National Institute for Public Health
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Ministry of Health, Welfare and Sport

Probit function technical support document

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substance name	CAS number
Dimethyl sulfate	**77-78-1**

This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

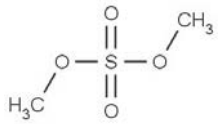
This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have had the opportunity to comment on the derivation of the proposed probit function. The status of this document has now been raised to "interim", pending a decision on its formal implementation.

The decision on actual implementation depends on the results of a further consequence analysis.

Detailed information on the procedures for the derivation, evaluation and formalization of probit functions is available at http://www.rivm.nl/.

Technical support document dimethyl sulfate

1. Substance identification



CAS-number:	77-78-1
IUPAC name:	Sulfuric acid, dimethyl ester
Synonyms:	Dimethyl sulfate, dimethyl monosulfate; methyl sulfate; sulfuric acid dimethyl ester, DMS
Molecular formula:	$(CH_3)_2SO_4$
Molecular weight:	126.13 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	188°C - decomposes (at 101.3 kPa)
Vapour pressure:	0.07 kPa (at 20°C)
Saturated vapour conc:	700 ppm = 3673 mg/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.191 ppm (at 20°C and 101.3 kPa)
	1 ppm = 5.247 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H-301-314-317-330-341-350

2. Mechanism of action and toxicological effects following acute exposure

Acute effects: Dimethyl sulfate (DMS) is a potent alkylating agent. The main target organs and tissues for inhalation exposure to DMS are the cornea, conjunctiva, skin and respiratory tract. DMS dissolves in the mucous membranes of the respiratory tract and eyes to form methanol and methyl sulphate, and further to sulphuric acid, a strong acid that produces coagulative necrosis. The health endpoints are all related to the corrosive properties of DMS. Symptoms of high exposure are laboured breathing, secretions from nose, mouth and eyes and prostration.
Damage occurs in the respiratory system, particularly the upper respiratory tract resulting in mucus secretion, upper airway and/or pulmonary oedema and laryngospasm. The resulting hypoxemia will cause CNS and cardiovascular (myocardial ischemia) effects. Respiratory damage proceeds to inflammation, degeneration and necrosis of affected tissue, atelectasis, emphysema and finally death.
Long-term effects: Chronic exposure produces essentially the same type of health effects. IARC considers dimethyl sulfate probably carcinogenic to humans (Group 2A).

3. Human toxicity data

Lethality after single inhalation exposures to DMS has been documented (NRC 2010). No informative reports on human toxicity following acute inhalation exposure were identified in which details about both health effects and the exposure have been documented in sufficient detail.

4. Animal acute toxicity data

During the literature search the following technical support documents and databases were consulted:

1. AEGL final TSD, ERPG document and EU RAR and reference database for dimethyl sulfate, covering references before and including 1995.
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, ECHA, IUCLID, RTECS, IRIS and ToxNet with the following search terms:
   - Substance name and synonyms
   - CAS number
   - lethal*

- mortal*
- fatal*
- LC_{50}, LC
- probit

3. Unpublished data were sought through networks of toxicological scientists.

Animal lethal toxicity data focused on acute exposure are described in Appendix 1. A total of 5 studies were identified -with 11 datasets for 4 species- with data on lethality following acute inhalation exposure. no datasets was/were assigned status A for deriving the human probit function, 5 datasets was/were assigned status B and 6 were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

One study was identified in which sensory irritation of DMS was studied (Mathison 1995). In this study 15 min exposure to concentrations up to and including 42 mg/m^3 did not reduce the respiratory rate. At 115 mg/m^3 the respiratory rate decreased to 78% of control (a 22% reduction), without reduction of tidal volume.

5. Probit functions from individual studies

All available acute lethality data on dimethyl sulfate are displayed in Figure 1.

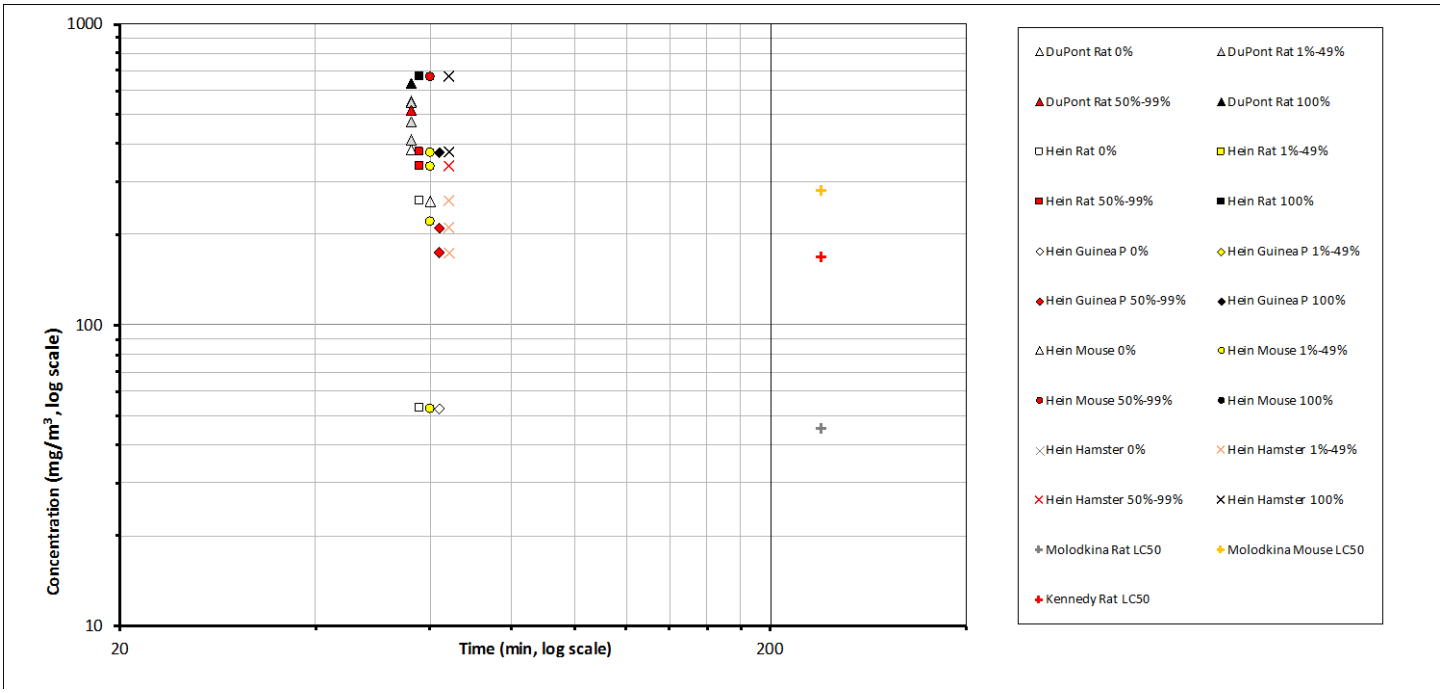


Figure 1 *All available acute lethality data for dimethyl sulfate (all observations at 60 min.).*

The data that were selected for primary analysis of the animal probit function are presented in Table 2 and Figure 3.

It was possible to derive a probit function for dimethyl sulfate based on the available studies with B1 quality by pooling data. Therefore, the probit function was derived using 60-minute data from the studies with B1 quality, none of which enabled to produce a concentration-time-lethality relationship.

Probit functions have been calculated and reported in Appendix 1 for each of the reported studies. The results of the calculations are presented in the table below.

Table 1 *Data selected for the initial analysis of the animal probit function of dimethyl sulfate.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50}, 60 minutes (mg/m^3) 95% C.I.	n-value 95% C.I.
B1.1	Rat	*60-min LC_{50}*	543 (504 – 601)	N/A
B1.2	Rat	*60-min LC_{50}*	343 (unable to estimate cfd-i)	N/A
B1.2	Mouse	*60-min LC_{50}*	693 (unable to estimate cfd-i)	N/A
B1.2	Guinea pig	*60-min LC_{50}*	151 (24.2 – 215)	N/A
B1.2	Hamster	*60-min LC_{50}*	264 (203 – 339)	N/A

The data of studies B1.1 (rats) and B1.2 (rats, mice, guinea pigs, hamsters) are presented graphically below.

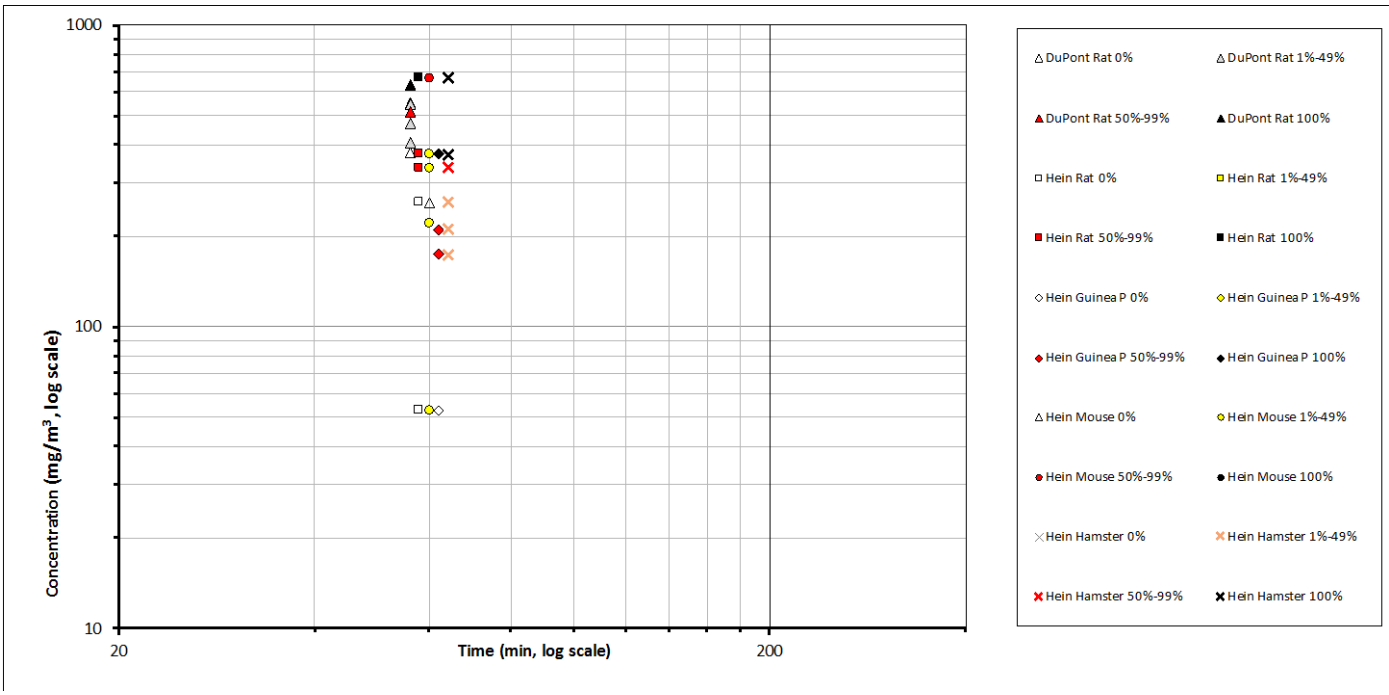


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of dimethyl sulfate (all observations at 60 min.).*

Data from studies B1.1 and B1.2 (all species) were selected for the final dataset for the derivation of the animal probit function. The final data eligible for calculating the animal probit function contains 5 datasets from 2 studies and includes data from 4 animal species.

Table 2 *Data selected for derivation of the animal probit function of dimethyl sulfate.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50}, 60 minutes (mg/m^3) 95% C.I.	n-value 95% C.I.
B1.1	Rat	*60-min LC_{50}*	543 (504 – 601)	N/A
B1.2	Rat	*60-min LC_{50}*	343 (unable to estimate cfd-i)	N/A
B1.2	Mouse	*60-min LC_{50}*	693 (unable to estimate cfd-i)	N/A
B1.2	Guinea pig	*60-min LC_{50}*	151 (24.2 – 215)	N/A
B1.2	Hamster	*60-min LC_{50}*	264 (203 – 339)	N/A

The data of the selected datasets are presented graphically below.

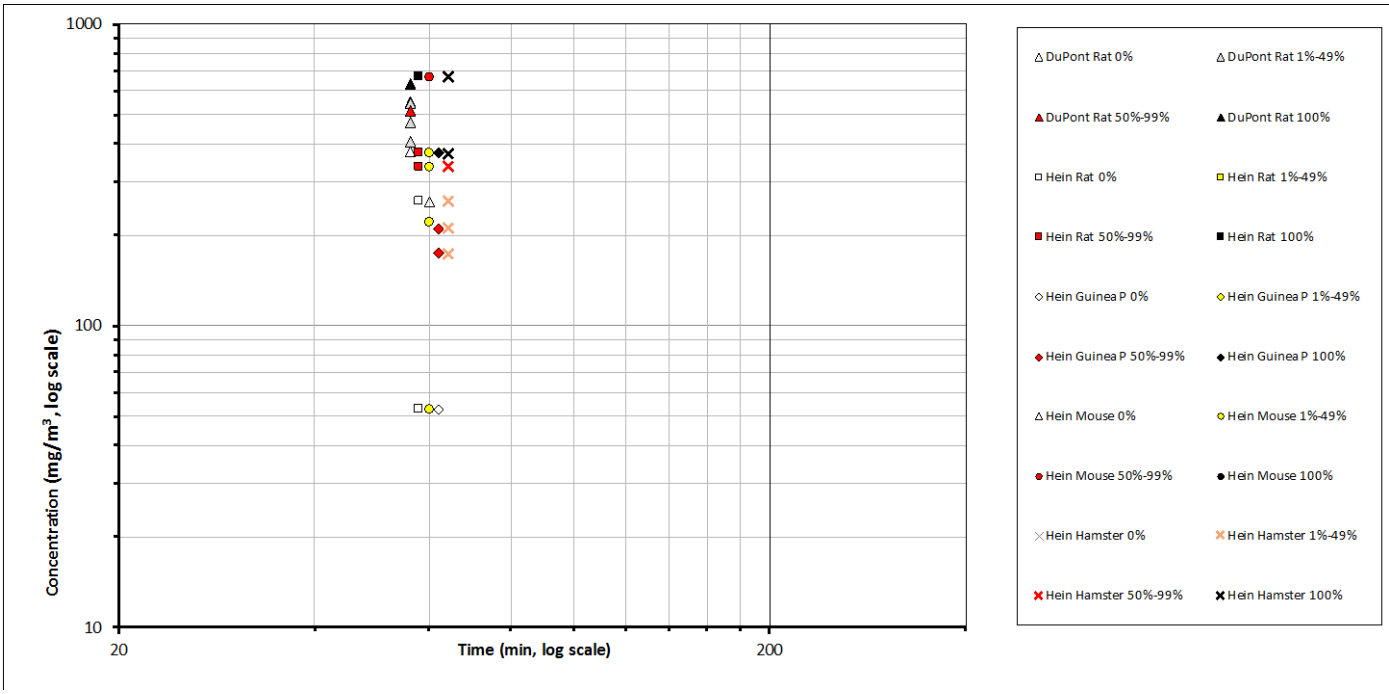


Figure 3 *Final data selected for derivation of the animal probit function of dimethyl sulfate (all observations at 60 min., identical to figure 2).*

6. Derivation of the human probit function

There were no A-quality studies available. To derive the human probit function the results from studies B1.1 and B1.2 (all species) have been used to derive a point of departure.

First, the species-specific geometric mean LC_{50}-values were calculated. The geometric mean LC_{50} value for the rat was 432 mg/m^3; for the other species, only data from study B1.2 (Hein) were available. The 60-minute geometric mean LC_{50} value was 330.4 mg/m^3.

In absence of information on the n-value, the default n-value of 2 was used for the probit function.

The human equivalent LC_{50} was calculated by applying the following assessment factors:

Table 3 *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation	3	No reason to deviate from the default factor of 3.
Nominal concentration	1	Chamber concentration was analysed. The maximum tested concentration in the studies was <25% of the saturated vapour concentration.
Adequacy of database:	1	2 reasonable B1 studies, support from C-studies that the mouse is less susceptible than the rat.

The estimated human equivalent 60-minute LC_{50} value is 330.4 / 3 = **110 mg/m^3**.

No reliable experimentally determined n-value was available, so the default n-value of **2** was used. Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1**.

The human probit function is then calculated on the human equivalent 60 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1 \times \ln(110^2 \times 60)$ resulting in the a-value of **-8.498**.

$Pr = -8.50 + 1 \times \ln(C^2 \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically acceptable basis. The probit function is based on 2 studies in the rat, mouse, guinea pig and hamster with B1 quality, with a total of 225 animals exposed for 60 minutes to concentrations ranging from 50 to 670 mg/m^3.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 34 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 24 mg/m^3.

Table 4 *LC-vales calculated with the derived probit function compared with existing acute inhalation exposure guidelines.*

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
0.1% lethality, this probit	33	24
1% lethality, this probit	49	34
AEGL-3 (2006, interim)	12	8.4
ERPG-3		N/A
LBW (2016)	30	24

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are roughly equal to the LBW values. AEGL values were set with higher assessment factors to allow a wider margin of safety for susceptible individuals, and are lower than the levels derived with this probit function.

Appendix 1 Animal experimental research

Study ID: B1.1

Author, year: ***DuPont 1971***
Substance: dimethyl sulfate
Species, strain, sex: male CHR-CD rats
Number/sex/conc. group: 6 / concentration, 5 groups
Age and weight: 'young', initial bodyweight 250-285 grams
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*GLP did not exist at the time*
Study carried out according to OECD 403 guideline(s)	*OECD guideline 403 did not exist at the time; many relevant details missing*
Stability of test compound in test atmosphere	*No information provided. Concentrations are well below the saturated vapour concentration. Aerosol formation due to (partial) hydrolysis cannot be excluded.*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*No information provided, but probably whole body.*
Type of restrainer	*No information, probably whole-body exposure.*
Pressure distribution	*No information provided.*
Homogeneity of test atmosphere in breathing zone of animals	*The test sample was delivered by a syringe drive into a stainless steel T-tube whose internal temperature was 180 °C. A metered stream of air passing through the T-tube carried the resultant vapours to the exposure chamber.*
Number of air changes per hour	*No information provided.*
Equilibration time (t95)	*No information to calcutate t95.*
Start of exposure relative to equilibration	*No information provided.*
Actual concentration measurement	*Chamber atmosphere was analyzed 2-3 times during the 1-hour exposures with a 'turbidimetric procedure'. Sampling location unspecified.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*No information provided*
Assessment of Reliability	***B1*** *Only 1 exposure duration, relevant details are missing (a.o. presence or absence of aerosol)*

This study was designed to assess the acute lethality and to assess the benefits of 3 medical treatments to prevent acute mortality. All data from the first study, and the data for animals that did not receive antidote treatment in the second study have been pooled.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Exposed	Fatal
CHR-CD rat	630		60	6	6
	551		60	6	1
	472		60	6	2
	472		60	6	1
	304		60	6	0[1]
Antidote study[2]	630		60	6	5
	546		60	6	2
	514		60	6	3
	409		60	6	1
	378		60	6	0

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, 2016) as

$Pr = a + b \times \ln C$

with C for concentration in mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male).

Species	a	b
Rat	-28.5	5.32

The LC_{50} value calculated with this model is as follows:

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I.
60	543 (504 – 601)

There were no data that allowed assessment of sex differences in the response to DMS. No C × t probit function could be calculated from these data alone.

[1] These animals were sacrificed after 1, 2 and 7 days for pathological examination and have been excluded from the probit calculation.
[2] Only data from animals without antidote treatment.

Study ID: B1.2

Author, year:	***Hein 1969***
Substance:	dimethyl sulfate
Species, strain, sex:	female Wistar rats, 100-300 gram female NMRI mice, 17-24 grams young guinea pigs, sex unknown, 250-300 grams female golden hamsters, 25-50grams
Number/sex/conc. group:	5-20 per group
Age and weight:	the animals' age was not specified
Observation period:	3 weeks

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*GLP did not exist at the time*
Study carried out according to OECD 403 guideline(s)	*OECD guideline 403 did not exist at the time*
Stability of test compound in test atmosphere	*No information provided. Concentrations are well below the saturated vapour concentration. Aerosol formation due to (partial) hydrolysis cannot be excluded.*
Use of vehicle (other than air)	*Filtered and dried room air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A (whole body)*
Pressure distribution	*No information provided.*
Homogeneity of test atmosphere in breathing zone of animals	*DMS was dripped on a large surface and allowed to vaporize in an air stream that was introduced into the chamber.*
Number of air changes per hour	*100-500 ℓ/h for a 224 ℓ chamber, which equals 9-11 air changes/h.*
Equilibration time (t95)	*1.3 - 6.7 minutes*
Start of exposure relative to equilibration	*After some equilibration time (unspecified) animals were placed through a lock, according to the author 'without appreciable drop of concentration'.*
Actual concentration measurement	*Chamber atmosphere was sampled 12 times per hour in the animal's breathing zone. DMS was trapped on an absorption tube and analysed with gas chromatography.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*No information provided.*
Assessment of Reliability	***B1*** *Only 1 exposure duration, otherwise reasonably well conducted study.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Exposed	Fatalities
Rat	666		60	5	5
Rat	373		60	5	3
Rat	336		60	5	3
Rat	257		60	5	0
Rat	52		60	5	0
Mouse	666		60	10	8
Mouse	373		60	10	2
Mouse	336		60	10	2
Mouse	257		60	20	0
Mouse	220		60	20	1
Mouse	52		60	20	1
Guinea Pig	373		60	5	5
Guinea Pig	210		60	5	3
Guinea Pig	173		60	5	4
Guinea Pig	52		60	5	0
Hamster	666		60	5	5
Hamster	373		60	5	5
Hamster	336		60	5	3
Hamster	257		60	5	2
Hamster	210		60	5	1
Hamster	173		60	5	1

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, 2016) as

$Pr = a + b \times \ln C$

with C for concentration in mg/m^3.

Species	a	b
Rat	-3.10	6.56
Mouse	-2.23	1.11
Guinea pig	-6.61	2.31
Hamster	-11.1	2.88

There were no data that allowed assessment of sex differences in the response to DMS. No C × t probit function could be calculated from these data alone.

None of the models fit very well, with the hamster data producing the best model fit. The data did not allow to calculate a confidence interval for the LC_{50} values in rats and mice, and an almost meaningless cfd-i for guinea pigs. The only trustworthy LC_{50} value was obtained for hamsters. The LC_{50} values as calculated by the author (method of Litchfield and Wilcoxon) have been added for comparison.

Species	LC_{50} 1 hr (mg/m^3) 95%-C.I. This review	LC_{50} 1 hr (mg/m^3) 95%-C.I. According to DuPont
Rat	343 (unable to estimate cfd-i)	336 (299 - 376)
Mouse	693 (unable to estimate cfd-i)	514 (402 - 630)
Guinea pig	151 (24.2 – 215)	168 (123 - 230)
Hamster	264 (203 – 339)	294 (196 - 441)

Study ID: C studies

BASF (1968) exposed rats (6 or 12/group) to 'saturated' vapour (3670 mg/m^3 if truly saturated) at 20°C. The mortality was documented after 3, 10, 30 and 60 minutes (unclear if this refers to mortality during exposure or including a post-exposure observation period). The mortality was 0/6 for 3 minutes, 0/12 for 10 minutes, 12/12 for 30 minutes and 6/6 for 60 minutes.

Ghiringelli (1957) exposed rats, mice and guinea pigs to concentrations of 394 mg/m^3 and determined the LT_{50}. It is not clear if the animals died during or after exposure. The reported LT_{50} values were 16 minutes for rats and guinea pigs and 40 minutes for mice. No concentration-time-mortality data were provided.

Smyth (1951) reported a range-finding study where 5/6 rats died within the 14-day observation period after receiving a single 4-hour exposure to 30 ppm (157 mg/m^3) DMS. The maximum duration for inhalation of saturated vapour without lethality was reported to be 2 minutes.

Molodkina (1979) found a 4-hour LC_{50} in rats of 45 mg/m^3 and in mice of 280 mg/m^3.

Kennedy (1991) found a 4-hour LC_{50} in rats of 168 mg/m^3. No details about the test design or the number of animals were given.

A number of range-finding studies with 1 or 2 animals, some very old (<1920) studies and secondary sources such as Flury and Zernik are not described.

Appendix 2 Reference list

AHLS. University of Arizona Emergency Medicine Research Center. Advanced Hazmat Life Support (AHLS). Provider Manual, 4th ed. Tucson, AZ, 2014.

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Smyth HF, CP Carpenter, CS Weil. Range-finding toxicity data: list IV. AMA Arch Indust Hyg Occup Med 1951;4:119-122.

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