak teratogenicity and fetotoxicity, at 106 mg/kg no effect (BASF (1976c, 1976d).
The NOAEL for both maternal and developmental toxicity in this study was 106 mg/kg bw/day.

For this study dose response modeling did not lead to satisfactory model fit for the effects observed. Thus no ED_{10} could be derived from this study.

Rat (prenatal development study, equivalent to OECD GL 414)

In a developmental toxicity study, groups of 22-25 pregnant rats were dosed with DMAC (purity 99.72%) at 0, 65, 160, 400 mg/kg bw/day by gavage on days 6 through 19 of gestation (Johannsen et al. 1987). (Study in agreement with OECD-guidelines). No treatment-related clinical signs were observed in the dams though animals in all groups including controls had red/swollen conjunctivae with associated swelling of the neck, indicating viral infection. Mean maternal body weight gain was reduced throughout the treatment period and until sacrifice at 400 mg/kg bw (statistically significant, $p \leq 0.05$) and at 160 mg/kg bw (slightly, not statistically significant). Examinations at Caesarean section revealed a slight increase in post-implantation loss at 400 mg/kg bw. The authors indicate that this was probably due to a relatively high incidence of early resorptions and a statistically significant increase in the number of late resorptions (because implantations were not decreased but viable fetuses were). Mean fetal bodyweight was decreased at 160 and 400 mg/kg bw (statistically significant only at 400 mg/kg).

Table 14: Findings at Caesarean section in pregnant rats dosed with DMAC (Johanssen et al. 1987)

	Dose (mg/kg bw/day)			
	0	65	160	400
No. of animals on study	28	28	28	28
No. gravid animals examined at Caesarean section	22	23	25	24
No. of dams with viable foetuses	22	22	23	24
No. of corpora lutea per dam**	16.4±2.87	16.8±3.17	16.5±2.99	17.2±2.78
No. of implantations per dam**	14.3± 2.48	14.0 ± 3.69	14.4 ± 4.00	14.8 ± 3.39
No. of post-implantation loss per dam**	1.2 ± 1.45	2.2 ± 2.57	0.7 ± 1.31	2.6 ± 1.80 *
No of dams with resorptions only	0	1	2	0
Group mean pre-implantation loss (%)[A]	9.0	16.5	9.1	14.3
Group mean post-implantation loss (%)[B]	8.6	15.5	5.0	17.8
No. of viable fetuses per dam**	13.0 ± 3.72	11.9 ± 4.95	13.7 ± 4.60	12.1 ± 3.25
No. of male foetuses	136	143	178	158
No. of female fetuses	151	130	165	134
Mean fetal body weight (g)**	3.5± 0.22	3.6± 0.28	3.3± 0.19	2.3± 0.25*

[A] (Total no. of corpora lutea – total no. of implantations)/Total no. of corpora lutea x 100
[B] (Total no. of implantations – total no. of viable fetuses)/Total no. of implantations x 100
* Statistically significant ($P<0.01$)
** Mean and standard deviation

Examinations for external malformations and visceral and skeletal abnormalities showed increased occurrence (statistically significant) of malformations at 400 mg/kg bw. The main finding was an increased occurrence of heart and/or blood vessel abnormalities (33 fetuses, 18 litters). The most common of these anomalies was a common truncus arteriosis and no ductus ateriosis. Other abnormalities with an increased occurrence (in more than one litter) included cleft palate (3 fetuses, 3 litters) and anasarca (5 fetuses, 2 litters). No clearly compound related malformations were found at lower dose levels. At 400 mg/kg bw there were also increased skeletal variations (associated with decreased body weight). This effect was also observed to a slight degree at 160 mg/kg bw.

Incidences of malformations and variations in fetuses from pregnant rats given DMAC (Johannsen et al. 1987):

FETAL MALFORMATIONS AND DEVELOPMENTAL VARIATIONS

	Dimethylacetamide (mg/kg/day)			
	0 (Control)	65	160	400
No. litters examined	22	22	23	24
No. fetuses examined externally	287	273	343	292
No. fetuses examined viscerally	142	136	164	146
No. fetuses examined skeletally	145	137	172	146
Malformations observed	No. fetuses (Litters)	No. fetuses (Litters)	No. fetuses (Litters)	No. fetuse (Litters)
Total fetuses (litters) with malformations	1 (1)	2 (1)	0 (0)	49 (21[a])
Encephalocele	1 (1)			
Fleshy protrusion from hard palate	1 (1)			1 (1)
Cleft palate		1 (1)		3 (3)
Micrognathia		2 (1)		1 (1)
Tail anomaly				1 (1)
Anasarca				5 (2)
Microphthalmia	1 (1)			
Heart and/or vessel anomaly				33 (18)
Situs inversus				1 (1)
Dysplasia (generalized)				1 (1)
Diaphragmatic hernia				1 (1)
Enlarged adrenals				3 (1)
Kidney anomaly with or without ureter anomaly				2 (1)
Malformed skull bone	1 (1)			
Atlas occipital defect				1 (1)
Malformed clavicle				1 (1)
Vertebral anomaly				3 (3)
Rib anomaly				3 (2)
Variations observed				
25 presacral vertebrae	4 (2)		14 (5)	18 (10)
14th rudimentary rib(s)	5 (4)	8 (6)	3 (3)	10 (7)
14th full rib(s)	1 (1)			11 (7)
12 full pair of ribs and a 13th rudimentary rib(s) with or without 13th full rib	3 (2)	2 (1)	13 (6)	5 (4)
7th cervical rib(s)	1 (1)	1 (1)	3 (2)	1 (1)
Hyoid unossified	1 (1)			
Sternebrae No. 5 and/or No. 6 unossified	42 (15)	19 (10)	35 (13)	128 (23)
Sternebrae Nos. 1–4 unossified	1 (1)	1 (1)		29 (13)
Entire sternum unossified				2 (2)
Skull reduced in ossification				14 (7)
Vertebrae reduced in ossification				30 (13)
Pubic unossified				1 (1)
Major vessel variations		3 (2)		9 (6)
Misplaced esophagus				2 (2)
Renal papillae not developed and/or distended ureter	10 (6)	8 (5)	5 (3)	6 (5)

[a] Significantly different from control group, $p < 0.01$.

The NOAEL for maternal toxicity and developmental toxicity in this study were 160 mg/kg bw/day and 65 mg/kg bw/day respectively.

Determination of the ED10-value:
Dose response modeling was done using the Benchmark dose software PROAST. This is described in Annex 3 to the present report. For this study the modeling was carried out for the parameters

'sum of malformations' and 'heart and great vessel abnormalities'. For details see Annex 3. As explained in Annex 3, this study was analyzed together with the main study of Haskell Labs (1997) because of their identical test species and similar experimental setups. The advantage of this pooling of data is a more precise end result, because it is based on more information. The analysis showed the Haskell Labs (1997) study to be more sensitive compared to the present study by Johanssen et al. (1987). Thus overall, the ED_{10} from the two studies together is based on the Haskell Lab (1997) data supported by the Johanssen et al. (1987) data. As is shown in Figure 4 of Annex 3, the preferred model for the two studies together indicates an ED_{10} for sum of malformations in the Johanssen et al. (1987) study of 358 mg/kg bw/day (90% confidence interval 339-378). For the heart and great vessel malformations in the Johanssen et al. (1987) the preferred model for both studies indicates an ED_{10} of 332 mg/kg bw/day (90% confidence interval 309-375, data not shown in figure).

ED_{10}-values were also estimated by interpolation of the Johannsen et al. (1987) data on heart and vessel malformations and sum of malformations. For details see Annex 4. The estimated ED_{10}-values are 264 mg/kg bw/day (heart and vessel malformations) and 400 mg/kg bw/day (sum of malformations).

Rat (pilot prenatal developmental toxicity study)
In a pilot developmental toxicity study groups of 8 mated Sprague-Dawley rats were dosed with DMAC (purity not reported) at 0, 62.5, 125, 250 or 500 mg/kg bw/day by gavage on days 7 through 21 of gestation (Haskell Lab 1997). On day 22 all rats were sacrificed and grossly necropsied. The fetuses were removed from the uterus and were weighed, sexed and examined for external alterations. In dams the following toxic effects were seen: decreased body weights (250 and 500 mg/kg), increased kidney weights (125, 250 and 500 mg/kg), increased liver weights (all dose levels). At 500 mg/kg marked embryolethality was observed with total litter resorption in 5/8 dams and increased resorptions in the 3 remaining litters; there were only 5 viable fetuses in this group which were very small and 4/5 of which had anasarca and domed heads. At 250 mg/kg reduced fetal weight and 3 fetuses with malformations (including imperforate anus, cleft palate, filamentous tail, domed head) were found (total number of fetuses in this group not reported). At 125 mg/kg there were 3 fetuses with cleft palate (total number of fetuses in this group not reported).

Rat (prenatal developmental toxicity study)
In the main prenatal developmental toxicity study following the above pilot study, groups of 24-25 pregnant Sprague Dawley rats received DMAC (purity not reported) by gavage at 0, 20, 65,150 or 400 mg/kg bw/day from gestation 7 through 21 (Haskell Lab 1997) with examination of the fetuses on day 22. After external investigation of all fetuses half of them were examined for soft tissue abnormalities by dissection and all of the fetuses were stained with alizarin for skeletal examination. (Study according to OECD-guidelines, according to GLP).
Mean maternal growth was reduced during the dosing period for animals dosed at 150 and 400 mg/kg (7 and 74% respectively). No effect on maternal body weight occurred at lower dose levels. In dams of the 400 mg/kg group absolute and relative kidney weight and relative liver weights were increased, but no histopathological evidence of organ damage and no changes in clinical chemistry were found. Developmental findings were as follows. At 400 mg/kg embryolethality occurred, as was shown by increased resorptions (3.1 per litter vs 0.5 in controls) and reduced litter size (10.4 vs 14.1 in controls). In addition at this dose level fetal weights were reduced, incidence of malformed fetuses at gross necroscopy was increased (69/250 from 17 litters), at visceral examination (133/206, 24 litters) and also at skeletal examination (13/250, 7 litters). The majority of malformations were of the head (synotia, naris atresia, micrognatia, cerebral ventricle distension),

cardiovascular system (heart and great vessels defects, pulmonary artery) and a variety of skeletal defects at low incidence.
At 150 mg/kg minimal developmental toxicity occurred. At this level there was a slight reduction in maternal bodyweight gain and in fetal body weight. No other effect was found except for one fetus with multiple malformations including naris atresia, heart and great vessels malformations and micrognatia.

The NOAEL for both maternal toxicity and developmental toxicity in this study was 65 mg/kg bw/day.

Tables with relevant results as taken from the study report:

REPRODUCTIVE OUTCOME[a]

		GROUP:	I	II	III	IV	V
		DAILY DOSE (mg/kg):	0	20	65	150	400
No. Mated			25	25	25	25	25
No. Pregnant			24	24	24	25	24
No. Deaths			0	0	0	0	0
No. With Total Resorptions			0	0	0	0	0
No. Early Deliveries			0	0	0	0	0
No. Litters			24	24	24	25	24
Means Per Litter							
Mean Corpora Lutea[b]			15.0	15.3	15.5	15.7	14.7
Implantations			14.5	14.3	14.8	15.0	13.5
Resorptions:	Total		0.5	0.6	0.6	0.5	3.1*
	Early		0.4	0.6	0.6	0.5	2.8*
	Late		0.0	0.0	0.0	0.0	0.3*
Dead Fetuses			0.0	0.0	0.0	0.0	0.0
Live Fetuses:[c]	Total		14.1	13.6	14.1	14.5	10.4*
	Males		7.3	6.8	6.8	7.3	5.8
	Females		6.8	6.8	7.3	7.2	4.6
Mean Fetal Weight:	Total		4.88	5.03	4.99	4.69*	3.22*
Sex Ratio[d]			0.51	0.50	0.49	0.51	0.58

[a] Individual data, standard deviations, and standard errors are presented in Appendix G.
[b] Statistical analyses are not conducted on mean corpora lutea data; these data are presented for information only.
[c] Statistical analyses are only conducted on the mean total number of live fetuses per litter. The mean numbers of males and females are presented for information only.
[d] Number male fetuses/total number fetuses per litter.
* Significant trend: $p \leq 0.05$.

INCIDENCE OF FETAL MALFORMATIONS[a]

GROUP:	I	II	III	IV	V
DAILY DOSE (mg/kg):	0	20	65	150	400
EXTERNAL					
No. Examined[b]	338[24]	327[24]	339[24]	362[25]	250[24]
No. Affected	1[1]	0[0]	1[1]	1[1]	69[17]
Mean percent affected	0.3	0.0	0.3	0.3	31.3
per litter (S.E.)	(0.28)		(0.35)	(0.31)	(6.49)
(S.D.)	(1.36)		(1.70)	(1.54)	(31.77)
Anus - Absent	...[c]	...	...	...	1(1)
Ear - Synotia	...	...	...	1(1)	15(7)*
Entire Body - Anasarca	1(1)	...	1(1)	...	28(8)*
Head - Micrognathia	...	...	...	1(1)	2(1)*
Limb - Short	1(1)	...	...	...	...
Paw - Adactyly	1(1)	...	...	...	...
Palate - Cleft	...	...	...	1(1)	...
Snout - Naris Atresia	...	...	...	1(1)	33(10)*
Tail					
- Absent	...	...	...	...	1(1)
- Vestigial	...	...	...	...	1(1)
VISCERAL					
No. Examined	184[24]	172[24]	177[24]	190[25]	206[24]
No. Affected	0[0]	0[0]	1[1]	1[1]	113[24]
Mean percent affected	0.0	0.0	0.6	0.5	57.3
per litter (S.E.)			(0.60)	(0.50)	(5.51)
(S.D.)			(2.92)	(2.50)	(26.98)
Heart &/or Greater Vessels					
- Malformation	...	...	1(1)	1(1)	113(24)*
HEAD					
No. Examined	183[24]	171[24]	177[24]	190[25]	206[24]
No. Affected	0[0]	0[0]	0[0]	5[2]	82[18]

INCIDENCE OF FETAL MALFORMATIONS[a]

GROUP:	I	II	III	IV	V
DAILY DOSE (mg/kg):	0	20	65	150	400
HEAD (CONT.)					
Mean percent affected	0.0	0.0	0.0	4.5	38.1
per litter (S.E.)				(4.01)	(6.72)
(S.D.)				(20.05)	(32.93)
Brain – Distended Lateral Ventricles[d]	...	...	...	4(1)	23(8)*
Severe	...	...	...	...	1(1)
Moderate	...	...	...	2(1)	15(7)
Slight	...	...	...	2(1)	7(2)
Mandible - Micrognathia	...	...	...	1(1)	...
Nares - Naris Atresia	...	...	...	1(1)	70(18)*
Palate - Cleft	...	...	...	1(1)	...
Tongue - Large	...	...	...	1(1)	...
SKELETAL					
No. Examined	338[24]	327[24]	339[24]	362[25]	250[24]
No. Affected	1[1]	0[0]	0[0]	0[0]	13[7]
Mean percent affected	0.3	0.0	0.0	0.0	5.1
per litter (S.E.)	(0.28)				(2.18)
(S.D.)	(1.36)				(10.68)
Rib - Fused	...	...	...	...	5(2)*
Sternebra - Non-fused	1(1)	...	...	...	2(2)
Vertebra					
- Absent	...	...	...	...	2(2)*
- Fused	...	...	...	...	2(1)
- Hemi	...	...	...	...	4(3)*
TOTAL NUMBER AFFECTED	2(2)	0(0)	1(1)	5(2)	167(24)
MEAN PERCENT AFFECTED	0.6	0.0	0.3	2.6	69.0
PER LITTER(S.E.)	(0.38)		(0.35)	(2.29)	(5.41)
(S.D.)	(1.88)		(1.70)	(11.47)	(26.50)

[a] Individual fetal alterations are presented in Appendix I.

[b] Number examined and affected, including the number affected with the listed malformations, are expressed as Fetuses [Litters] or Fetuses (Litters).

[c] For ease of reading, zeros have been replaced with ellipses for the listed malformations.

[d] Statistical analyses were performed on the combined data. The data broken down by severity are presented for information only.

* Significant trend (Jonckheere's test); $p \leq 0.05$.

Note: Statistical analyses are only conducted on the individual endpoints. The overall total and totals by exam are presented for information only.

Determination of the ED10-value:
Dose response modeling was done using the Benchmark dose software PROAST. This is described in Annex 3 to the present report. For this study the modeling was carried out for the parameters 'sum of malformations' and 'heart and great vessel abnormalities'. For details see Annex 3. As explained in Annex 3, this study was analyzed together with the study by Johanssen et al. (1987) because of their identical test species and similar experimental setups. The advantage of this pooling of data is a more precise end result, because it is based on more information. The analysis showed the Haskell Lab (1997) study to be more sensitive compared to the Johanssen et al. (1987) study. Thus overall the ED10 from the two studies together is based on the Haskell Lab (1997) data supported by the Johanssen et al. (1987) data.

Table 15: Incidences of selected malformations after oral dosing to rats on days 7-21 of gestation (Haskell Lab 1997)

	Dose (mg/kg body weight)				
	0	20	65	150	400
Sum of malformations (no. of fetuses/total no. of fetuses)	2/338 (0.6%)	0/327 (0%)	1/339 (0.3%)	5/362 (1.4%)	167/250 (67%)
ED_{10}	217 mg/kg bw (200-240)[a]				
Heart and great vessel abnormalities (no. of fetuses/total no. of fetuses)	0/184 (0%)	0/172 (0%)	1/177 (0.6%)	1/190 (0.5%)	113/206 (55%)
ED_{10}	244 mg/kg bw (220-322)[a]				

[a] lower 5% and upper 95% confidence bounds

ED_{10}-values were also estimated by interpolation of the Haskell Lab (1997) data on heart and vessel malformations and sum of malformations. For details see Annex 4. The estimated ED_{10}-values are mg/kg bw/day 194 (heart and vessel malformations) and 185 mg/kg bw/day (sum of malformations).

Rabbit (prenatal developmental study, equivalent to OECD GL 414)
In an early prenatal developmental study (BASF 1974) groups of 10 pregnant New Zealand White rabbits were given oral gavage doses of 0, 0.1, 0.3 or 0.9 ml DMAC per kg bw/day on days 6 through 18 of gestation. Based on the specific gravity of DMAC these dose levels are equal to 94, 282 or 846 mg/kg bw/day. At day 29 of gestation all dams were killed and fetuses were examined for external abnormalities. The numbers of implantation sites, resorptions (early, late) were recorded, as were the numbers of live and dead fetuses, fetal weights and fetal sex. The soft tissues of the fetuses were examined and skeletal tissues were stained and examined.
At 846 mg/kg marked maternal toxicity occurred with all dams dying before test end (no live fetuses in this group). At 282 mg/kg no maternal toxicity was found. At this dose level the numbers of dead fetuses were increased, as was the number of resorptions; post implantation loss was increased in this group (45% versus 11.5% in controls) and the incidence of malformed fetuses increased (3/61 fetuses: 1 exencephaly, 1 cleft palate, 1 renal cyst; versus 0/85 in controls). At the lowest dose level no differences with the control group were found (incidence 0/80) (BASF 1974).

Determination of the ED_{10}-value:
Dose response modeling was done using the Benchmark dose software PROAST. This is described in Annex 3 to the present report. For this study the modeling was carried out for the parameter 'sum of malformations'. For details see Annex 3. As explained in Annex 3, this study was analyzed together with the study by Merkle and Zeller (1980) because of their identical test species and similar experimental setups. The potential advantage of this pooling of data is an improved statistical end result. See the description of the determination of the ED_{10} below under the Merkle

and Zeller (1980)/BASF (1976e) study for the result obtained from the combined analysis of these two rabbit studies.

ED_{10}-values could not be estimated via interpolation of the data because there was only one effect-dose in this study.

Rabbit (prenatal developmental study, OECD GL 414)
In the study by Merkle and Zeller (1980) (also reported as BASF 1976e) groups of 10-12 pregnant rabbits were given oral gavage doses of 0, 100, 300 or 500 µl/kg bw/day DMAC (technical grade) on gestation days from day 6 through 18 of gestation. Based on the reported density for DMAC these dose levels equal 94, 282 and 470 mg/kg bw/day respectively. At day 28 of gestation all dams were killed and fetuses were examined for external abnormalities. The number of implantation sites, resorptions (early, mid-late, late) were recorded, as were the numbers of live and dead fetuses, fetal weights and fetal sex. The heads were fixed in Bouin's solution and examined according Wilson's technique. Skeletal tissues were examined via x-rays.
At 470 mg/kg maternal toxicity occurred (2/12 dams died, growth retardation, clinical signs). At lower dose levels there was no maternal toxicity. All embryos were resorbed at 470 mg/kg. At 282 mg/kg bw the number of live fetuses was decreased (39 versus 54 in controls) and the number of resorptions increased (early, mid and late; in total 26 resorptions versus 8 in controls); fetal weights were decreased in this group. The number of malformed fetuses was increased at this dose level (5 malformed fetuses in 3 litters, 2 fetuses with two malformations; the malformations were cleft palate, fused ribs, microphtalmia; versus 0/54 in controls). At 94 mg/kg no effects were found (incidence 0/65) (BASF 1976e; Merkle and Zeller 1980).

Determination of the ED_{10}-value:
Dose response modeling was done using the Benchmark dose software PROAST. This is described in Annex 3 to the present report. For this study the modeling was carried out for the parameter 'sum of malformations'. For details see Annex 3. As explained in Annex 3, this study was analyzed together with the study reported as BASF (1974) because of their identical test species and similar experimental setups. The advantage of this pooling of data is a more precise end result, because it is based on more information. The table gives the combined data for the two rabbit studies and the resulting ED_{10}.

Table 16: Selected malformations after oral dosing to rabbits on days 6-18 of gestation (BASF 1974; Merkle and Zeller 1980; BASF 1976e)

	Dose (mg/kg body weight)				
	0	94	282	470	846
Sum of malformations (no. of fetuses/total no. of fetuses)	0/139 (0%)	0/145 (0%)	8/100 (8%)	0/0* -	0/0* -
ED_{10}	284 mg/kg bw (271-332)[a]				

[a] lower 5% and upper 95% confidence bounds
* no live fetuses in this group

The ED_{10}-value was also estimated via interpolation of the Merkle and Zeller (1980) data on sum of malformations. The result was an ED10 of 239 mg/kg bw/day (for derivation see Annex 4).

Inhalation studies

Rat (prenatal developmental study, equivalent to OECD GL 414)
In a developmental study, groups of pregnant CrL:CD rats were exposed to DMAC (purity >99.9%) vapour concentrations of 0, 32, 100 or 281 ppm (equivalent to 0, 114, 355 or 997 mg/m^3) for 6

hours/day from day 6 through day 15 of gestation. These test concentrations are equivalent to daily doses of 31, 96 and 269 mg/kg bw/day, respectively.[3] On day 21 of gestation all animals were killed and submitted to gross macroscopy. The numbers of corpora lutea and implantations, resorptions (early, late) were recorded. The number of fetuses, their weights and sex were determined. External examinations for malformations were done on fetuses. One half of the fetuses were examined for visceral abnormalities (this included stunted and externally malformed fetuses). The heads of these fetuses were fixed in Bouin's solution and examined. Skeletal examination was done on all fetuses.
The only effects observed in this study were decreased maternal growth and decreased fetal weights at 281 ppm. No other effects were found (Haskell Lab 1983; Solomon et al. 1991).

Rat (prenatal developmental study, equivalent to OECD GL 414)
In a prenatal developmental study, groups of 10 pregnant rats were exposed to vapour of DMAC (purity >99.9%) at concentrations of 0, 100, 300, 450 or 600 ppm (equivalent to 0, 355, 1065, 1600 or 2130 mg/m^3) for 6 hours per day from day 6 through 19 (Okuda et al. 2006). These test concentrations are equivalent to daily doses of 95, 287, 432 and 575 mg/kg bw/day, respectively.[4] Dams were weighed regularly and were monitored for clinical signs. On day 20 of gestation all dams were necropsied. On the day of necropsy liver enzymes (ASAT, ALAT, LDH) were measured in dams. Liver histopathology was also done. The uterus was opened and the numbers of live and dead fetuses (including resorptions) and implantations were recorded. Fetal weights and sex were determined and the fetuses were examined externally for malformations. One half of the fetuses was examined for visceral malformations after fixation with Bouin's solution (Nishimura's technique). The other half were examined for skeletal malformations after staining with Alizarin red S.
Maternal growth was retarded at ≥300 ppm. Maternal liver effects (increased weights, increased incidence of swelling of centrilobular hepatocytes) were seen at ≥300 ppm. The numbers of intrauterine deaths was increased at 600 ppm only; the number of live fetuses was decreased at this dose level (no clear effect at lower concentrations). Fetal weights were decreased at ≥300 ppm. External examination for malformations revealed an increased incidence of anasarca at 600 ppm (4/99 fetuses in 3/9 litters versus zero incidence in all other groups). Visceral examinations showed increased incidences of malformations of the cardiovascular system at ≥300 ppm (no of fetuses, no. of litters):

Table 17: Visceral malformations in rat fetuses after inhalation exposure from day 6-19 (Okuda et al. 2006) (no. of fetuses and no. of litters respectively)

Visceral malformation	0	100 ppm	300 ppm	450 ppm	600 ppm
Ventrical septal defect	0/68 (10) (0%)	0/65 (10) (0%)	2/63 (2/10) (3.2%)	7/63 (6/10) (11%)	22/49 (8/8) (45%)
Persistent truncus arteriosis	0/68 (10) (0%)	0/65 (10) (0%)	0/63 (10) (0%)	2/63 (2/10) (3.2%)	12/49 (7/8) (24%)
Malpositioned subclavian branch	0/68 (10) (0%)	0/65 (10) (0%)	0/63 (10) (0%)	0/63 (10) (0%)	4/49 (3/8) (8.2%)
Retro-oesophagal subclavian	0/68 (10) (0%)	0/65 (10) (0%)	0/63 (10) (0%)	0/63 (10) (0%)	3/49 (3/8) (6.1%)
Total	0/68 (0/10) (0%)	0/65 (0/10) (0%)	2/63 (2/10) (3.2%)	7/63 (6/10) (11%)	23/49 (8/8) (47%)

[3] Calculated route-to-route based on the defaults as given in the REACH guidance R.8 (Table R.8-17). Mean body weight female rat 0.35 kg, inhalation volume 15.7 litres/hour. Calculation based on equal absorption of DMAC via inhalation and orally.

[4]Calculated route-to-route based on the defaults as given in the REACH guidance R.8 (Table R.8-17). Mean body weight female rat 0.35 kg, inhalation volume 15.7 litres/hour. Calculation based on equal absorption of DMAC via inhalation and orally.

Skeletal malformations were increased at 450 and 600 ppm (no. of fetuses, no. of litters):

Table 18: Skeletal malformations in rat fetuses after inhalation exposure from day 6-19 (Okuda et al. 2006) (no. of fetuses and no of litters respectively)

Skeletal malformation	0	100 ppm	300 ppm	450 ppm	600 ppm
Fused exoccupital	0/73 (10) (0%)	0/72 (10) (0%)	0/67 (10) (0%)	0/68 (10) (0%)	4/50 (4/9) (8%)
Fused cervical arch	0/73 (10) (0%)	0/72 (10) (0%)	0/67 (10) (0%)	4/68 (2/10) (5.9%)	2/50 (2/9) (4%)
Fused rib	0/73 (10) (0%)	0/72 (10) (0%)	0/67 (10) (0%)	0/68 (10) (0%)	2/50 (2/9) (4%)
Total	0/73 (0/10) (0%)	0/72 (0/10) (0%)	0/67 (0/10) (0%)	4/68 (2/10) (5.9%)	6/50 (6/9) (12%)

The study-authors conclude that the NOAEL for both maternal toxicity and developmental toxicity is 100 ppm in this study (Okuda et al. 2006).

Determination of the ED_{10}-value:
Dose response modeling was done using the Benchmark dose software PROAST. This is described in Annex 3 to the present report. For this study the modeling was carried out for the parameter total heart and great vessel malformations and for the different individual heart/vessel malformations listed in Table 17. For details see Annex 3. Table 19 gives the resulting ED_{10}-values as inhalation test concentrations. Using the defaults as given in the REACH guidance a route-to-route calculation was done to estimate the corresponding oral dose levels.[5]

Table 19: ED_{10}-values calculated from the inhalation study in rats by Okuda et al. (2006)

	ED_{10} as mg/m^3	ED_{10} as oral dose in mg/kg bw/day
Ventrical septal defect	1440 (1280-1650)[a]	387 (344-444)
Persistent truncus arteriosis	1840 (1690-1970)	495 (455-530)
Malpositioned subclavian branch	2140 (2040-2560)	576(549-689)
Retro-oesophagal subclavian	2160 (2170-2240)	581(584-603)
Total heart/great vessel malformations	1440 (1280-1660)	387(344-447)

[a] lower 5% and upper 95% confidence bounds

ED_{10}-values were also estimated by interpolation of Okuda et al. (2006) data on heart and vessel malformations. For details see Annex 4. The estimated ED_{10}-value equals 413 mg/kg bw/day.

Rabbit (prenatal developmental study, OECD GL 414)
In a prenatal developmental study, groups of 15 pregnant Himalayan rabbits were exposed to DMAC vapours at concentrations of 0, 200, 700 or 2000 mg/m^3 for 6 hours per day on days 7 through 19 of gestation (Klimisch and Hellwig 2000; BASF 1989). On day 29 of gestation all animals were killed and fetuses were examined externally. Dams were observed for clinical signs and maternal bodyweights were recorded. At termination the weights of the uterus were measured, the numbers of corpora lutea and implantations were counted. The numbers of fetuses were determined, fetal weight and sex. External examination for malformations was done. Trunks of the

[5] Calculation based on the defaults as given in the REACH guidance R.8 (Table R.8-17). Mean body weight female rat 0.35 kg, inhalation volume 15.7 litres/hour. Calculation based on equal absorption of DMAC via inhalation and orally.

fetuses were fixed in ethanol and processed for staining. The heads of the fetuses were fixed in Bouin's solution and evaluated according to Wilson's technique.
No signs of maternal toxicity were found. At 2000 mg/m^3 placental and fetal weights were decreased. External examination of fetuses showed cleft palate in 2 fetuses (2 litters) at 2000 mg/m^3. The incidence of soft tissue malformations was slightly higher at 2000 mg/m^3 (total incidence 8/78 in 7/14 litters versus 4/66 in 3/13 litters in controls; malformations different from controls were heart and great vessel abnormalities: truncus arteriosis communis in 1/78 fetuses versus 0/66 in controls, great vessel malformation in 1/78 fetuses versus 0/66 in controls, septal defects in 4/78 fetuses of 4/14 litters versus 2/66 in controls en 2/13 litters). In addition an increased incidence of separated origin on the carotids (classified as a soft tissue variation) was observed at 2000 mg/m^3 (incidences 66/78, 42/95, 26/86 and 28/66 at 2000, 700, 200 and 0 mg/m^3 respectively). Total incidences of skeletal variations were increased at 2000 and 700 mg/m^3 (present in 44/78, 17/95, 7/86 and 7/66 fetuses at 2000, 700, 200 and 0 mg/m^3 respectively). Conclusions by study-authors: inconclusive evidence for a weak teratogenic effect at 2000 mg/m^3, fetotoxicity at 700 and 2000 mg/m^3. NOAEL for maternal toxicity is 2000 mg/m3; the developmental NOAEL is 200 mg/m^3 (BASF 1989; Klimisch and Hellwig 2000)

Dermal studies

Reports were supplied on a dermal developmental study in rats and a dermal developmental study in rabbits. These studies were carried during the 1970's by Industrial Bio-Test Laboratories, a lab known to have provided fraudulent reports to sponsors during this period. In absence of independent verification of the study reports in question, these data are not considered further here.

4.11.2.2 Human information

Not available.

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

DMAC is currently classified for developmental toxicity. The present CLH only aims at removing the current SCL, not on changing the classification for reproductive toxicity. Therefore, no summary and justification of the current classification is required. Only a justification of removing the SCL is required.

The criteria for setting SCLs for reproductive toxicity focus on the ED_{10} after oral exposure for effects fulfilling the classification criteria. The following DMAC studies were selected for analysis:
- a single dose oral developmental study in mice (BASF 1975);
- an oral developmental study in mice with dosing from day 9-15 of gestation (BASF 1976a, 1976b);
- two oral developmental studies in rats (Johanssen et al. 1987 and Haskell Lab 1997);
- two oral developmental studies in rabbits (BASF 1974 and Merkle and Zeller 1980);
- an inhalation developmental study in rats (Okuda et al. 2006).

Developmental effects selected as fulfilling the classification criteria and included for the analysis of ED_{10}-values were various malformations (including cleft palate, exencephalia, brachygnathia, visceral malformations, fused ribs) seen in studies in mice, rats and rabbits and heart and great vessel malformations seen in several rat studies. The ED_{10}-values were determined using

Benchmark dose software (PROAST). This analysis is presented in Annex 3. Table 20 below provides a summary of the results.

In the dose response modelling, studies with the same test species and similar experimental setups were analysed together. The advantage of this pooling of data is a more precise end result, because it is based on more information. This was done for two rat studies (Johanssen et al. 1987 and Haskell Lab 1997) and for two rabbit studies (BASF 1975 and Merkle and Zeller). Details can be found in Annex 3. For the rat inhalation study the resulting ED10-values were converted to oral dose levels using default values for ventilation volume and body weight for female rats from the REACH guidance.

Table 20: ED_{10}-values for effects fulfilling the reproductive toxicity classification criteria, as derived from several animal developmental toxicity studies

Study	**Endpoint**	**ED_{10} (mg/kg bw/day)**	
		BMD-analysis	**Linear interpolation**
Mouse, oral single dose (BASF 1975)	Total malformations (visceral + skeletal)*	597 (572-630)**	596
Mouse oral study (BASF 1976a, 1976b);	Cleft palate	844 (581-912)	597
	Fused ribs	484 (435-539)	463
Rat oral study (Johanssen et al. 1987)	Sum of malformations (head, whole body, heart, vessels, skeleton)	358 (339-378)	400
	Heart and great vessels malformations	332 (309-375)	264
Rat oral studies pooled (Haskell Lab 1997 with Johanssen et al. 1987)	Sum of malformations (head, whole body, heart, vessels, skeleton)	217 (200-240)	185 (Haskell Lab study only)
	Heart and great vessels malformations	244 (220-322)	194 (Haskell Lab study only)
Rabbit oral studies (BASF 1974, Merkle and Zeller 1980)	Sum of malformations (cleft palate, fused ribs, microphtalmia)	284 (271-332)	239 (Merkel and Zeller study) For BASF 1974 no interpolation possible
Rat inhalation study (Okuda et al. 2006)	Total heart/great vessel malformations	387(344-447)***	413***

* The available report only reported the sum of visceral and skeletal malformations
** Between brackets the lower 5% and upper 95% confidence bounds
*** Estimated oral value, route-to-route recalculated from inhalation value

4.11.5 Comparison with criteria

Currently, DMAC is classified with a specific concentration limit (SCL) of 5% for Repr. 1B, H360D. A revision of the specific concentration limit (SCL) for developmental toxicity (Repr. 1B; H360D) according the 'Guidance on the Application of the CLP Criteria', is proposed as follows:

Based on the available data from the oral animal studies on developmental toxicity, the reproductive toxicity dose descriptor ED_{10} (effective dose with a 10% effect level above the background) was established for a number of effects warranting classification. Table 20 presents the calculated ED_{10}-values. The oral rat data lead to the lowest ED_{10}-values for total malformations and heart and great vessels malformations respectively. The ED10-values obtained via BMD-analysis are statistically more reliable than those obtained via interpolation since BMD analysis uses all data-points to determine the ED10 (instead of only 2 data-points) and also provides information on the uncertainty. The lowest ED_{10}-value of 217 mg/kg bw via BMD-analysis for effects warranting classification (total malformations) is determinative for the overall ED_{10} for the substance. This ED_{10}-value corresponds to the medium potency group (i.e. boundaries: 4 mg/kg bw/day < ED_{10}-value < 400 mg/kg bw/day).

According to the 'Guidance on the Application of the CLP Criteria' (paragraph 3.7.2.5.5) modifying factors (i.e. for type of effect or severity, data availability, dose-response relationship, modes or mechanism of action, toxicokinetics, and bio-accumulation of substances) may be applied to account for case-specific data situations which indicate that the potency group for a substance as obtained by the preliminary assessment should be changed. The type of effect produced by DMAC in animal studies (various structural malformations) should be considered as severe. But the ED_{10} is not close to the border of a higher potency group (not close to 4 mg/kg bw/day). Therefore, a modifying factor would not change the potency group. The available data for DMAC are considered more than adequate compared to the REACH requirements and do not justify adaptation of the potency group. DMAC shows a steep dose response relationship, which constitutes a further reason for not adapting the potency group. No conclusive information is available on the mechanism of action of DMAC for the induction of developmental effects. In rats the effects determining the ED_{10} were observed at dose levels also causing maternal toxicity. According to CLP Guidance paragraph 3.7.2.5.5 this should already have been taken into consideration during the classification and should not be used again to justify a higher SCL. It should be noted that in the mouse and rabbit structural malformations occurred without maternal toxicity. DMAC is not an accumulating substance as indicated by the available information in the registration dossier. There are no data available indicating that DMAC animal data would not be relevant for humans.

In conclusion, based on the available data, no modifying factors which might affect the preliminary potency, are considered necessary. Therefore, DMAC is considered a medium potency reproductive toxicant.

Based on the above ED_{10}-analysis removal of the existing SCL of 5% is proposed. DMAC is classified for Repr. 1B, H360D. According to the criteria in the 'Guidance on the Application of the CLP Criteria' (as described in tables 3.7.2.5.4 and 3.7.2.5.5 of this guidance) the GCL applies to DMAC.

4.11.6 Conclusions on classification and labelling

No conclusion on the classification is required as this proposal only concerns a change in SCLs for reproductive toxicity.

Based on the information available for DMAC showing multiple ED_{10}-values for developmental effects between 4 and 400 mg/kg bw/day and no modifying factors affecting the preliminary potency, DMAC is of medium potency and the current SCL of 5% for developmental toxicity of DMAC should be reduced to the appropriate level of 0.3% which is the GCL.

4.12 Other effects

Not applicable

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

4.12.1.2 Immunotoxicity

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not applicable

5.1 Degradation

5.1.1 Stability

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

5.1.2.3 Simulation tests

5.1.3 Summary and discussion of degradation

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

5.2.2 Volatilisation

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

5.3.1.2 Measured bioaccumulation data

5.3.2 Summary and discussion of aquatic bioaccumulation

5.4 Aquatic toxicity

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

5.4.1.2 Long-term toxicity to fish

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

5.4.2.2 Long-term toxicity to aquatic invertebrates

5.4.3 Algae and aquatic plants

5.4.4 Other aquatic organisms (including sediment)

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

6 OTHER INFORMATION

7 REFERENCES

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8 ANNEXES

Annex 1.

Justification of current classification for developmental toxicity

[redacted]

EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Environment Institute
European Chemicals Bureau

DMA
A034

ECBI/35/98 Rev. 1
01.09.98

SUMMARY RECORD

Commission Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity

[redacted]

Meeting at Arona, 24-25 June 1998

The meeting was chaired by **Dr A. Smith.** A list of participants is attached (for participants only).

1. Adoption of the agenda.

The draft agenda was adopted. A copy of the adopted agenda is attached.

2. Discussion of Reproductive toxicity

[redacted]

Fenarimol (P548)
CAS No.: 60168-88-9 EC No.: 262-095-7 Annex I Index No: 650-900-00-8
Lead Country: Denmark
At issue: Fertility and developmental toxicity

The **Specialised Experts** were asked to consider whether fenarimol should be classified as toxic to reproduction in Category 3 (Xn, R62; Possible risk of impaired fertility). A key issue was to decide if the fenarimol induced effects on fertility in rats and mice were of human relevance. The **Specialised Experts** also discussed whether it would be appropriate to classify fenarimol for developmental effects.

On reviewing the single- and multiple generation studies available, it was observed that fertility in both rats and mice was sensitive to fenarimol. There was also evidence that gestation length was increased and of parturition difficulties in these species at relatively high doses of fenarimol. Detailed studies in rats showed a sensitivity to fenarimol even during development and that the effect on fertility was mediated through the males.

1

[Further info on Fenarimol deleted]

Dimethylacetamide (A034)
CAS No.: 127-19-5 EC No.: 204-826-4 Annex I Index No: 616-011-004
Lead Country: Germany
At issue: Developmental toxicity

The **Specialised Experts** were asked to consider whether findings of specific malformations in exposed rats and rabbits justified classification for effects on development in Category 2 or Category 3.

In 2 oral gavage studies in rats, the reproducible finding of heart and great vessel malformations was noted to occur in the presence of some maternal toxicity. It was noted however, that similar cardiac and large vessel malformations were observed in rabbits after inhalation exposure. The **Specialised Experts** noted that the incidence of these malformations seen in rabbits was not statistically significant, but felt that it should be considered as qualitatively significant. In particular, the observation that half of the litters at the highest exposure level included malformed foetuses was of concern. Although this exposure concentration was relatively high, there were no signs of maternal toxicity. It was also commented that signs of embryotoxicity were seen in this inhalation study.

It was noted that specific malformations had not been seen in a rabbit gavage study. However, the **Specialised Experts** noted the relatively low group sizes employed and the possibility that the methodology had not allowed for detection of heart and great vessel malformations. It was also noted that no specific malformations had been observed in studies involving inhalation exposure of rats to dimethylacetamide.

The **Specialised Experts** felt that the findings in rat and rabbit dermal studies were also relevant. Embryolethality was observed in rats at dermal levels that did not lead to maternal toxicity.

Having reviewed each of the studies available, all but one of the **Specialised Experts** concluded that dimethylacetamide should be classified for reproductive toxicity with Repr Cat 2; R61. The remaining expert concluded that the available information did not allow for a decision between Category 2 and Category 3 to be reached. In addition, written comments received from two further Specialised Experts who did not attend the meeting had concluded that classification with Repr Cat 2; R61 and Repr Cat 3; R63, respectively were the most appropriate positions.

Due to the high dosages required to cause the developmental effects of concern, the **Specialised Experts** considered a suggestion from the DE specialised expert to take this relatively low potency into account by setting a specific concentration limit of 5% for R61. The proposal was based on the lowest dose with a relevant effect from the most sensitive oral rat study. There was some discussion about this suggestion and it was noted that the rabbit inhalation study might better be taken as the most sensitive study. Overall, whilst this approach was of some interest, it was felt that the suggestion needed to be documented clearly and considered in more detail before any agreement should be reached on this issue.

Conclusion:
The **Specialised Experts** recommended that dimethylacetamide should be classified for

reproductive toxicity and that Repr Cat 2; R61 was appropriate. Notably, signs of specific developmental toxicity were observed after dermal, inhalation and oral routes of administration. They recommended also that the use of potency considerations to set a specific concentration limit for R61 should be reviewed for dimethylacetamide.

Annex 2

German Proposal for current specific concentration limit of 5%

ECBI /14/95 Add. 13

Dimethylacetamide

Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
Federal Institute for Occupational Safety and Health

A 034

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	AS 2.3 -341 23-262/98	29.09.1998

Subject: DMAC

Meeting of the Classification and Labelling Working group, TOP 3.2

In enclose a german proposal for the application of concentration limits to substances which cause developmental toxicity. The concept is illustrated by two examples: DMF und DMAC. As DMAC is on the agenda under TOP 3.2 I hope it is possible to disuss or proposal for DMAC in Connection with the conpet under that TOP

Kind regards

Dr. Elke Kahler-Jenett

Attachment: DMAC1.doc

CONCENTRATION LIMITS FOR SUBSTANCES CLASSIFIED AS TOXIC TO REPRODUCTION/DEVELOPMENTAL TOXICITY IN PREPARATIONS

For substances being toxic to the fetal development it is assumed that there exists a threshold below which the substance exerts no such activity as is known for other systemically toxic endpoints. toxicity.

Therefore the following general procedure for the derivation of concentration limits in preparations is proposed:

Concentration limits in reparations are derived by applying the No. 4.2.3.3 of Annex IV of directive 93/21/EWG to preparations analogously, i.e. 1000 mg/kg bw of a preparation should contain amounts of the developmentally toxic substance which correspond at maximum to the NOEL of a valide study.
In case of existence of several different NOELs the highest one in the most sensible relevante spezies (including human data for substances of category I) should be taken as a basis in general.
In case of inhalative toxicity data the appropriate NOEL is calculated by multiplication of the NOEC with the corresponding respiratory volume of the species. As far as there are no detailed substance specific data a complete resorption of the amount of inhaled substance is assumed.

The concentration limit in % in preparations is received by dividing the NOEL by the limit-dose followed by multiplication with 100.

For illustration the proposed procedure is applied to two concrete examples: N,N-dimethyl acetamide (DMAC) and dimethyl formamide (DMF).

[Information on DMF deleted]

Derivation of a substance-specific concentration limit for the classification and labeling of preparations containing DMAC (127-19-5) classified as toxic to the fetal development

In the German MAK-documentation dating from 1990 [1] the following NOECs and NOELs, respectively, are mentioned for different application routes of dimethyl acetamide:

a) inhalative:		
rat	NOEC 360 mg/m^3/6h (ca. 104 mg/kg bw/d)	LOEC 1020 mg/m^3/6h
rabbit	NOEC 700 mg/m^3/6h (ca. 66 mg/kg bw/d)	LOEC 2000 mg/m^3/6h
b) dermal:		
rat	NOEL 500 mg/kg bw/d	LOEL 1000 mg/kg bw/d
rabbit	NOEL 250 mg/kg bw/d	LOEL 500 mg/kg bw/d
c) oral:		
rat	NOEL 106 mg/kg bw/d	LOEL 320 mg/kg bw/d
rat	NOEL 160 mg/kg bw/d	LOEL 400 mg/kg bw/d
mouse	NOEL 401 mg/kg bw/d	LOEL 602 mg/kg bw/d
rabbit	NOEL 94 mg/kg bw/d	LOEL 282 mg/kg bw/d.

The inhalation study with rabbits represents the relevant animal experiment leading to a NOEC of 700 mg/m^3/6h being equivalent to a maximal daily dose of 66 mg/kg bw (100 % resorption assumed) according to the following calculation:

DMAC concentration in air: 700 mg/m^3/6h
Respiratory volume/rabbit: 0.26 l/kg bw/min [2]; equivalent to 94 l/kg bw/6 h
Daily intake of substance: 0.094 x 700 = 65.8 mg/kg bw/d.

This NOEL is in the same order of magnitude as the one derived from the oral study in rabbits (94 mg/kg KGW/Tag).

Calculation of concentration limit in preparations:
65.8 : 1000 x 100 = 6.58 %

Taking the limit-dose as a basis (1000 mg/kg bw/d) the NOEL would not yet be reached by a DMAC content of 5 % in the preparation.

Concentration limit for DMAC in preparations: 5 %
(deviation from Annex I No. 6.1 of Directive 88/379/EWG).

[1] Greim, H. (Hrsg.): Gesundheitsschädliche Arbeitsstoffe. Toxikologisch-arbeitsmedizini- sche Begründung von MAK-Werten: N,N-Dimethylacetamid (Kapitel MAK-Werte und Schwangerschaft). VCH, Weinheim 1990

[2] Snipes, M.B.: Long-term retention and clearance of particles inhaled by mammalian species. Crit. Rev. Toxicol. 20, 175-211 (1989).

Annex 3

Determination of the ED10-value

The ED_{10} value (as used for reprotoxicity SCLs) is the lowest dose which induces reproductive toxic effects which fulfill the criteria for classification for reproductive toxicity with an incidence or magnitude of 10% after correction for the spontaneous incidence. According to the ECHA guidance (ECHA, 2012) the ED_{10} may be obtained either directly or by linear interpolation from experimental data or estimated using benchmark dose (BMD) software. The use of BMD software will result in a more precise estimate of the ED_{10} because all data from the dose-response curve are used. Here, we will derive the ED_{10} using the benchmark dose software PROAST, which is developed by RIVM and available at www.rivm.nl/proast.

The application of the BMD approach is performed according to the guidelines as set by EFSA (2009) and involves the following steps:
1. Specification of type of dose-response data
2. Specification of the relevant (benchmark) response (BMR)
3. Selection of candidate dose-response model(s)
4. Identification of acceptable models
5. Estimating the ED_{10}

These steps are discussed below.

1. Specification of type of dose-response data
Endpoints not showing dose response relationships are normally not used for deriving a BMD. The decision to disregard endpoints has been done by visual inspection of the data. Response data may be of various types: as an incidence (quantal data, non-parametric data), a magnitude (continuous data, parametric data) or both (ordinal data). The distinction between data types is important for statistical reasons (such as assumption of underlying statistical distribution), but also for the interpretation of the BMR.

In the case of DMAC several effects on reproduction are observed in various studies, and the classification is based on the weight of evidence of all results. Therefore, all effects identified in the CLH-report have been analyzed. These effects are all quantal data and are analyzed accordingly. For quantal data the number of affected individuals and the sample size are needed for each dose group.

2. Specification of the relevant (benchmark) response (BMR)
For quantal data the BMR is defined in terms of an increase in the incidence of the lesion / response scored, compared with the background response. The common way of doing this is either by additional risk or extra risk. According to ECHA guidance (ECHA, 2012), the relevant BMR is 10%, which is in the case of DMAC defined in terms of extra risk. The dose corresponding to the 10% extra risk is termed BMD_{10} or ED_{10}.

3. Selection of candidate dose-response models
Different models, which fit the data equally well, can result in different ED_{10}s, reflecting model uncertainty. To take this aspect of uncertainty into account, various models need to be fitted to the same dataset. The usual suit of quantal models, containing the two-stage, log-logistic, Weibull, log-probit, gamma, logistic, probit, exponential and Hill models, is applied here. To avoid the models having undesirable properties, certain constraints are imposed on the model parameters. For instance, since quantal responses are usually between 0% and 100% response, the background response parameter (*a*) is constrained to be between (fractions) 0 and 1. For more details see EFSA (2009).

4. Identification of acceptable models

The PROAST software takes care of fitting a model, which means finding the values of the unknown parameters in the model that make the associated dose-response curve approach the data as closely as possible. This is called the best fit and is achieved by maximizing the log-likelihood. The BMD approach does not aim to find the single statistically best estimate of the BMD but rather all plausible values that are compatible with the data; therefore, the goal is not to find the single best fitting model, but rather to find those models with an acceptable fit.

The acceptance of a fitted model is based on two principles. The first principle is that from a nested family of models (i.e. the exponential and Hill families) only one member is accepted, by comparing the log-likelihoods of the various members in that family, using the likelihood ratio test. When a member with fewer parameters does not show a significantly poorer fit, then this member will be preferred.

The second principle is that any fitted model should provide reasonable description of the dose-response data, according to a goodness-of-fit test with a P value greater than 0.05. There are several types of goodness-of-fit tests. The likelihood ratio test is the recommended choice here. In the likelihood ratio test, the log-likelihood value associated with a fitted model is compared with, and tested against, the log-likelihood value associated with the so-called "full model". The full model simply consists of the observed (mean) responses at each applied dose. Hence, the number of parameters equals the number of dose groups. If a model's fit is not significantly worse than that of the full model, then the model may be accepted. The likelihood ratio test may be used to test if additional parameters in nested models result in a significant improvement of the fit. See Slob (2002) or EFSA (2009) for more details.

5. Estimating the ED_{10}

For each identified critical endpoint, the set of models is applied. Subsequently, for each of the accepted models the ED_{10} is derived. The lowest ED_{10} from this range can be considered to be the overall ED_{10}.

When the experimental data provide sufficient information on the dose-response relationship, the various models that fit the data will have similar shapes and will yield a narrow range of ED_{10} values. In some circumstances, the dose response relationship may not be well defined. For instance, there may be large gaps between consecutive response levels. In such datasets the various models that fit the data (according to the statistical criteria discussed above) may assume different shapes, and consequently the ranges of ED_{10} values obtained may be wide. These ED_{10} values would not provide a secure basis for establishing an SCL. Criteria to judge the adequacy of the dose-response data on the basis of the range of ED_{10} values obtained have so far not been established. As a general rule, dose-response data should not result in a range of ED_{10} values from different accepted models that substantially exceeds one order of magnitude. When this value is exceeded, several options are available and should be considered on a case-by-case basis, e.g. re-evaluating the set of models.

Results

For each study the dose response data of the critical endpoints are analyzed and reported by one table and one figure. In the table the number of parameters (npar) and loglikelihood (loglik) are given for the identification of acceptable models by the likelihood ratio test (see 4. above). The results of the null and full models are included for this reason as well. The lowest ED_{10} obtained from the accepted models is printed in bold.
The $ED_{10}L$ and $ED_{10}U$, reported for the accepted models, are the lower 5^{th} and upper 95^{th} percent confidence limits of the ED_{10} and are equivalent to the BMDL and BMDU. These confidence limits are indicative for the quality of the experimental data. Poor experimental data will result in a large confidence interval.
The figure illustrates the dose-response data (including 90%-CI) and curve of the model providing the lowest ED_{10}.

As an example the results of the sum of malformation data in mice (BASF 1975) (Table1) are discussed in more detail.
- All models are highly significantly better than the no-response (null) model: the log-likelihood values are around 250 units higher, where an increase of only less than 4 units would have been sufficient.
- For both the (nested) exponential and Hill family of models, the fifth model (m5) is significantly better than their family member with less parameters. For more details see Slob (2002).
- Only two of the nine models passed the goodness of fit test: the other models show log-likelihoods that are significantly lower than the log-likelihood of the full model.
- The ratio between $ED_{10}L$ and $ED_{10}U$ is relatively small (approx. 1.1), indicating experimental data of good quality.
-The associated ED_{10}s (and their confidence intervals) for the two accepted models are similar, indicating that these dose-response data are suitable for deriving an ED_{10}.

The studies of Johanssen et al. (1987) and main study of Haskell Lab (1997) are analyzed together because of their similar experimental setups. The same goes for the two rabbit studies referenced as BASF (1974) and Merkle and Zeller (1980). Analysis is performed to test for differences in background response (parameter *a*) and sensitivity of the experiment (parameter *b*). When these parameters are listed in the covariate column of the table then this indicates a significant difference in background and/or sensitivity between both studies. For more details see Slob (2002).

For the sum of malformations and heart and vessel malformations in the Johanssen et al. (1987) and Haskell Lab (1997) studies show a significant difference in sensitivity between studies. In the Haskell Lab study the effect is more pronounced. Hence the ED_{10}s listed in the table are based on this study.

The BASF (1974) and Merkle and Zeller (1980) studies did not show any differences. Therefore the reported ED_{10}s are based on both studies.

Note that the animals in the Okuda et al. (2006) experiment were exposed via inhalation (mg/m^3) in contrast to all other experiments providing dose-response data, in which animals were orally exposed (mg/kg bw/day).

Table 1: ED_{10}s obtained from the sum of malformations data on day 9 in mice (BASF, 1975)

Model	npar	loglik	Accept	ED_{10} (mg/kg bw/dy)	ED_{10}L	ED_{10}U
null	1	-663.77	--	NA	NA	NA
full	5	-362.35	--	NA	NA	NA
two-stage	3	-425.5	No	293	NA	NA
log-logist	3	-401.21	No	522	NA	NA
Weibull	3	-414.42	No	462	NA	NA
log-probit	3	-397.54	No	542	NA	NA
gamma	3	-409.81	No	512	NA	NA
logistic	2	-457.63	No	755	NA	NA
probit	2	-448.38	No	714	NA	NA
exponential (m5)	4	-363.29	Yes	**597**	572	630
Hill (m5)	4	-363.8	Yes	598	560	642

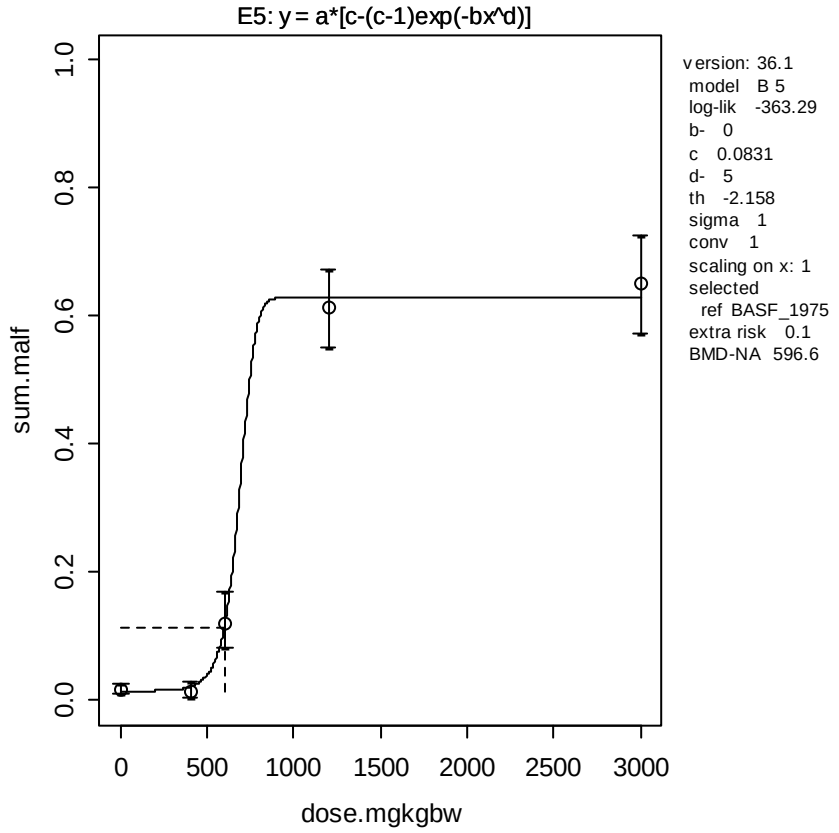

Figure 1: Dose response curve (exponential model, m5) of sum of malformations on day 9 in mice. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from BASF (1975).

Table 2: ED_{10}s obtained from the cleft palate data in mice (BASF, 1976)

model	npar	loglik	Accept	ED_{10} (mg/kg bw/dy)	ED_{10}L	ED_{10}U
null	1	-148.15	--	NA	NA	NA
full	4	-115.19	--	NA	NA	NA
two-stage	3	-128.85	No	384	NA	NA
log-logist	3	-116.4	Yes	1054	1011	1087
Weibull	3	-116.4	Yes	1048	817	1089
log-prob	3	-116.4	Yes	926	609	981
gamma	3	-116.4	Yes	969	919	1021
logistic	2	-120.8	No	512	NA	NA
probit	2	-121.92	No	487	NA	NA
exponential (m3)	3	-116.41	Yes	963	645	1000
Hill(m3)	3	-116.43	Yes	**844**	581	912

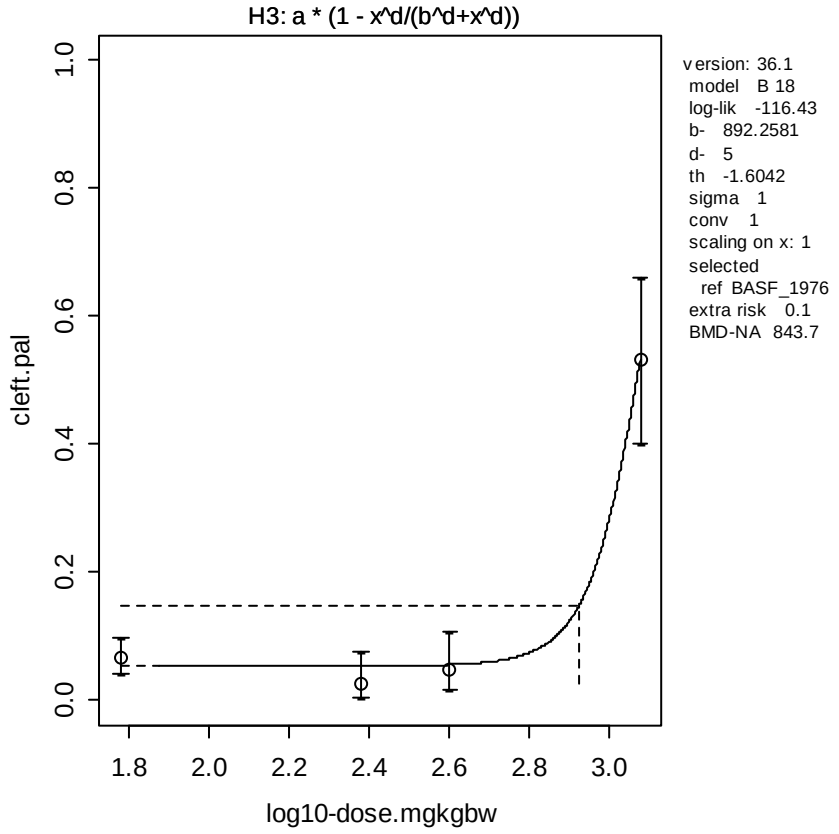

Figure 2: Dose response curve (Hill model, m3) of cleft palate in mice. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from BASF (1976).

Table 3: ED_{10}s obtained from the fused ribs data in mice (BASF, 1976)

model	npar	loglik	Accept	ED_{10} (mg/kg bw/dy)	ED_{10}L	ED_{10}U
null	1	-288.26	--	NA	NA	NA
full	4	-87.2	--	NA	NA	NA
two-stage	3	-146.15	No	203	NA	NA
log-logist	3	-87.21	Yes	505	449	571
Weibull	3	-87.26	Yes	537	471	612
log-prob	3	-87.32	Yes	**484**	435	539
gamma	3	-87.22	Yes	499	447	558
logistic	2	-87.75	Yes	599	539	663
probit	2	-87.62	Yes	539	491	590
exponential (m2)	2	-87.62	Yes	540	488	594
Hill(m3)	3	-87.21	Yes	503	447	575

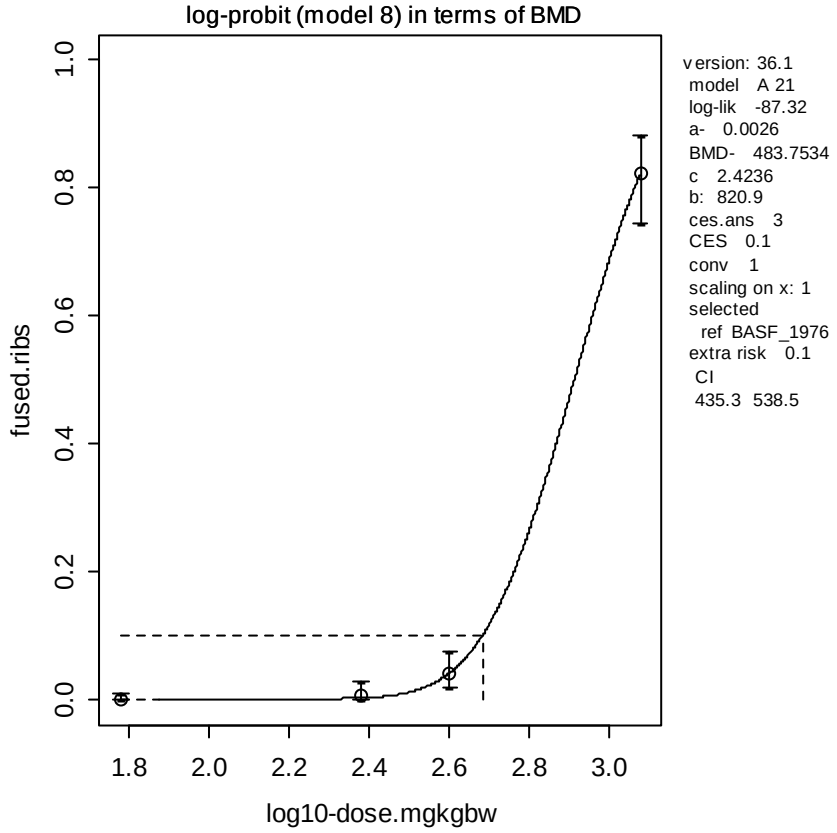

Figure 3: Dose response curve (log-probit model) of fused ribs in mice. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from BASF (1976).

Table 4: ED_{10}s obtained from the sum of malformations data in rat (Johanssen et al 1987, Haskell Lab 1997).

model	covar	npar	loglik	accept	ED_{10} (mg/kg bw/dy)	ED_{10}L	ED_{10}U	level
null	NA	1	-788.79	--	NA	NA	NA	--
full	NA	9	-354.93	--	NA	NA	NA	--
two-stage	b	4	-498.61	No	89.5	NA	NA	haskell_1997
log-logist	b	4	-358.41	Yes	236	214	269	haskell_1997
Weibull	b	4	-358.65	Yes	250	226	286	haskell_1997
log-prob	b	4	-358	Yes	**217**	200	240	haskell_1997
gamma	b	4	-358.14	Yes	226	208	250	haskell_1997
logistic	b	3	-361.71	No	247	NA	NA	haskell_1997
probit	ab	4	-365.42	No	222	NA	NA	haskell_1997
exponential (m3)	b	4	-359.32	Yes	277	242	341	haskell_1997
Hill(m3)	b	4	-358.59	Yes	237	213	275	haskell_1997

Covariate = reference

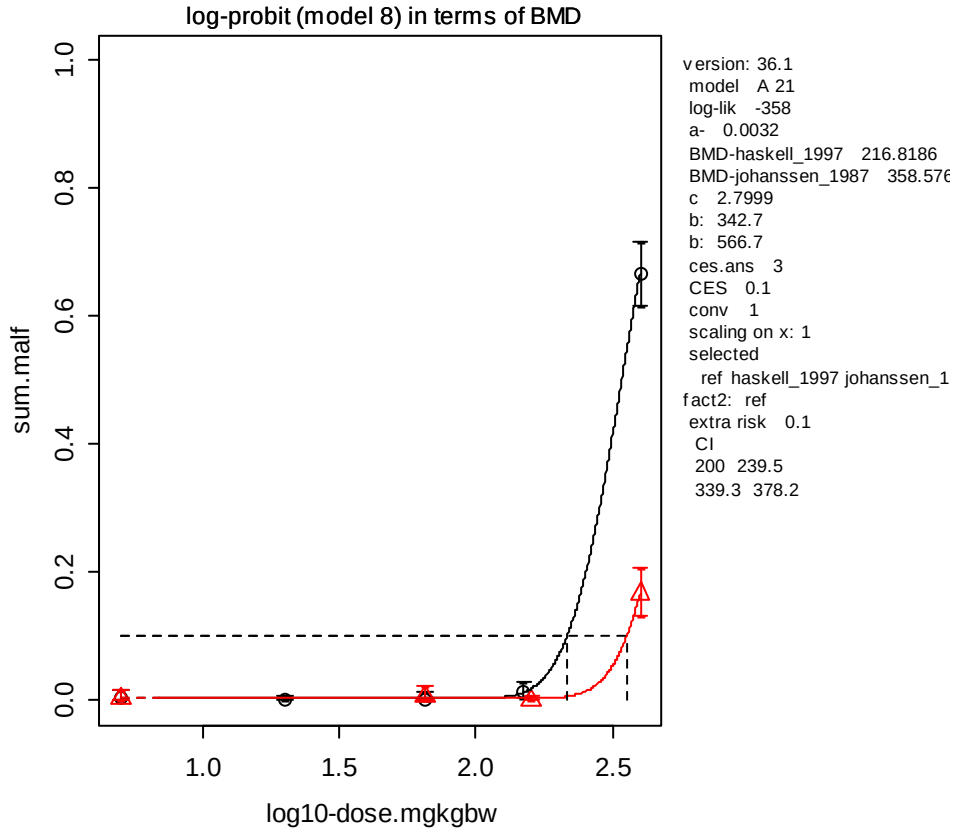

Figure 4: Dose response curve (log-probit model) of sum of malformations in rat. The horizontal dashed line represents 10% extra risk and the vertical dashed lines are located at the ED_{10}. Data are from Haskell Lab (1997, circles) and Johanssen et al. (1987, triangles).

Table 5: ED_{10}s obtained from the heart & vessel malformations data in rat (Johanssen et al 1987, Haskell Lab 1997).

model	covar	npar	Loglik	accept	ED_{10} (mg/kg bw/dy)	$ED_{10}L$	$ED_{10}U$	level
null	NA	1	-484.97	--	NA	NA	NA	--
full	NA	9	-232.26	--	NA	NA	NA	--
two-stage	b	4	-304.85	no	92.9	NA	NA	haskell_1997
log-logist	b	4	-234.16	yes	269	231	400	haskell_1997
Weibull	b	4	-234.21	yes	284	252	363	haskell_1997
log-prob	b	4	-234.04	yes	**244**	220	322	haskell_1997
gamma	b	4	-234.09	yes	255	226	325	haskell_1997
logistic	b	3	-234.13	yes	288	265	310	haskell_1997
probit	a	3	-233.81	yes	255	NA	NA	haskell_1997
exponential (m2)	a	3	-233.81	yes	255	232	277	haskell_1997
Hill(m3)	a	4	-233.42	yes	268	224	301	haskell_1997

Covariate = reference

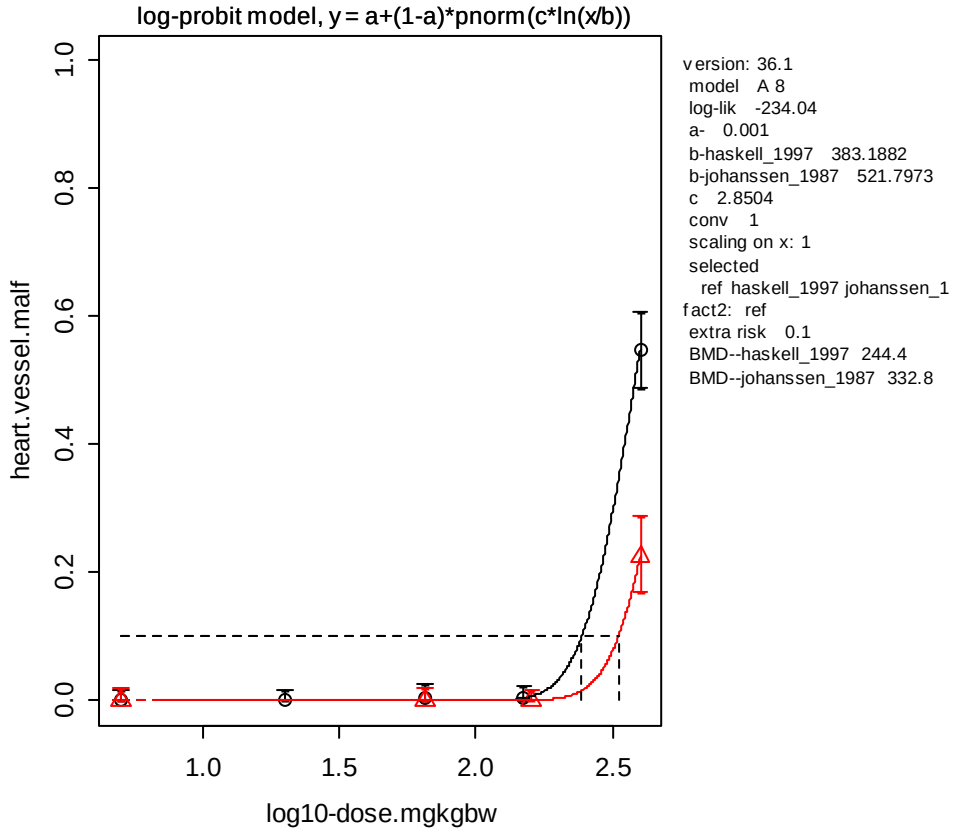

Figure 5: Dose response curve (log-probit model) of heart & vessel malformations in rat. The horizontal dashed line represents 10% extra risk and the vertical dashed lines are located at the ED_{10}. Data are from Haskell Lab (1997, circles) and Johanssen et al. (1987, triangles).

Table 6: ED_{10}s obtained from sum of malformations data in rabbit (BASF 1974, Merkle and Zeller 1980).

model	covar	npar	loglik	accept	ED_{10} (mg/kg bw/dy)	$ED_{10}L$	$ED_{10}U$
null	NA	1	-38.89	--	NA	NA	NA
full	NA	6	-26.9	--	NA	NA	NA
two-stage	--	3	-31.14	No	536	NA	NA
log-logist	--	3	-27.88	Yes	286	264	386
Weibull	--	3	-27.88	Yes	286	265	382
log-prob	--	3	-27.88	Yes	290	259	416
gamma	--	3	-27.88	Yes	289	262	392
logistic	--	2	-27.88	Yes	**284**	271	332
probit	--	2	-27.88	Yes	287	NA	NA
exponential (m2)	--	2	-27.89	Yes	292	267	346
Hill(m2)	--	2	-28.81	Yes	347	263	477

Covariate = reference

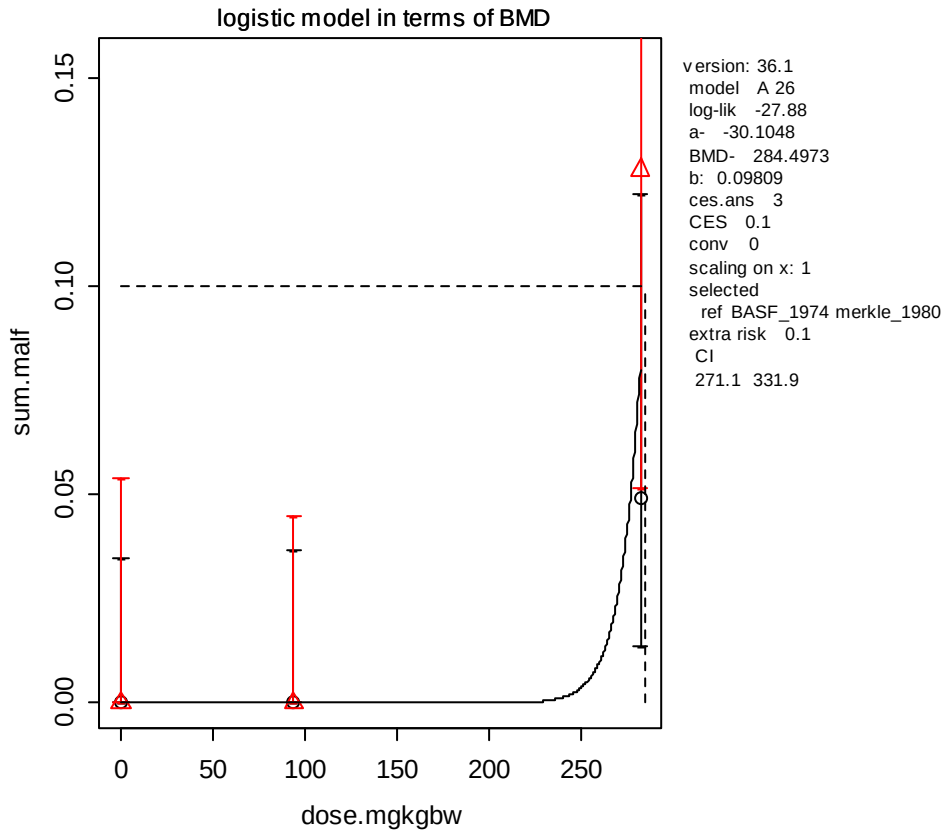

Figure 6: Dose response curve (logistic model) of sum of malformations in rabbit. The horizontal dashed line represents 10% extra risk and the vertical dashed lines are located at the ED_{10}. Data are from BASF (1974, circles) and Merkle and Zeller (1980, triangles).

Table 7: ED_{10}s obtained from the heart and vessel malformations data in rat (Okuda et al. 2006).

model	npar	loglik	Accept	ED_{10} (mg/m^3)	ED_{10}L	ED_{10}U
null	1	-102.74	--	NA	NA	NA
full	5	-64.72	--	NA	NA	NA
two-stage	3	-78.67	No	866	NA	NA
log-logist	3	-65.35	Yes	1480	1310	1640
Weibull	3	-65.14	Yes	1490	1320	1650
log-prob	3	-65.9	Yes	**1440**	1280	1660
gamma	3	-65.63	Yes	1460	1290	1640
logistic	2	-65.05	Yes	1540	1380	1670
exponential (m2)	2	-65.19	Yes	1490	1330	1620
Hill(m3)	3	-65.6	Yes	1490	1300	1680

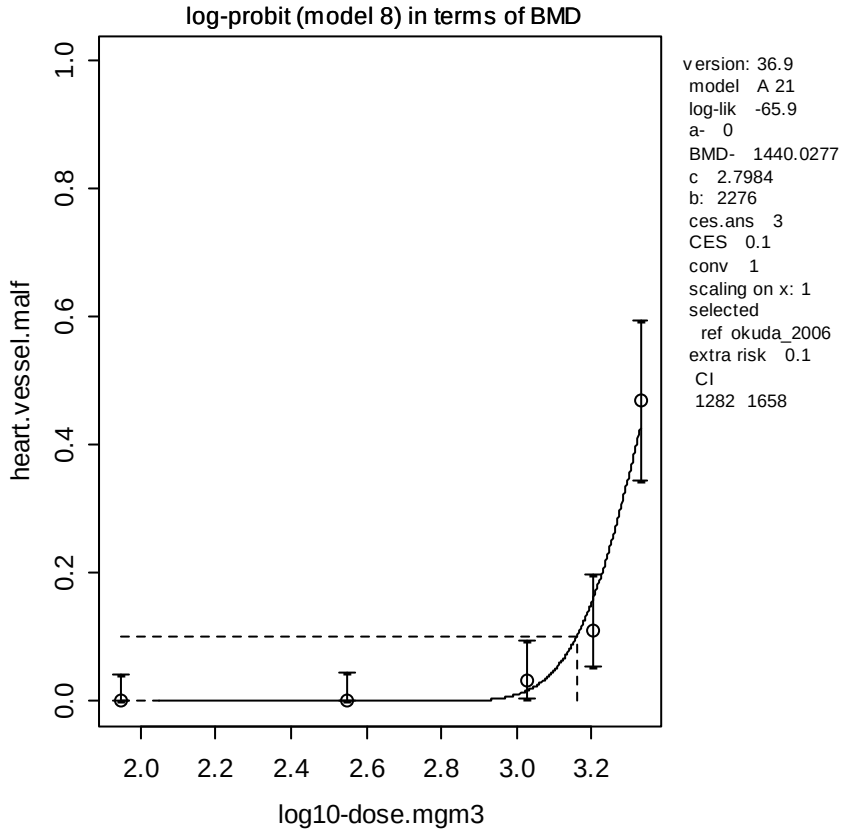

Figure 7: Dose response curve (log-probit model) of heart & vessel malformations in rat. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from Okuda et al. (2006).

Table 8: ED_{10}s obtained from the ventrical septal defect data in rat (Okuda et al. 2006).

model	npar	loglik	accept	ED_{10} (mg/m^3)	$ED_{10}L$	$ED_{10}U$
null	1	-100.56	--	NA	NA	NA
full	5	-64.55	--	NA	NA	NA
two-stage	3	-77.43	no	898	NA	NA
log-logist	3	-65.09	yes	1480	1310	1650
Weibull	3	-64.91	yes	1490	1320	1640
log-prob	3	-65.57	yes	**1440**	1280	1650
gamma	3	-65.33	yes	1460	1290	1630
logistic	2	-64.87	yes	1540	1380	1670
exponential (m2)	2	-64.95	yes	1500	1340	1630
Hill(m3)	3	-65.33	yes	1480	1300	1670

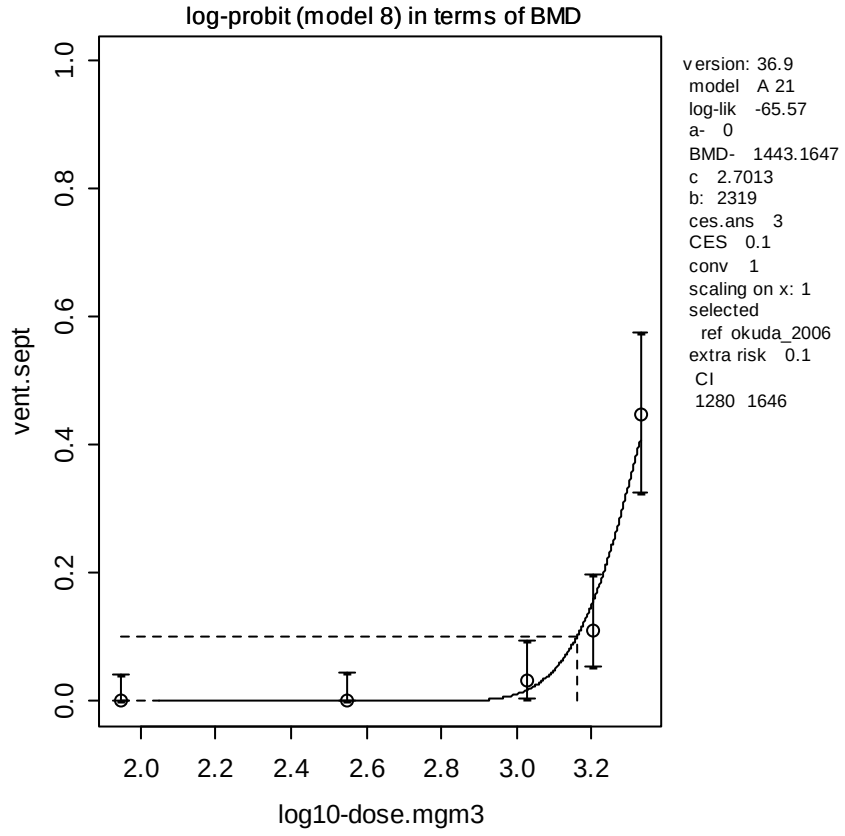

Figure 8: Dose response curve (log-probit model) of ventrical septal defect in rat. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from Okuda et al. (2006).

Table 9: ED_{10}s obtained from the persistent truncus arteriosis data in rat (Okuda et al. 2006).

model	npar	loglik	accept	ED_{10} (mg/m^3)	$ED_{10}L$	$ED_{10}U$
null	1	-56.95	--	NA	NA	NA
full	5	-36.14	--	NA	NA	NA
two-stage	3	-45.97	no	2110	NA	NA
log-logist	3	-36.22	yes	1870	1720	1990
Weibull	3	-36.23	yes	1880	1730	2000
log-prob	3	-36.16	yes	**1840**	1690	1970
gamma	3	-36.17	yes	1850	1700	1980
logistic	2	-56.82	no	150000	NA	NA
exponential (m2)	2	-36.32	yes	1850	1720	1990
Hill(m3)	3	-36.17	yes	1850	1700	1980

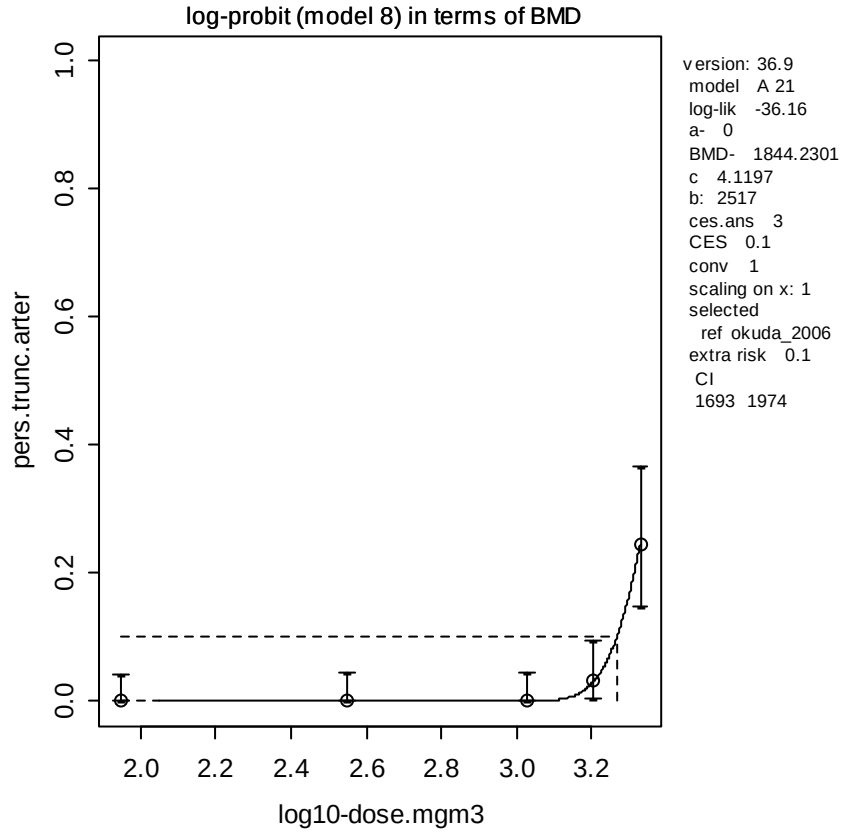

Figure 9: Dose response curve (log-probit model) of persistent truncus arteriosis in rat. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from Okuda et al. (2006).

Table 10: ED_{10}s obtained from the malpositioned subclavian branch data in rat (Okuda et al., 2006).

model	npar	loglik	accept	ED_{10} (mg/m^3)	$ED_{10}L$	$ED_{10}U$
null	1	-21.35	--	NA	NA	NA
full	5	-13.85	--	NA	NA	NA
two-stage	3	-18.12	no	7670	NA	NA
log-logist	3	-13.85	yes	**2140**	2050	2510
Weibull	3	-13.85	yes	**2140**	2060	2500
log-prob	3	-13.85	yes	**2140**	2040	2600
gamma	3	-13.85	yes	**2140**	2040	2560
logistic	2	-21.34	no	1340000	NA	NA
exponential (m2)	2	-14.53	yes	2250	2030	2710
Hill(m3)	3	-13.86	yes	2150	2040	2590

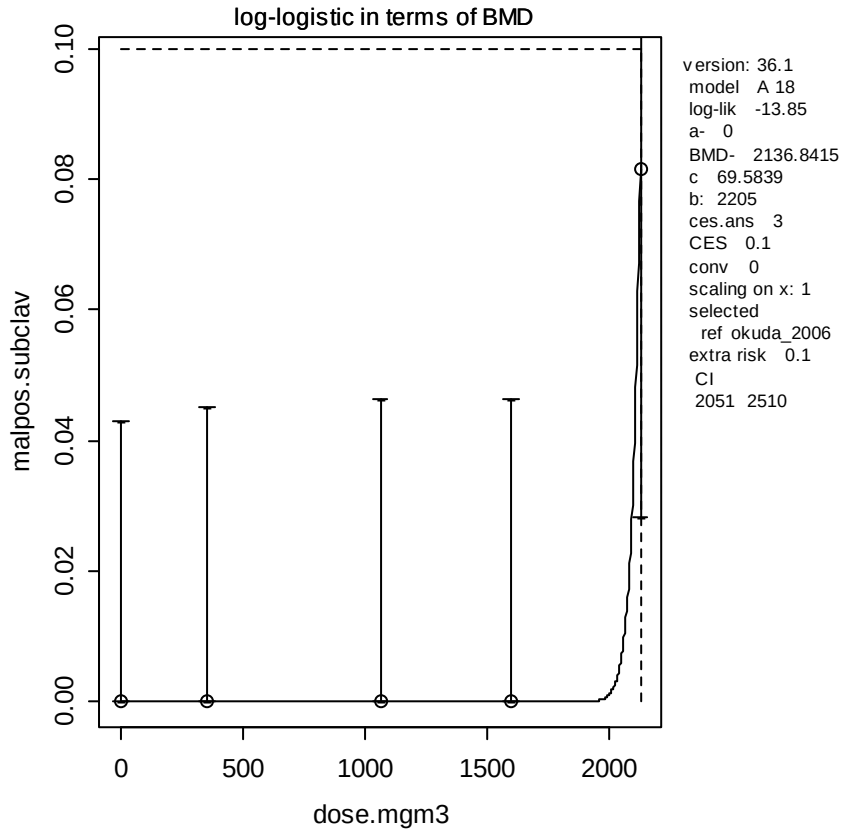

Figure 10: Dose response curve (log-logistic model) of malpositioned subclavian branch in rat. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from Okuda et al. (2006).

Table 11: ED_{10}s obtained from the retro-oesophagal subclavian data in rat (Okuda et al. 2006).

model	npar	loglik	Accept	ED_{10} (mg/m^3)	$ED_{10}L$	$ED_{10}U$
null	1	-16.88	--	NA	NA	NA
full	5	-11.29	--	NA	NA	NA
two-stage	3	-14.47	No	10300	NA	NA
log-logist	3	-11.29	Yes	4070	2120	4070
Weibull	3	-11.29	Yes	4070	2120	4070
log-prob	3	-11.29	yes	2170	2110	4110
Gamma	3	-11.29	yes	**2160**	2170	2240
logistic	2	-16.88	no	2280000	NA	NA
exponential (m2)	2	-11.79	yes	2350	2090	3110
Hill(m3)	3	-11.29	yes	2190	2100	2310

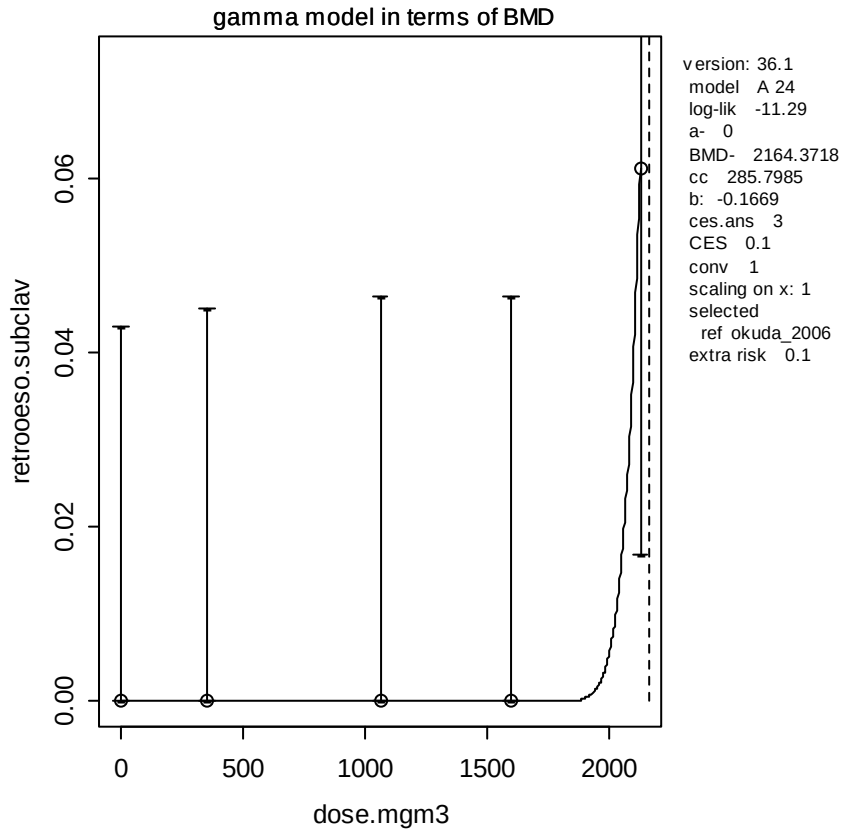

Figure 10: Dose response curve (gamma model) of the retro-oesophagal subclavian in rat. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from Okuda et al. (2006).

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Annex 4. Calculations of ED10 values by linear interpolation.

Mouse oral (BASF 1975)

- ***Malformations (visceral + skeletal) after single oral dose on day 9:***

Dose-level (mg/kg bw/day)	No. of malformations	Percent effect
0	15/838	1.8
400	4/300	1.3
600	22/181	12
1200	115/187	61
3000	75/115	65

- Effect-level at control is 1.8 %. Effect-level at ED_{10} is 11.8% (i.e.1.8% + 10%)
- 400 mg/kg bw/day < ED10 < 600 mg/kg bw/day
- An increase of 200 mg/kg bw/day (i.e. 600-400 mg/kg bw/day) ~ an increase in malformations incidence of 10.7% (i.e.12-1.3%)
- 1% change ~18.7 mg/kg bw/day increase in dose
- ED10 = 400 + (11.8%- 1.3%) *18.7 = 596 mg/kg bw/day

Mouse oral (BASF 1976a)

- ***Selected malformations, cleft palate:***

Dose-level (mg/kg bw/day)	Cleft palate (number of fetuses)	Percent effect
0	16/239	6.7
240	2/80	2.5
400	4/83	4.8
1200	24/45	53

- Effect-level at control is 6.7 %. Effect-level at ED10 is 16.7% (i.e.6.7% + 10%)
- 400 mg/kg bw/day < ED10 < 1200 mg/kg bw/day
- An increase of 800 mg/kg bw/day (i.e. 1200-400 mg/kg bw/day) ~ an increase in cleft palate incidence of 48.2% (i.e. 53-4.8%)
- 1% change ~16.6 mg/kg bw/day increase in dose
- ED10 = 400 + (16.7%- 4.8%) *16.6 = 598 mg/kg bw/day

- ***Selected malformations, fused ribs:***

Dose-level (mg/kg bw/day)	Fused ribs (number of fetuses)	Percent effect
0	1/476	0.2
240	1/160	0.6
400	7/169	4.1
1200	79/96	82

- Effect-level at control is 0.2 %. Effect-level at ED10 is 10.2% (i.e.0.2% + 10%)
- 400 mg/kg bw/day < ED10 < 1200 mg/kg bw/day
- An increase of 800 mg/kg bw/day (i.e. 1200-400 mg/kg bw/day) ~ an increase in fused ribs incidence of 77.9% (i.e.82-4.1%)
- 1% change ~ 10.3 mg/kg bw/day increase in dose
- ED10 = 400 + (10.2%- 4.1%) *10.3 =463 mg/kg bw/day

Rat oral (Johannsen et al. 1987)

- ***Heart and vessel malformations***

Dose-level (mg/kg bw/day)	Heart and vessel malformations (number)	Percent effect
0	0/142	0
65	0/136	0
160	0/164	0
400	33/146	23

- Effect-level at control is 0 %. Effect-level at ED10 is 10% (i.e. 0% + 10%)
- 160 mg/kg bw/day< ED_{10} < 400 mg/kg bw/day
- Increase in dosing of 240 mg/kg bw/day (i.e. 400-160 mg/kg bw/day) ~ an increase of 23% (i.e. 23-0%) in number of heart and vessel malformations
- 1% change ~10.4 mg/kg bw/day increase in dose
- ED10 = 160 + 10* 10.4 = 264 mg/kg bw/day

- ***Sum of malformations***

Dose-level (mg/kg bw/day)	Sum of malformations (number)	Percent effect
0	1/287	0.3
65	2/273	0.7
160	0/336	0
400	49/492	10

- Effect-level at control is 0.3 %. Effect-level at ED10 is 10.3% (i.e. 0.3% + 10%)
- ED_{10} at 400 mg/kg bw/day

Rat oral (Haskell Lab 1997)

- ***Heart and vessel malformations***

Dose-level (mg/kg bw/day)	Heart and vessel malformations (number)	Percent effect
0	0/184	0

20	0/172	0
65	1/177	0.6
150	1/190	0.5
400	113/206	55

- Effect-level at control is 0 %. Effect-level at ED10 is 10%
- 150 mg/kg bw/day < ED_{10} < 400 mg/kg bw/day
- Increase in dosing of 250 mg/kg bw/day (i.e. 400-150 mg/kg bw/day) ~ an increase of 54.5% (i.e. 55-0.5%) in number of heart and vessel malformations
- 1% change ~4.6 mg/kg bw/day increase in dose
- ED10 = 150 + (10 – 0.5) * 4.6 = 194 mg/kg bw/day

- ***Sum of malformations***

Dose-level (mg/kg bw/day)	**Sum of malformations**	**Percent effect**
0	2/338	0.6
20	0/327	0
65	1/339	0.3
150	5/362	1.4
400	167/250	67

- Effect-level at control is 0.6 %. Effect-level at ED10 is 10.6% (i.e. 0.6% + 10%)
- 150 mg/kg bw/day < ED_{10} < 400 mg/kg bw/day
- Increase in dosing of 250 mg/kg bw/day (i.e. 400-150 mg/kg bw/day) ~ an increase of 65.6% (i.e. 67-1.4%) in sum of malformations
- 1% change ~3.8 mg/kg bw/day increase in dose
- ED10 = 150 + (10.6 - 1.4) * 3.8 = 185 mg/kg bw/day

Rabbit oral (BASF 1974)

- ***Sum of malformations***

Dose-level (mg/kg bw/day)	**Sum of malformations**	**Percent effect**
0	0/85	0
94	0/80	0
282	3/61	4.9
846	No data	-

- Effect-level at control is 0 %. Effect-level at ED10 is 10%
- ED_{10} > 282 mg/kg bw/day
- no interpolation possible

Rabbit oral (Merkle and Zeller 1980; BASF 1976[e])

- ***Sum of malformations***

Dose-level (mg/kg bw/day)	Sum of malformations	Percent effect
0	0/54	0
94	0/65	0
282	5/39	13
470	No data	-

- Effect-level at control is 0 %. Effect-level at ED10 is 10%
- 94 <ED_{10} < 282 mg/kg bw/day
- Increase in dosing of 188 mg/kg bw/day (i.e. 282- 94 mg/kg bw/day) ~ an increase of 13% (i.e. 13-0%) in sum of malformations
- 1% change ~ 14.5 mg/kg bw/day increase in dose
- ED10 = 94 + (10 - 0) * 14.5 = 239 mg/kg bw/day

Rat inhalation (Okuda et al. 2006)

- ***Total heart/great vessels malformations***

Test concentration (ppm)	Dose-level (mg/kg bw/day)	Total heart/great vessel malformations	Percent effect
0	0	0/68	0
100	95	0/65	0
300	287	2/63	3.2
450	432	7/63	11
600	575	23/49	47

- Effect-level at control is 0 %. Effect-level at ED10 is 10%
- 287 mg/kg bw/day < ED_{10} <432 mg/kg bw/day
- Increase in dosing of 145 mg/kg bw/day (i.e. 432-287 mg/kg bw/day) ~ an increase of 7.8 % (i.e. 11-3.2%) in total heart/great vessel malformations
- 1% change ~18.6 mg/kg bw/day increase in dose
- ED10 = 287 + (10 – 3.2) * 18.6 =413 mg/kg bw/day

References to Annex 4

See the reference list to the main document.