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

Probit function technical support document

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substance name	CAS number
Hydrogen sulfide	**7783-06-4**

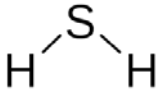
This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

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/en/Topics/P/Probit_functions.

Technical support document Hydrogen sulfide



1. Substance identification

CAS-number:	7783-06-4
IUPAC name:	hydrogen sulfide
Synonyms:	hydrosulfuric acid, sulfur hydrate, sulfurated hydrogen, dihydrogen monosulfide
Molecular formula:	H_2S
Molecular weight:	34.08 g/mol
Physical state:	gas (at 20°C and 101.3 kPa)
Boiling point:	-60°C (at 101.3 kPa)
Vapour pressure:	1880 kPa (at 20°C)
Saturated vapour conc:	N/A
Conversion factor:	1 mg/m^3 = 0.705 ppm (at 20°C and 101.3 kPa)
	1 ppm = 1.42 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H330

2. Mechanism of action and toxicological effects following acute exposure[1]

Acute effects: The main target organs and tissues for inhalation exposure to hydrogen sulfide are the respiratory system and the nervous system. Hydrogen sulfide causes ocular and respiratory irritation and may cause lung oedema. High exposure levels result in chemical asphyxia, which mainly affects nervous cells and (cardio)myocytes. The lack of oxygen in the brain may result in neurological symptoms and brain damage. Lethality results from respiratory arrest.
Long-term effects: Lack of oxygen in the brain due to acute exposure to hydrogen sulfide may result in permanent brain damage. There is no information available concerning the health consequences of chronic exposure to hydrogen sulfide.

3. Human toxicity data

No informative reports on health effects in humans following acute inhalation exposure were identified. Such reports are considered informative if both health effects as well as the exposure have been documented in sufficient detail.

Several reports of exposure of humans to lethal concentrations of hydrogen sulfide are available. Death resulted from respiratory failure, which was preceded by respiratory insufficiency, pulmonary oedema, coma or cyanosis. Usually loss of consciousness occurred rapidly after inhalation of hydrogen sulfide. None of the available reports provided adequate information concerning the level and duration of exposure. The highest volunteer exposure reported (Bhambhani et al. (1996b), cited in AEGL (2010)) showed no treatment-related effects after 30 minutes at 10 ppm (14.2 mg/m^3) exposure on oxygen uptake, carbon dioxide production, respiratory exchange ratio, heart rate, blood pressure, arterial blood oxygen, and carbon dioxide tensions.

4. Animal acute toxicity data

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

[1] AEGL 2010

1. AEGL final TSD (2010), ERPG document and EU RAR and reference database for hydrogen sulfide, covering references before and including 1995.
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, IUCLID, ECHA, RTECS, IRIS and ToxNet with the following search terms:
   - Hydrogen sulfide 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 9 studies were identified -with 11 datasets for 3 species- with data on lethality following acute inhalation exposure. 3 datasets were assigned status A for deriving the human probit function, 4 datasets were assigned status B1 and 4 were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

A single study was identified in which sensory irritation was studied. In this study, the following RD_{50} values were observed:

Table 1 *Sensory irritation data for hydrogen sulfide*

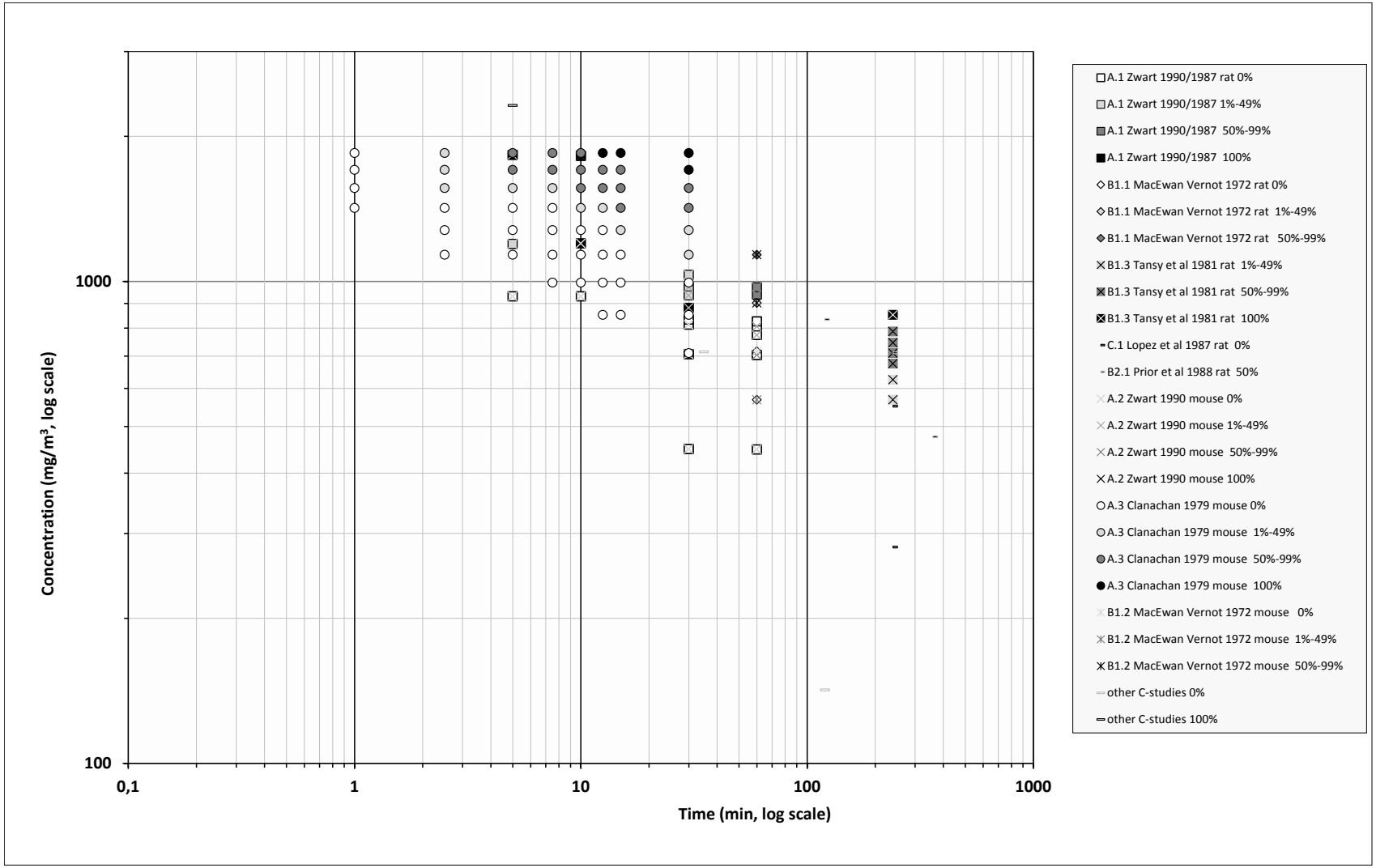
Species/strain	RD_{50} (mg/m^3)	Exposure duration (min)	Author/year
Rat	235^P	30	Arts, 2000

P: a plateau was reached

5. Probit functions from individual studies

All available acute lethality data on hydrogen sulfide are displayed in Figure 1.

1



2
3 ***Figure 1*** *All available acute lethality data for hydrogen sulfide.*

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

The rat A.1, B1.1 and B1.3 studies and the mouse A.2, A.3 and B1.2 studies were selected for derivation of the animal probit function for hydrogen sulfide. Study B2.1 was not included for the initial analysis, as, according to the guideline, B2-studies can only be used if there are no suitable A- or B1-studies.

To enable intra-species pooling, LC_{50}-values of rat B1-studies were scaled to 30-minutes using the n-value of study A.1 (8.27) and LC_{50}-values of mouse B1-study were scaled using the average mouse n-value of studies A.2 and A.3 (4.76) with the following formula (section 6):

$$LC_{50,c} = LC_{50,test}\left(\frac{t_{test}}{t_c}\right)^{(1/n)}$$

With $LC_{50,c}$ = scaled LC_{50} value for common exposure duration t_c
$LC_{50,test}$ = observed LC_{50} value for tested exposure duration
t_c = common exposure duration for intra-species pooling
t_{test} = tested exposure duration
n = species specific (for rat/mouse) n-value

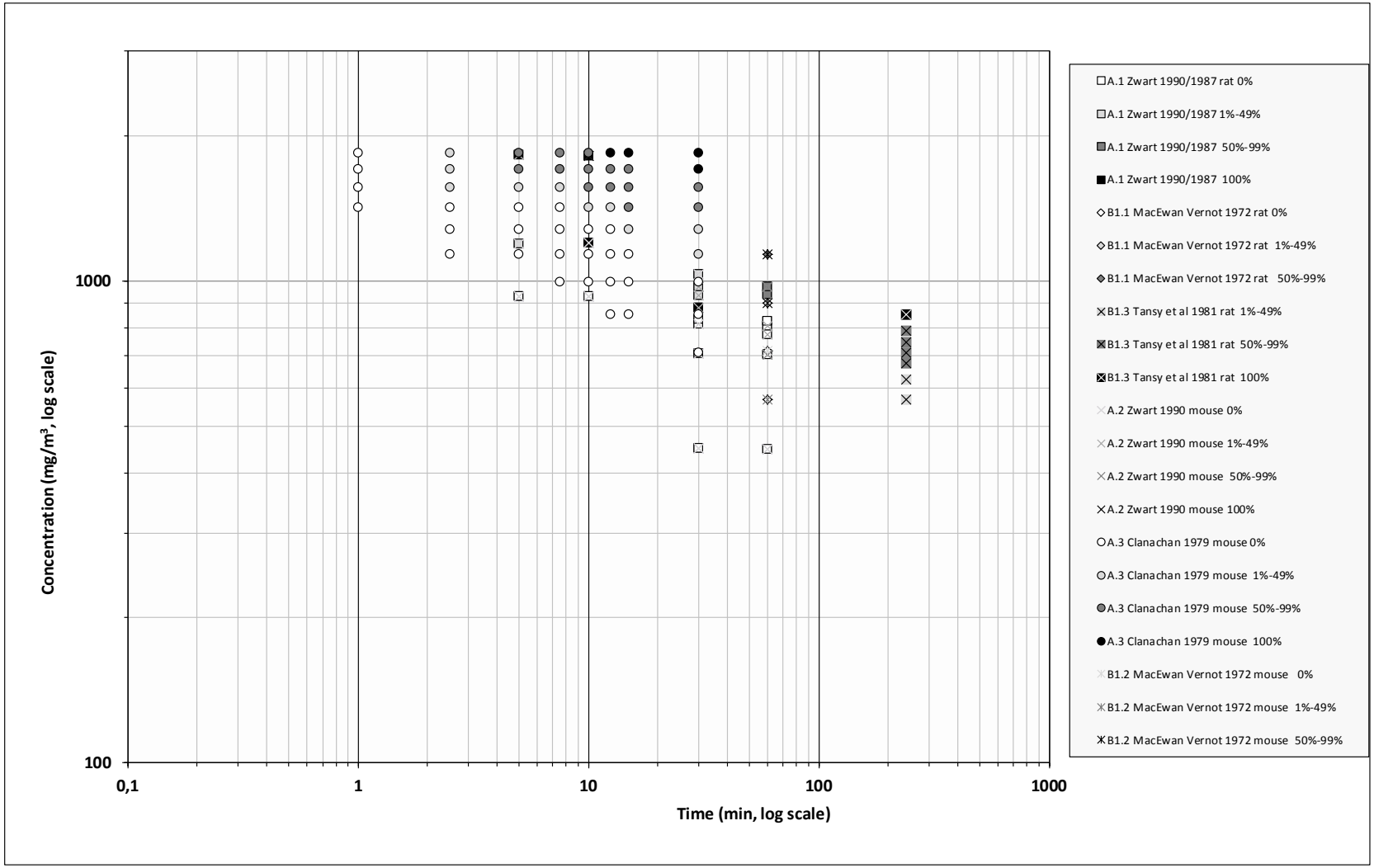
Probit functions have been calculated and reported in Appendix 1 for each of the reported studies. The results of the calculations are presented in Table 2.

Table 2 *Data selected for initial analysis of the animal probit function of hydrogen sulfide.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} at tested exposure duration (mg/m^3) 95% C.I.	LC_{50}, 30 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
A.1	Rat	-57.6 + 8.54xlnC + 1.03×lnt		1011 (968 - 1094)	8.27 (4.65 – 11.88)
B1.1	Rat	*60-min LC_{50}*	1012 (932-1100)	*1100*	
B1.3	Rat	*240-min LC_{50}*	638 (584-673)	*820*	
A.2	Mouse	-40.8 + 5.62×lnC + 1.88×lnt		1114 (1063 - 1193)	2.99 (2.50 – 3.48)
A.3	Mouse	-70.3 + 9.77xlnC + 1.50xlnt		1325 (1293 - 1355)	6.53 (5.75 – 7.31)
B1.2	Mouse	*60-min LC_{50}*	919 (787-1193)	*1063*	

The data of the rat A.1, B1.1 and B1.3 studies and the mouse A.2, A.3 and B1.2 are presented graphically below.

1



2

3 ***Figure 2*** *Data selected for the initial analysis for the derivation of the animal probit function of hydrogen sulfide.*

Based on criteria outlined in the guideline the data from rat A.1 and B1.1 studies and the mouse A.2, A.3 and B1.2 studies were selected for the final dataset for the derivation of the animal probit function. Studies A.1 and A.2 included multiple exposure durations ranging from 5-60 min, study A.3 included multiple exposure durations ranging from 1-30 min. As a point of departure for the human probit function, a 30-60 min LC_{50} is preferred. The derivation of a geometric mean LC_{50} value as a point of departure for a probit function requires LC_{50} values for the same exposure duration. Therefore, as the extrapolation from an exposure duration of 240-min to a 30-60 min value is believed to be too uncertain, the 240-min rat study of Tansy et al. (1981; study B1.3) was not included for the final dataset for the derivation of the animal probit function. In addition, an exposure duration of 240 min in rat study B1.3 is more than a factor 2-3 outside the range of exposure durations included in the rat A.1 C x t study.

Figure 3 provides an overview of LC_{50} values and LC_{50}-time relationships for all studies in the final analysis. The data that were selected for final analysis of the animal probit function are presented in Table 3 and Figure 4.

The final data for calculating the animal probit function contains 5 datasets from 3 studies and includes data from 2 animal species.

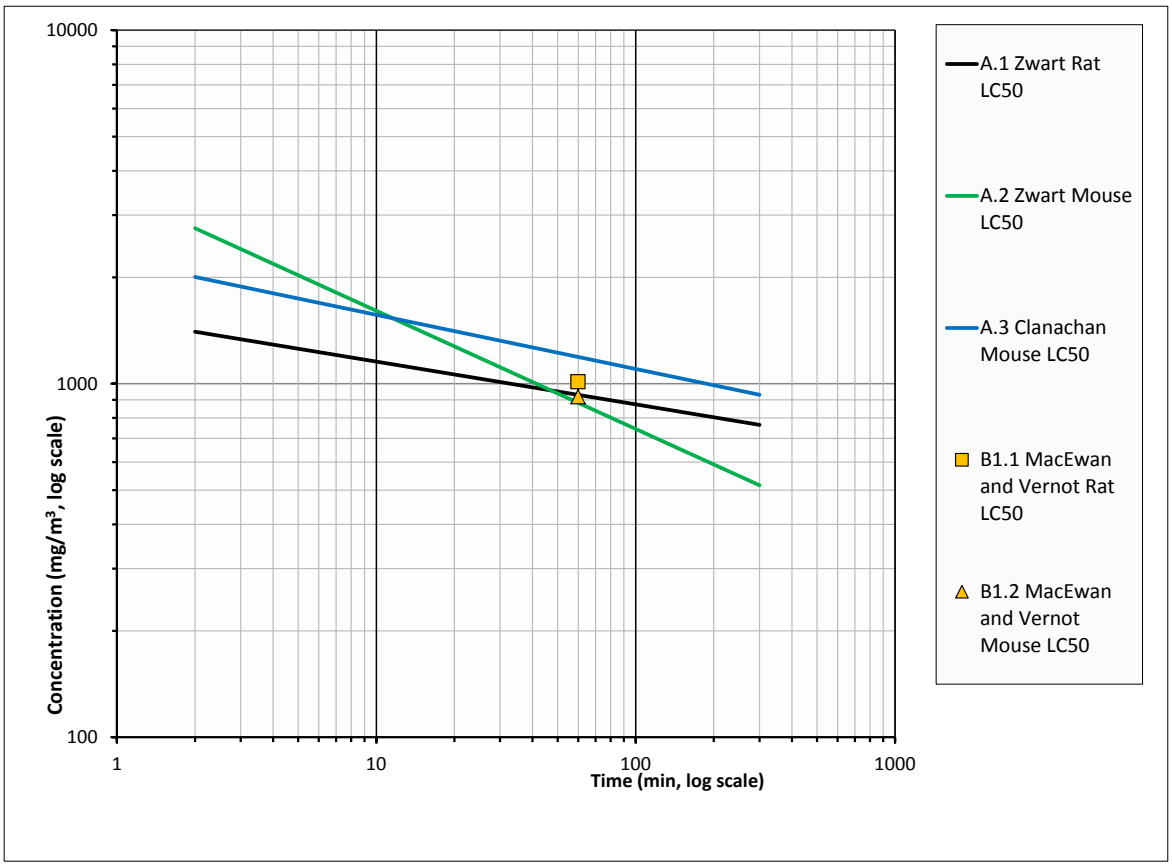


Figure 3 *LC_{50} values of A and B1 datasets for hydrogen sulfide, over time where available.*

Table 3 *Data selected for the derivation of the animal probit function of hydrogen sulfide.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} at tested exposure duration (mg/m^3) 95% C.I.	LC_{50}, 30 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
A.1	Rat	-57.6 + 8.54xlnC + 1.03×lnt		1011 (968 - 1094)	8.27 (4.65 – 11.88)
B1.1	Rat	*60-min LC_{50}*	1012 (932-1100)	*1100*	
A.2	Mouse	-40.8 + 5.62×lnC + 1.88×lnt		1114 (1063 - 1193)	2.99 (2.50 – 3.48)
A.3	Mouse	-70.3 + 9.77xlnC + 1.50xlnt		1325 (1293 - 1355)	6.53 (5.75 – 7.31)
B1.2	Mouse	*60-min LC_{50}*	919 (787-1193)	*1063*	

The data of the selected datasets are presented graphically below.

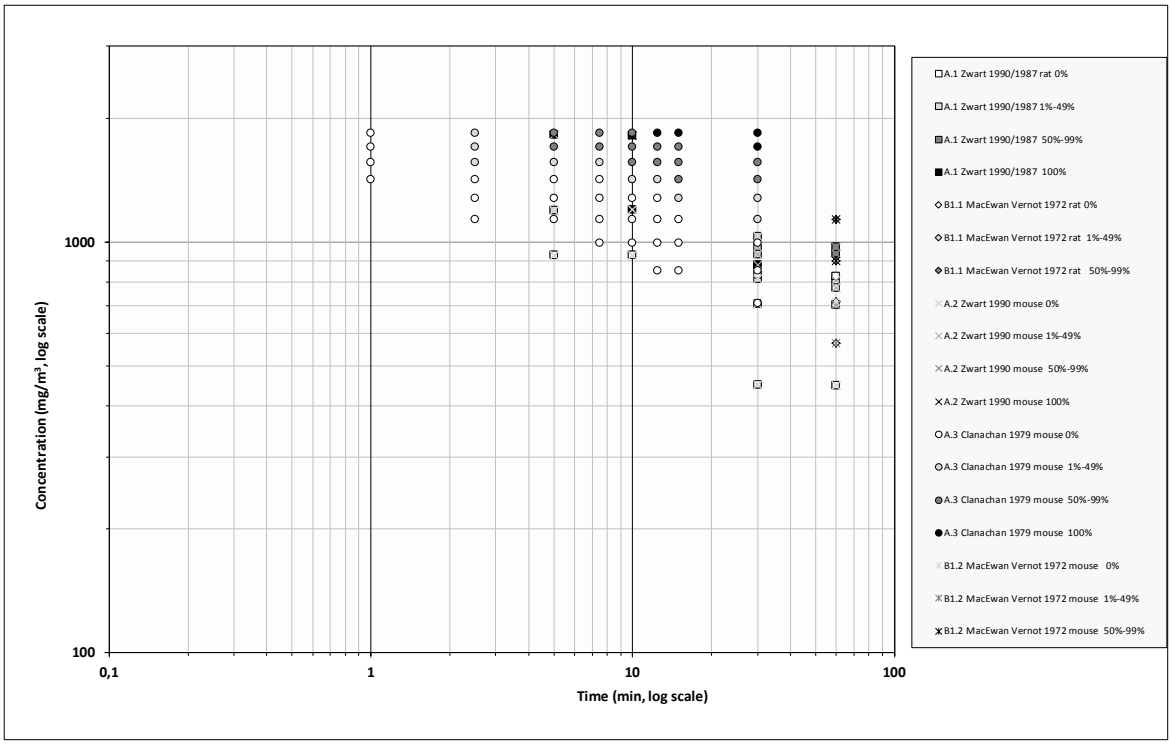


Figure 4 Final data selected for derivation of the animal probit function of hydrogen sulfide.

6. Derivation of the human probit function

To derive the human probit function the results from rat A.1 and B1.1 studies and the mouse A.2, A.3 and B1.2 studies have been used to derive a point of departure as outlined above.

First, the arithmetic mean n-value was calculated from rat study A.1 and mouse studies A.2 and A.3.

The species-specific n-value was 8.27 for the rat, 4.76 for the mouse. The mean n-value across species is the arithmetic mean of the species-specific mean n-values, and was calculated to be 6.52.

Second, the LC_{50}-values of all applicable A- and B1-studies were calculated for a common exposure duration of 30 minutes. To enable this intra-species pooling, LC_{50}-values of B1-studies were scaled using the species specific n-value of 8.27 for rat and 4.76 for mouse with the following formula:

$$LC_{50,c} = LC_{50,test}\left(\frac{t_{test}}{t_c}\right)^{(1/n)}$$

With $LC_{50,c}$ = scaled LC_{50} value for common exposure duration t_c
$LC_{50,test}$ = observed LC_{50} value for tested exposure duration
t_c = common exposure duration for intra-species pooling
t_{test} = tested exposure duration
n = species specific (for rat/mouse) n-value

Finally, the species-specific geometric mean LC_{50}-values were calculated from all available (time-scaled) LC_{50} values of rat A.1 and B1.1 studies and the mouse A.2, A.3 and B1.2 studies. The species-specific 30-min LC_{50}-value were 1055 and 1162 mg/m^3 for the rat and mouse, respectively, and these were subsequently geometrically averaged. The overall formula for the geometric mean of time-scaled LC_{50}-values is as follows:

$$\overline{LC_{50}} = \left[\prod_{j=1}^{s}\left(\prod_{i=1}^{m} LC_{50,i}\right)^{1/m}\right]^{(1/s)}$$

With $\overline{LC_{50}}$ = geometric mean LC_{50}-value across species
$LC_{50,i}$ = LC_{50}-value of study i.
m = number of observations on LC_{50}-values within a species (i=1…m).
s = number of species for which LC_{50}-values are pooled (j= 1…s).

The Point of Departure for the human probit function is a 30-minute geometric mean animal LC_{50} value of 1107 mg/m^3 and an arithmetic mean n-value of 6.52.

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

Table 4 *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	Default.
Nominal concentration	1	Studies with analytically determined concentrations available.
Adequacy of database:	1	Three A studies and two B1 studies available with sufficient number of C x t datasets

The estimated human equivalent 30-minute LC_{50} value is 1107 / 3 = **369 mg/m^3**.

The experimentally determined n-value was **6.52** (arithmetic mean n-value of rat study A.1 and mouse studies A.2 and A.3). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **0.31**.

The human probit function is then calculated on the human equivalent 30 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 0.31 \times \ln(369^{6.52} \times 30)$ resulting in the a-value of **–7.87**.

$Pr = -7.87 + 0.31 \times \ln(C^{6.52} \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically sound basis. The probit function is based on one study in the rat with A quality, one study in rat with B1 quality, two studies in the mouse with A quality and one study in mouse with B1 quality. Further, these data included in total 143 C x t combinations, including durations from one minute to 60 minutes and lethality in the range of 0-100%.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 67 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 98 mg/m^3.

Table 1 *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	75	67
1% lethality, this probit	109	98
AEGL-3[2] (2010, final)	84	71
ERPG-3[2] (1991)	-	142
LBW (2015)	84	72

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are similar.

[2] AEGL and ERPG values were converted from ppm to mg/m^3 with the conversion factor calculated in section 1. Therefore, the AEGL and ERPG values in mg/m^3 can deviate slightly from those reported in the AEGL and ERPG TSDs.

Appendix 1 Animal experimental research

Study ID: A.1

Author, year: Zwart et al. (1990), Zwart (1987)
Substance: hydrogen sulfide
Species, strain, sex: rat, SPF Wistar-derived, male and female
Number/sex/concentration group: 5
Age and weight: 5-6 weeks, 150-170 g (males) and 130-140 g (females) at arrival at lab
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	Yes
Study carried out according to OECD 403 guideline(s)	No statement of compliance with OECD guideline 403 provided
Stability of test compound in test atmosphere	Not specified
Use of vehicle (other than air)	No
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	N/A
Pressure distribution	Not specified
Homogeneity of test atmosphere in breathing zone of animals	An adjustable flow of test substance was mixed with airflow.
Number of air changes per hour	Approximately 100-150 air changes/hour (Content exposure cylinder (length: 0.9 m, r: 0.075 m) 15.9 l; air flow 25-40 l/min)
Equilibration time (t95)	1.2-1.9 min
Start of exposure relative to equilibration	Not specified. However, the shortest exposure duration in this study (i.e. 5 min) corresponds to 3 x t95 (i.e. 3.6-5.7 min).
Actual concentration measurement	Test atmosphere was sampled via ports at the entry and exit of inhalation chamber. Concentrations were measured continuously, by IR analysis.
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	N/A
Assessment of Reliability	**A** Multiple concentration levels and durations were tested, resulting in a more or less concentration related mortality of 0-100%.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	931	N/A	5	0/5	0/5
Rat	1196	N/A	5	2/5	0/5
Rat	1831	N/A	5	5/5	5/5
Rat	931	N/A	10	0/5	0/5
Rat	1199	N/A	10	3/5	5/5
Rat	1821	N/A	10	5/5	5/5
Rat	449	N/A	30	0/5	0/5
Rat	706	N/A	30	0/5	0/5
Rat	813	N/A	30	0/5	0/5
Rat	833	N/A	30	0/5	0/5
Rat	881	N/A	30	4/5	5/5
Rat	935	N/A	30	0/5	1/5
Rat	972	N/A	30	2/5	0/5
Rat	1032	N/A	30	2/5	1/5
Rat	448	N/A	60	0/5	0/5
Rat	703	N/A	60	0/5	0/5
Rat	774	N/A	60	0/5	0/5
Rat	806	N/A	60	0/5	0/5
Rat	826	N/A	60	0/5	0/5
Rat	939	N/A	60	3/5	4/5
Rat	972	N/A	60	3/5	4/5

Probit function

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

$Pr = a + b \times lnC + c \times lnt + d \times S$

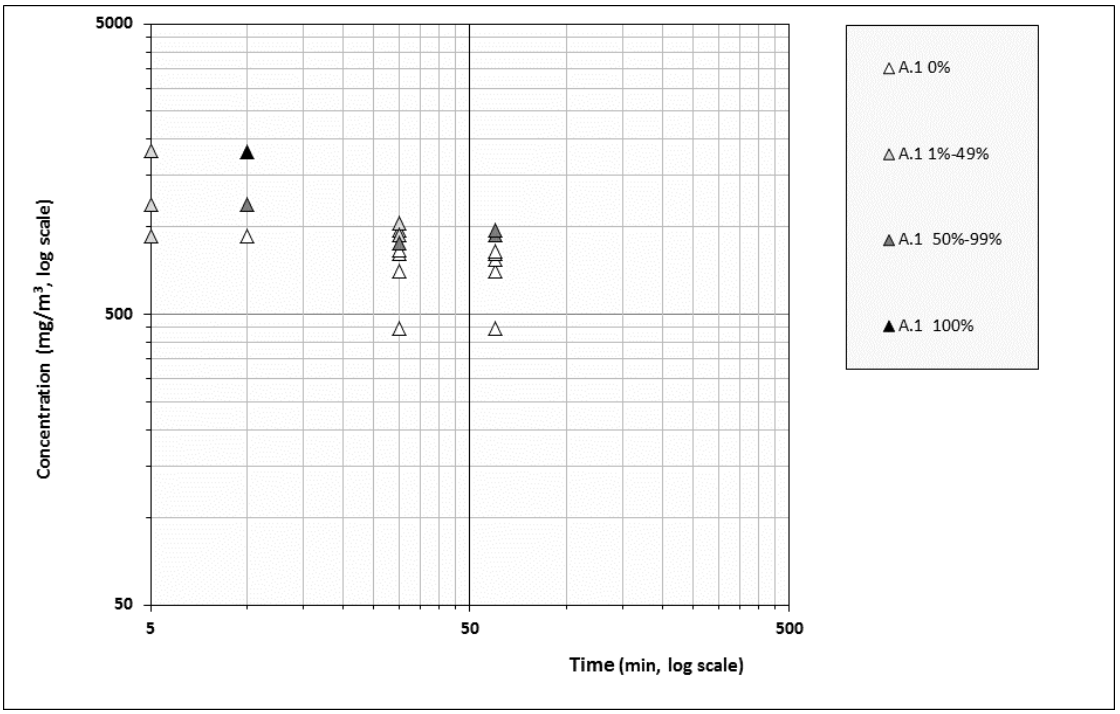
with C for concentration in mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male).

Probit function	Species	a	b	c	d	n-value
Sex as variable	Rat	-57.6	8.55	1.03	0.061	8.27 (4.62-11.93)
Sexes combined	Rat	-57.6	8.54	1.03	-	8.27 (4.65 – 11.88)

The LC_{50} values for both sexes did not differ by more than a factor of 2. This does not support the proposition that sex differences exist in the lethal response. For this reason, the data from both sexes were pooled and analysed to derive the animal probit function.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Male	LC_{50} (mg/m^3) 95%-C.I. Female	LC_{50} (mg/m^3) 95%-C.I. Combined
10	1159 (1066 – 1301)	1150 (1058 - 1288)	1155 (1077 - 1276)
30	1015 (954 - 1119)	1077 (948 - 1108)	1011 (968 - 1094)
60	933 (867 - 1045)	926 (861 - 1035)	930 (877 - 1024)

A graphical overview of the data is presented below. Each concentration-time combination (with 5 male and 5 female animals) represents one point in the plot.


5000
500
50
5
50
500
Concentration (mg/m³, log scale)
Time (min, log scale)
A.1 0%
A.1 1%-49%
A.1 50%-99%
A.1 100%

1
2

Study ID: A.2

Author, year: Zwart et al., 1990

Study details of study A.2 are the same as the study details of A.1 (based on personal communication with TNO Zeist, The Netherlands, where the study was performed).The mouse study A.2 was performed simultaneously with the rat study A.1.

Substance: hydrogen sulfide
Species, strain, sex: mouse, Swiss, male and female
Number/sex/concentration group: 5
Age and weight: 7-8 weeks, 23-27 g (females) and 28-34 g (males) at arrival at lab
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	Yes
Study carried out according to OECD 403 guideline(s)	No statement of compliance with OECD guideline 403 provided
Stability of test compound in test atmosphere	Not specified
Use of vehicle (other than air)	No
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	N/A
Pressure distribution	Not specified
Homogeneity of test atmosphere in breathing zone of animals	An adjustable flow of test substance was mixed with airflow.
Number of air changes per hour	Approximately 100-150 air changes/hour (Content exposure cylinder (length: 0.9 m, r: 0.075 m) 15.9 l; air flow 25-40 l/min)
Equilibration time (t95)	1.2-1.9 min
Start of exposure relative to equilibration	Not specified. However, the shortest exposure duration in this study (i.e. 5 min) corresponds to 3 x t95 (i.e. 3.6-5.7 min).
Actual concentration measurement	Test atmosphere was sampled via ports at the entry and exit of inhalation chamber. Concentrations were measured continuously, by IR analysis.
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	N/A
Assessment of Reliability	**A** Multiple concentration levels and durations were tested, resulting in a more or less concentration related mortality of 0-100%.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	

Mouse	931	N/A	5	0/5	0/5
Mouse	1196	N/A	5	0/5	0/5
Mouse	1831	N/A	5	1/5	2/5
Mouse	931	N/A	10	0/5	0/5
Mouse	1199	N/A	10	0/5	0/5
Mouse	1821	N/A	10	4/5	5/5
Mouse	449	N/A	30	0/5	0/5
Mouse	706	N/A	30	0/5	0/5
Mouse	813	N/A	30	0/5	0/5
Mouse	881	N/A	30	1/5	1/5
Mouse	935	N/A	30	0/5	1/5
Mouse	972	N/A	30	1/5	2/5
Mouse	1032	N/A	30	0/5	0/5
Mouse	448	N/A	60	0/5	0/5
Mouse	703	N/A	60	0/5	3/5
Mouse	774	N/A	60	0/5	2/5
Mouse	806	N/A	60	2/5	1/5
Mouse	939	N/A	60	3/5	4/5
Mouse	972	N/A	60	4/5	2/5

Probit function

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

$Pr = a + b \times lnC + c \times lnt + d \times S$

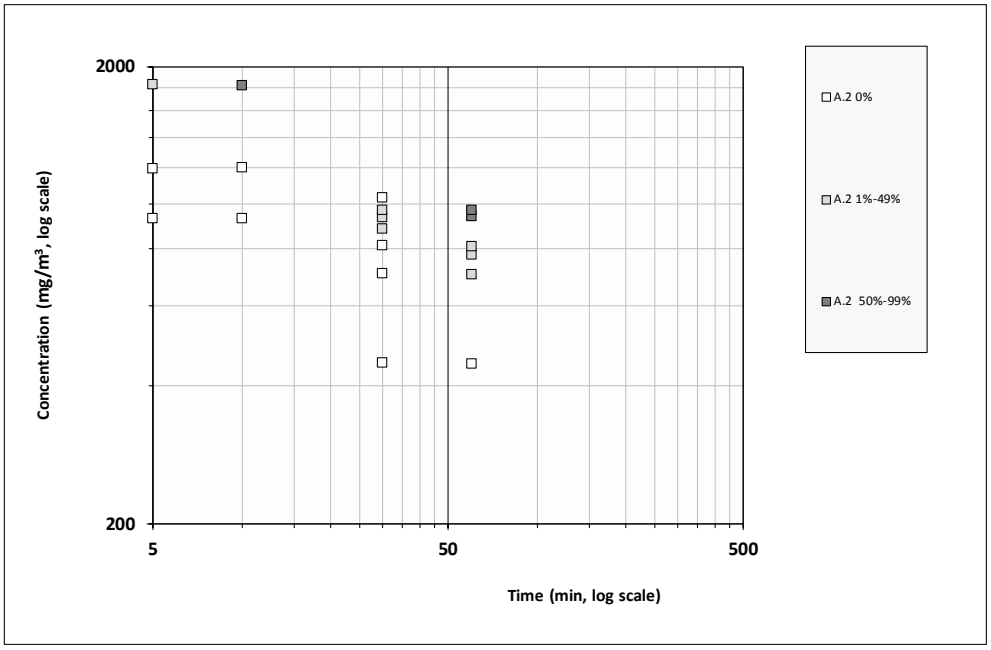
with C for concentration in mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male).

Probit function	Species	a	b	c	d	n-value
Sex as variable	Mouse	-41.8	5.77	1.92	-0.43	3.00 (2.52 – 3.49)
Sexes combined	Mouse	-40.8	5.62	1.88	-	2.99 (2.50 – 3.48)

The LC_{50} values for both sexes did not differ by more than a factor of 2. This does not support the proposition that sex differences exist in the lethal response. For this reason, the data from both sexes were pooled and analysed to derive the animal probit function.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Male	LC_{50} (mg/m^3) 95%-C.I. Female	LC_{50} (mg/m^3) 95%-C.I. Combined
10	1668 (1527 - 1877)	1547 (1423 – 1715)	1608 (1492 - 1778)
30	1157 (1083 – 1271)	1073 (1011 - 1159)	1114 (1063 - 1193)
60	919 (852 – 1016)	852 (794 - 929)	883 (832 - 957)

A graphical overview of the data is presented below. Each concentration-time combination (with 5 male and 5 female animals) represents one point in the plot.


2000
200
5
50
500
Concentration (mg/m³, log scale)
Time (min, log scale)
A.2 0%
A.2 1%-49%
A.2 50%-99%

1
2
3

Study ID: A.3

Author, year: Clanachan, 1979

Substance: hydrogen sulfide
Species, strain, sex: mouse, BALB/CCR, male and female
Number/sex/concentration group: 20 or 46 (sexes combined)
Age and weight: 15-25 g
Observation period: 5 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	No GLP statement provided
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	Not specified
Use of vehicle (other than air)	No
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	N/A
Pressure distribution	Not specified
Homogeneity of test atmosphere in breathing zone of animals	Test atmosphere was generated by diluting air with the test substance, which is passed into a 120 l chamber. Animals were placed in a wired cage on slides in a near smaller chamber. By moving the wired cage from the smaller chamber into the exposure chamber exposure was initiated. The smaller chamber can be sealed off.
Number of air changes per hour	20 l/min in a 120 l chamber. This approximates 10 air changes per hour.
Equilibration time (t95)	18 min
Start of exposure relative to equilibration	The two gas flows (air and test substance) were allowed to mix thoroughly before passing into the gassing chamber. The exposure chamber was then allowed to equilibrate for at least 45 min before exposure of each group of animals.
Actual concentration measurement	No continuous measurement of the substance concentration was made. The dynamic dilution and flow rates were monitored continuously every 3-4 minutes before and during the experiments with a dry gas meter for air and soap bubble flow meter for the substance.
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	N/A

Assessment of Reliability	**A** Study data were suitable to derive a probit function. Multiple concentration levels and durations were tested, resulting in clearly concentration related mortality of 0-100%. Although no analytical concentration measurements were performed, the exposure method provides sufficient confidence in the results. Although a relatively short post-exposure period was included (5 days), data from other studies indicated that death occurred on the first day after exposure.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		Male+female
				Dead/tested
Mouse	1420	N/A	1	0/20
Mouse	1562	N/A	1	0/20
Mouse	1704	N/A	1	0/20
Mouse	1846	N/A	1	0/20
Mouse	1136	N/A	2.5	0/20
Mouse	1278	N/A	2.5	0/20
Mouse	1420	N/A	2.5	0/20
Mouse	1562	N/A	2.5	1/20
Mouse	1704	N/A	2.5	2/20
Mouse	1846	N/A	2.5	3/20
Mouse	1136	N/A	5	0/20
Mouse	1278	N/A	5	0/20
Mouse	1420	N/A	5	0/20
Mouse	1562	N/A	5	4/20
Mouse	1704	N/A	5	13/20
Mouse	1846	N/A	5	12/20
Mouse	994	N/A	7.5	0/20
Mouse	1136	N/A	7.5	0/20
Mouse	1278	N/A	7.5	0/20
Mouse	1420	N/A	7.5	0/20
Mouse	1562	N/A	7.5	8/20
Mouse	1704	N/A	7.5	14/20
Mouse	1846	N/A	7.5	17/20
Mouse	994	N/A	10	0/20
Mouse	1136	N/A	10	0/46
Mouse	1278	N/A	10	0/46
Mouse	1420	N/A	10	9/46
Mouse	1562	N/A	10	25/46
Mouse	1704	N/A	10	34/46
Mouse	1846	N/A	10	44/46
Mouse	852	N/A	12.5	0/20
Mouse	994	N/A	12.5	0/20

Mouse	1136	N/A	12.5	0/20
Mouse	1278	N/A	12.5	0/20
Mouse	1420	N/A	12.5	6/20
Mouse	1562	N/A	12.5	13/20
Mouse	1704	N/A	12.5	17/20
Mouse	1846	N/A	12.5	20/20
Mouse	852	N/A	15	0/20
Mouse	994	N/A	15	0/20
Mouse	1136	N/A	15	0/20
Mouse	1278	N/A	15	2/20
Mouse	1420	N/A	15	14/20
Mouse	1562	N/A	15	13/20
Mouse	1704	N/A	15	19/20
Mouse	1846	N/A	15	20/20
Mouse	710	N/A	30	0/20
Mouse	852	N/A	30	0/20
Mouse	994	N/A	30	0/20
Mouse	1136	N/A	30	1/20
Mouse	1278	N/A	30	7/20
Mouse	1420	N/A	30	12/20
Mouse	1562	N/A	30	17/20
Mouse	1704	N/A	30	20/20
Mouse	1846	N/A	30	20/20

Probit function

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

$Pr = a + b \times lnC + c \times lnt + d \times S$

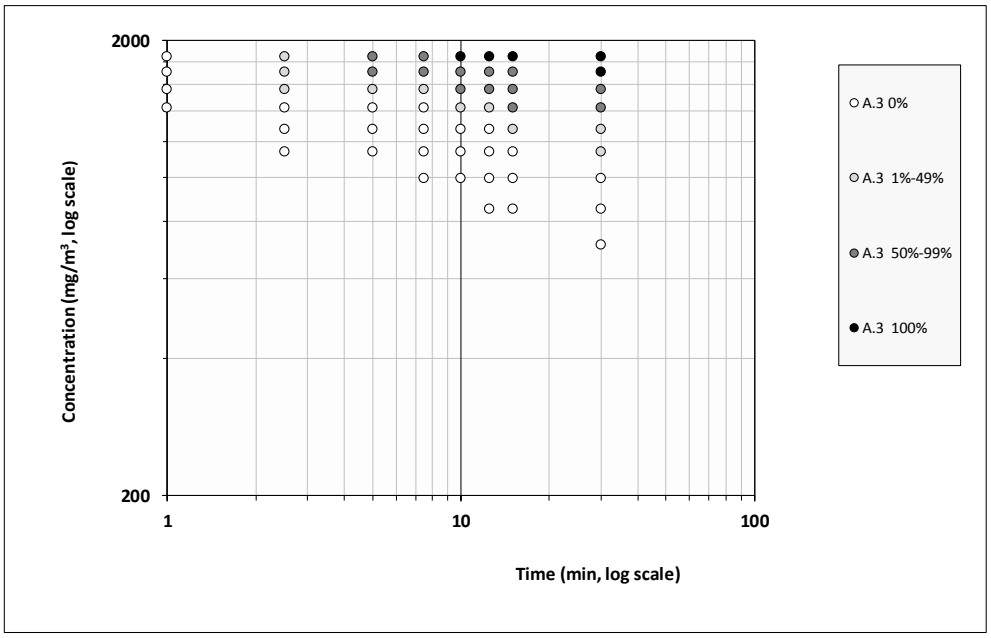
with C for concentration in mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male).

Probit function	Species	a	b	c	d	n-value
All data	Mouse	-70.3	9.77	1.50	-	6.53 (5.75 – 7.31)
Excl. 1-7.5 min data	Mouse	-69.1	9.68	1.26	-	7.67 (5.55 – 9.80)

Analysis of all data and data excluding the 1-7.5 min exposure durations resulted in similar LC_{50} values. Moreover, the exposure concentrations during these short-term exposure durations are expected to be > 95% of $C_{equilibrium}$. Therefore, the data from all exposure durations were used to derive the animal probit function.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. All data	LC_{50} (mg/m^3) 95%-C.I. Excl. 1-7.5 min
10	1567 (1549 - 1586)	1555 (1526 - 1583)
30	1325 (1293 - 1355)	1347 (1304 - 1391)
60	1191 (1149 - 1233)	1231 (1164 - 1301)

A graphical overview of the data is presented below. Each concentration-time combination (with 10-23 male and female animals combined) represents one point in the plot.


2000
200
1
10
100
Concentration (mg/m³, log scale)
Time (min, log scale)
A.3 0%
A.3 1%-49%
A.3 50%-99%
A.3 100%

1

Study ID: B1.1

Author, year: MacEwan and Vernot, 1972
Substance: hydrogen sulfide
Species, strain, sex: rat, CFE (Sprague-Dawley), male
Number/sex/concentration group: 10
Age and weight: 200-300 g
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
Stability of test compound in test atmosphere	Stable ('the actual concentrations during exposure were unchanged from nominal, indicating little or no reactivity of H_2S with chamber materials')
Use of vehicle (other than air)	No
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	N/A
Pressure distribution	Not specified
Homogeneity of test atmosphere in breathing zone of animals	Test atmosphere was generated by mixing H_2S gas with an air supply system
Number of air changes per hour	30 l/min in a 30 l glass bell jar results in 60 air changes per hour
Equilibration time (t95)	3 min
Start of exposure relative to equilibration	Not specified
Actual concentration measurement	Continuous measurements with ion-specific electrode
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	N/A
Assessment of Reliability	**B1** Well-performed study, limited to one exposure duration.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		Male
				Dead/tested
Rat	568	N/A	60	0/10
Rat	716	N/A	60	0/10
Rat	902	N/A	60	1/10
Rat	1136	N/A	60	9/10

Probit function

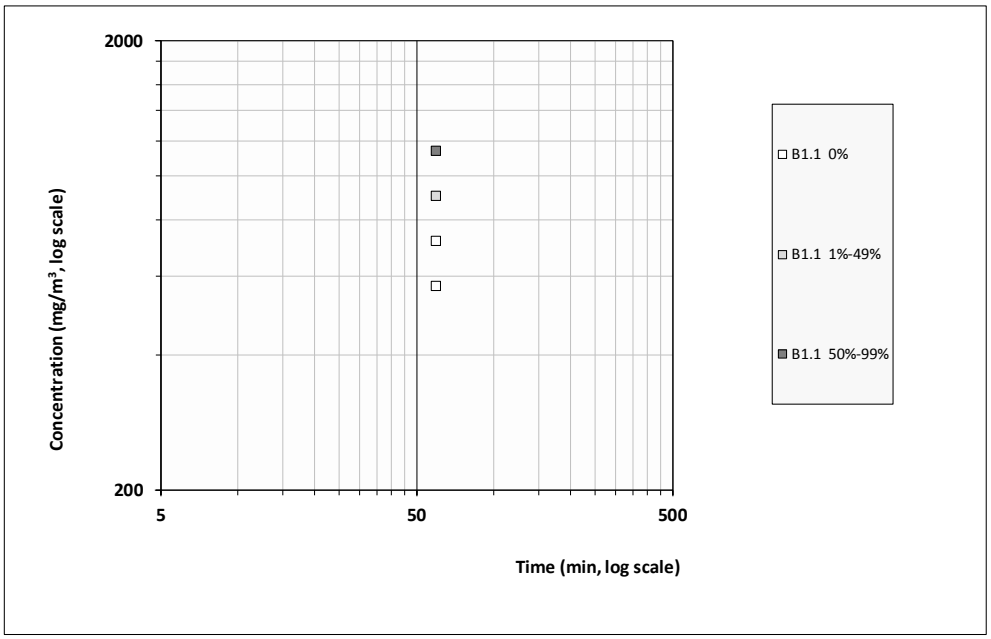
The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, 2016) as
$Pr = a + b \times \ln C$
with C for concentration in mg/m^3.

Probit function	Species	a	b	n-value
	Rat	-72.0	11.1	-

No C × t probit function could be calculated from these data alone.

The probit function resulted in the following LC_{50} value for 60 minutes exposure duration: 1012 mg/m^3 (95%-C.I.: 932-1100 mg/m^3).

A graphical overview of the data is presented below. Each concentration-time combination (with 10 male animals) represents one point in the plot.



Study ID: B1.2

Author, year: MacEwan and Vernot, 1972
Substance: hydrogen sulfide
Species, strain, sex: mouse, CFI (ICR), male
Number/sex/concentration group: 10
Age and weight: 20-30 g
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
Stability of test compound in test atmosphere	Stable ('the actual concentrations during exposure were unchanged from nominal, indicating little or no reactivity of H_2S with chamber materials')
Use of vehicle (other than air)	No
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	N/A
Pressure distribution	Not specified
Homogeneity of test atmosphere in breathing zone of animals	Test atmosphere was generated by mixing H_2S gas with an air system
Number of air changes per hour	30 l/min in a 30 l glass bell jar results in 60 air changes per hour
Equilibration time (t95)	3 min
Start of exposure relative to equilibration	Not specified
Actual concentration measurement	Continuous measurements with ion-specific electrode
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	N/A
Assessment of Reliability	**B1** Well-performed study, limited to one exposure duration.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		Male
				Dead/tested
Mouse	568	N/A	60	2/10
Mouse	716	N/A	60	0/10
Mouse	902	N/A	60	5/10
Mouse	1136	N/A	60	8/10

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

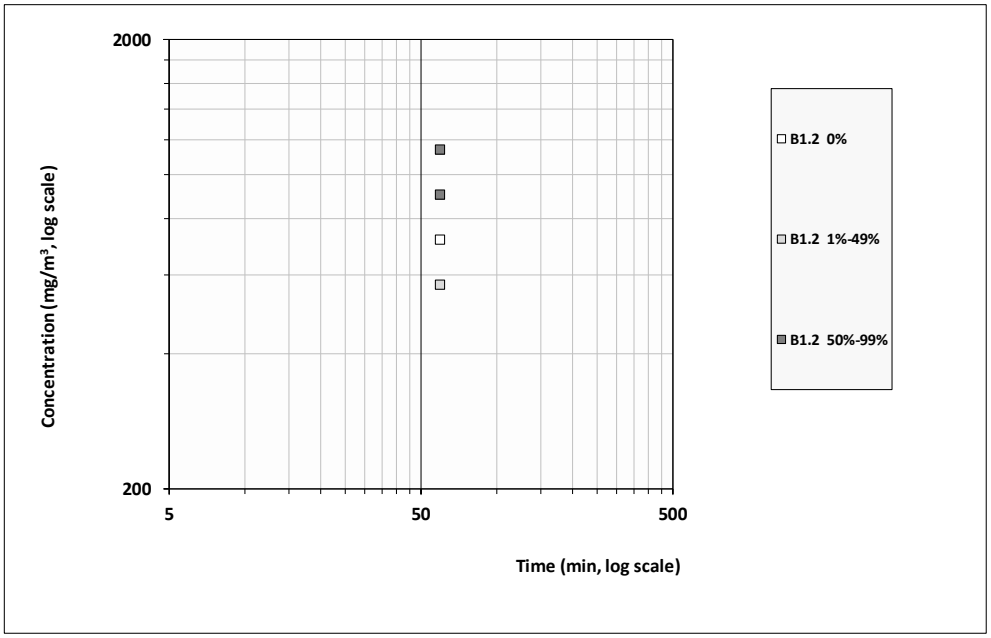
Pr = a + b×lnC
with C for concentration in mg/m^3.

Probit function	Species	a	b	n-value
	mouse	-15.3	2.97	-

No C × t probit function could be calculated from these data alone.

The probit function resulted in the following LC_{50} value for 60 minutes exposure duration: 919 mg/m^3 (95%-C.I.: 787-1193 mg/m^3).

A graphical overview of the data is presented below. Each concentration-time combination (with 10 male animals) represents one point in the plot.



Study ID: B1.3

Author, year: Tansy et al., 1981

Substance: hydrogen sulfide
Species, strain, sex: rat, Sprague-Dawley, male and female
Number/sex/concentration group: 10 (sexes combined)
Age and weight: age unknown, 90-100 g (on day of receipt at lab)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	No GLP statement provided
Study carried out according to OECD 403 guideline(s)	No statement of compliance with OECD guideline 403 provided
Stability of test compound in test atmosphere	Not specified
Use of vehicle (other than air)	No
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	N/A
Pressure distribution	Slightly negative pressure in test chamber
Homogeneity of test atmosphere in breathing zone of animals	Not specified, but exposure chamber was designed to ensure uniform spatial distributions.
Number of air changes per hour	Unknown
Equilibration time (t95)	No data available to calculate t95
Start of exposure relative to equilibration	Not specified
Actual concentration measurement	Not specified
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	N/A
Assessment of Reliability	**B1** Well-performed study, limited to one exposure duration.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		Male
				Dead/tested
Rat	0	N/A	240	0/10
Rat	568	N/A	240	3/10
Rat	625	N/A	240	3/10
Rat	675	N/A	240	7/10
Rat	710	N/A	240	8/10
Rat	746	N/A	240	8/10
Rat	787	N/A	240	9/10
Rat	852	N/A	240	10/10

Probit function

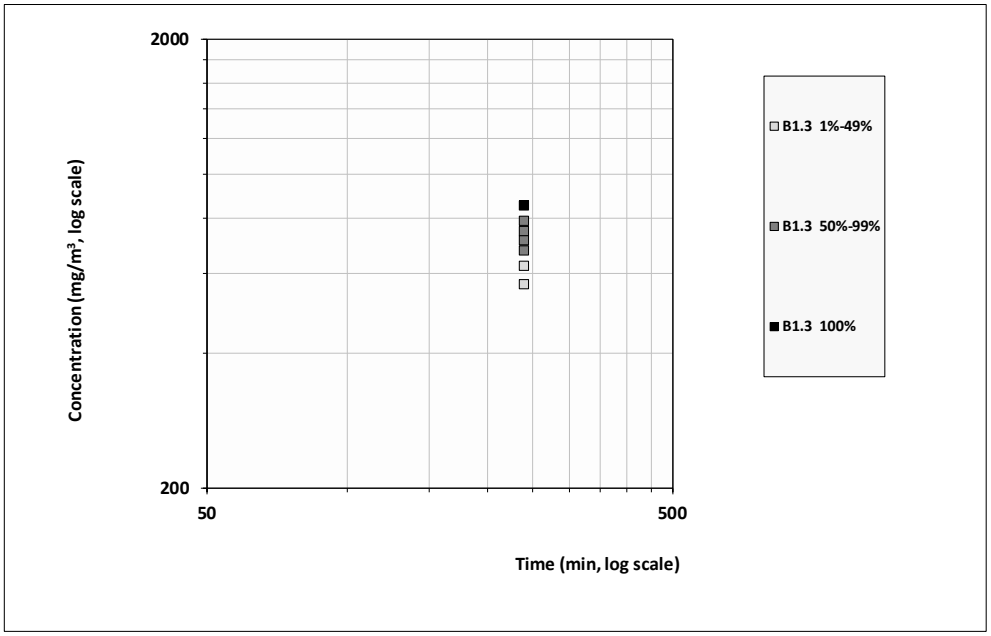
The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, 2016) as
Pr = a + b×lnC
with C for concentration in mg/m^3.

Probit function	Species	a	b	n-value
	rat	-37.5	6.58	-

No C × t probit function could be calculated from these data alone.

The probit function resulted in the following LC_{50} value for 240 minutes exposure duration: 638 mg/m^3 (95%-C.I.: 584-673 mg/m^3).

A graphical overview of the data is presented below. Each concentration-time combination (with 10 male animals) represents one point in the plot.



Study ID: B2.1

Author, year: Prior et al., 1988

Substance: hydrogen sulfide
Species, strain, sex: rat, Fischer-344, Sprague-Dawley and Long Evans, male and female
Number/sex/concentration group: 72 males and females at 4 hour exposure group, 72 males and 84 females for the 2h and 6h exposure group
Age and weight: 7 to 8 weeks old at start of exposure
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	According to Canadian Council on animal care. No GLP declaration provided.
Study carried out according to OECD 403 guideline(s)	No statement of compliance with OECD guideline 403 provided
Stability of test compound in test atmosphere	Stable
Use of vehicle (other than air)	No
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	N/A
Pressure distribution	-74.6 Pa during exposures
Homogeneity of test atmosphere in breathing zone of animals	Flows of hydrogen sulfide (99.5% commercial product) was mixed with air before entering exposure chamber of 69.3 ±0.2L. See figure below adopted from Prior et al. 1988
Number of air changes per hour	Not specified
Equilibration time (t95)	No data available to calculate t95
Start of exposure relative to equilibration	Not specified
Actual concentration measurement	Approximately four times per hour by gas chromatography with flame photometric detector
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	N/A
Assessment of Reliability	**B2** Few study details are available. LC_{50} values (incl. confidence intervals) are provided for 3 exposure durations.

Results

Species	Exposure duration (min)	LC_{50} (mg/m^3) 95%-C.I. Sexes combined (study authors)
Rat (all strains)	120	834 (C.I. could not be estimated)

Rat (all strains)	240	711 (677-774)
Rat (all strains)	360	476 (462-490)

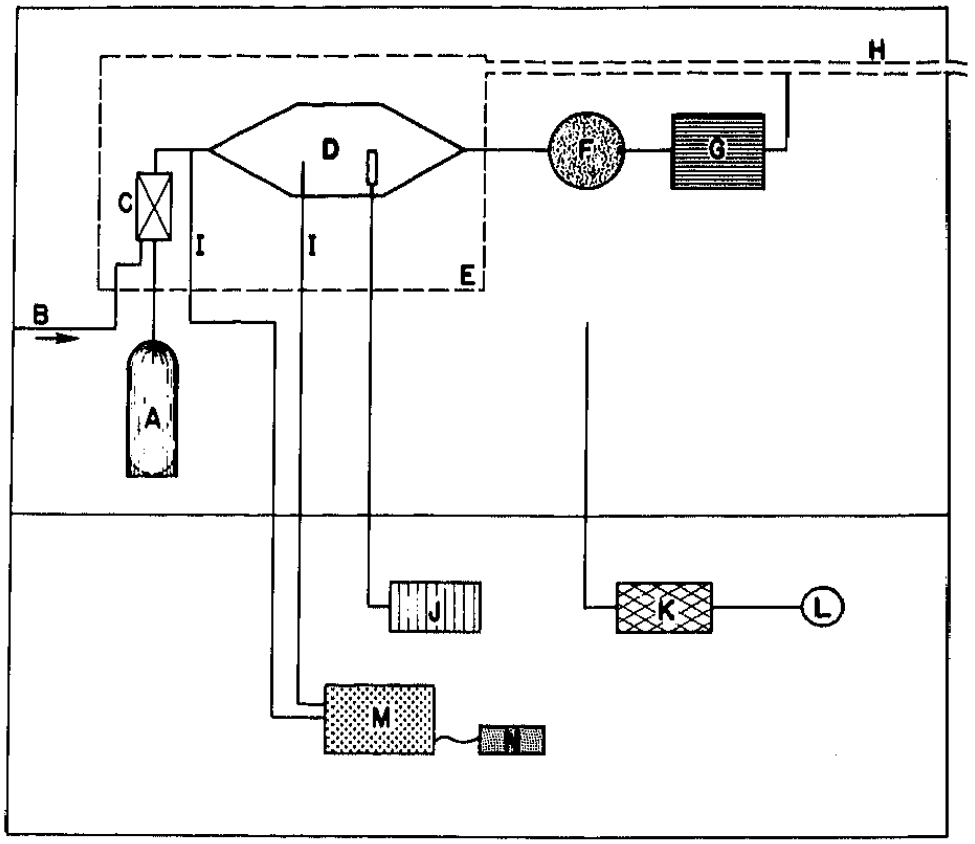
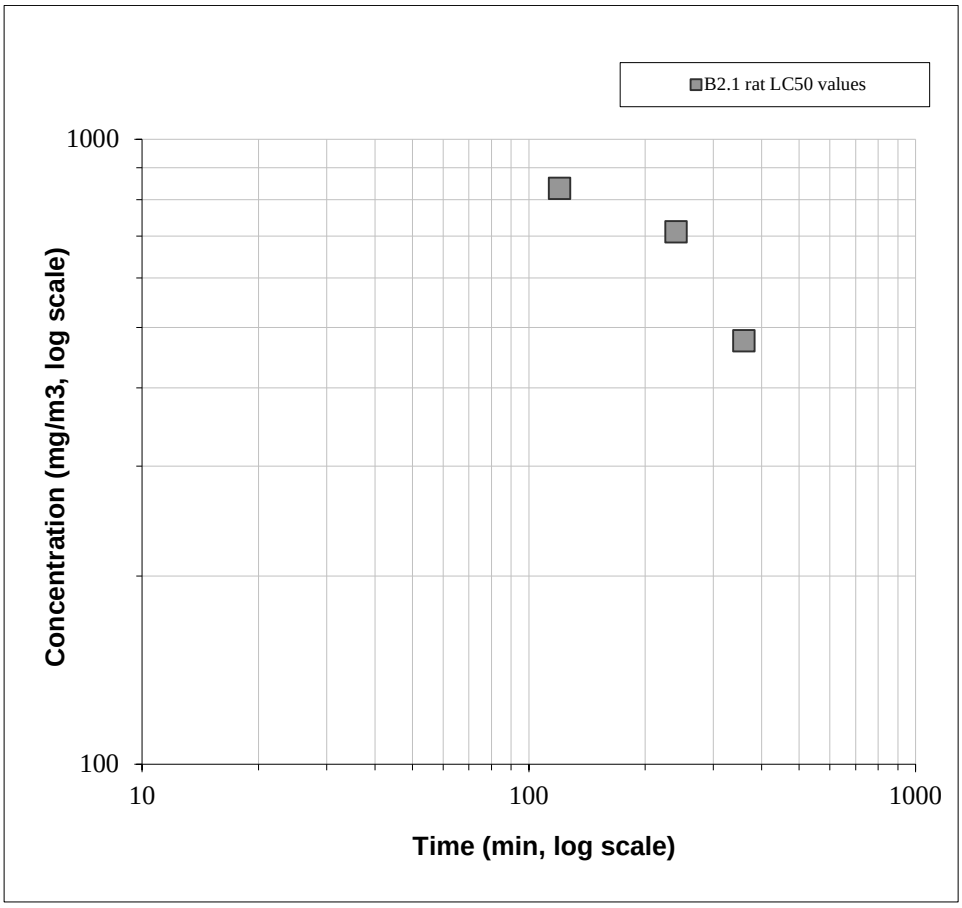


Fig. 1. Schematic of acute inhalation exposure system. (Only one of the two exposure chambers is depicted). A — gas cylinder; B — air; C — flow controllers; D — exposure chamber; E — fume hood; F — vacuum pump; G — scrubber; H — exhaust to outside; I — sample line; J — relative humidity and temperature monitor; K — H_2S monitor; L — alarm; M — gas chromatograph; N — computer.

Probit function

No probit function could be calculated from these data alone.

A graphical overview of the LC_{50} values is presented below.


B2.1 rat LC50 values
1000
100
Concentration (mg/m3, log scale)
10
100
1000
Time (min, log scale)

1

Study ID: C.1

Author, year: Lopez et al., 1987
Substance: hydrogen sulfide
Species, strain, sex: rat, Fischer-344, male
Number/sex/concentration group: 4
Age and weight: 12 weeks old, weight unknown
Observation period: 44 hours

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	No GLP statement provided
Study carried out according to OECD 403 guideline(s)	No statement of compliance with OECD guideline 403 provided
Stability of test compound in test atmosphere	Stable
Use of vehicle (other than air)	No
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	N/A
Pressure distribution	Negative pressure of -74.6 Pa in test chamber
Homogeneity of test atmosphere in breathing zone of animals	Test atmosphere was generated by passing hydrogen sulfide through a regulator and flowmeter and then mixed with filtered air prior to introduction in the chamber.
Number of air changes per hour	17 l/min or approximately 15 air changes per hour (volume chamber: 69 l)
Equilibration time (t95)	12 min
Start of exposure relative to equilibration	Not specified
Actual concentration measurement	samples were analysed three times per hour by gas chromatography
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	N/A
Assessment of Reliability	**C** Limited to one exposure duration. Too short observation period considered. No lethality occurred and an LC_{50} could not be derived.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		Male
				Dead/tested
Mouse	0	N/A	240	0/4
Mouse	13.6	N/A	240	0/4
Mouse	281	N/A	240	0/4

Mouse	551	N/A	240	0/4

Probit function

Given the 0% lethality for all exposure concentration-duration combinations, a probit function cannot be derived based on these data.

Study ID: other C studies

Savolainen et al., (1980) exposed 5 female NMRI mice to a concentration of 142 mg/m^3 for two hours. None of the animals died during the exposure period or observation period of 4 days.

Lopez et al., (1989) scored the types of oedema observed after exposure to a predetermined lethal concentration in five male Sprague-Dawley rats. All animals died within three minutes after a 5 min-exposure to 2318 mg/m^3 H_2S.

In 1966, Lund studied the toxicity of H_2S in one rhesus monkey. At the concentration of 714 mg/m^3 for 35 minutes the monkey did not survive. No further details were given.

Appendix 2 Reference list

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2 concentration-time-mortality relationships to replace LC50 values. Inhal. Toxicol.
3 1990; 2: 105-17.