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
Sulfuric Acid **Sulfur trioxide** **Oleum**	**7664-93-9** **7446-11-9** **8014-95-7**

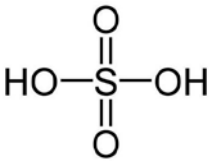
This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

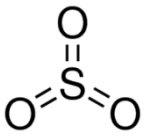
This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have 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 sulfuric acid





1. Substance identification

CAS-number:	7664-93-9 Sulfuric acid
	7446-11-9 Sulfur trioxide
	8014-95-7 Oleum
IUPAC name:	sulfuric acid
Synonyms:	Dihydrogen sulfate, oil of vitriol, battery acid.
Molecular formula:	H_2SO_4, H_2SO_3
*** All physical-chemical data below are for sulfuric acid ***	
Molecular weight:	98.1 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	315°C (at 101.3 kPa)
Vapour pressure:	0.1 Pa (at 20°C)
Saturated vapor conc:	1.0 ppm = 4.081 mg/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.245 ppm (at 20°C and 101.3 kPa)
	1 ppm = 4.081 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H314

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

Special considerations: The AEGL document states that studies of the thermodynamics of clouds generated from spills of SO_3 and oleum (H_2SO_4 containing up to 80% free SO_3) found that, while the conversion from SO_3 to H_2SO_4 is very fast, the content of atmospheric moisture immediately above the pool is insufficient for complete and rapid reaction to sulfuric acid mist. Close to the source clouds will contain SO_3 vapour, H_2SO_4 vapour and H_2SO_4 aerosol. Typically some 50-100 m downwind from the source only sulfuric acid will be present in the aerosol cloud.
The probit function in this document is for the sulphuric acid (aerosol) that ultimately results from an airborne release of any of these 3 substances.
Species specificity: Guinea pigs are far more susceptible to pulmonary damage by sulfuric acid inhalation than other species (mice, rats, monkeys, rabbits). Guinea pigs respond with a reflex airway constriction mediated by the parasympathetic nervous system, a mechanism which has not been demonstrated to occur in humans..
Inhalation exposure produces lethality in guinea pigs at 12 mg/m^3 for 8 hours, while occupational exposures up to 35 mg/m^3 have been reported with only mild respiratory effects (NAC/AEGL, 2008).
For these reasons, the guinea pig is considered not to be a suitable animal model to predict the acute health effects of sulfuric acid inhalation in humans. Therefore, the results of the extensive body of studies in guinea pigs were not included in this document.

Acute effects: The main target organs and tissues for inhalation exposure to H_2SO_4 are the cornea, conjunctiva, skin and respiratory tract. H_2SO_4 dissolves in the mucous membranes of the respiratory tract and eyes to form sulfuric acid, a strong acid that produces coagulative necrosis. The health endpoints are all related to the irritative and corrosive properties of H_2SO_4. 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

[1] Interim AEGL.TSD for sulfuric acid (2008), AHLS Provider manual 3rd ed. 2003.

(myocardial ischemia) effects. Lethality results when the 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. Reactive Airways Dysfunction Syndrome, an acquired asthma-like condition has been described to develop after single high exposure to H_2SO_4. Symptoms occur within minutes to hours after the initial exposure and may persist as non-specific bronchial hyper-responsiveness for months to years. IARC classifies occupational exposure to strong-inorganic-acid mists containing sulfuric acid as carcinogenic to humans (Group 1).

3. Human toxicity data

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.
In humans, sulfuric acid causes irritation of the respiratory tract. In one study, workers of two battery manufactories (n=33) were continuously exposed at either 26-35 mg/m^3 in one plant or at approximately 13 mg/m^3 in the second plant. This is the highest human exposure concentration found in literature. No lethality was reported, but there were some health effects seen. The incidence of chronic bronchitis was higher in exposed workers compared to non-exposed control workers, but the difference was not statistically significant. The pH of the saliva was 7 before and 6.95 after the shift in controls, whereas in exposed workers the pH dropped from 6.9 to 6.7. The VC was not affected by exposure, although the FEV_1 decreased with 82 ml during the shift of exposed workers. Dental erosion was evident in exposed workers (El-Sadik et al., 1972).

4. Animal acute toxicity data

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

1. AEGL interim TSD, ERPG document and EU RAR and reference database for sulfuric acid, 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:
    - 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 four studies were identified -with nine datasets for five species- with data on lethality following acute inhalation exposure. Two datasets were assigned status A for deriving the human probit function, no datasets were assigned status B and seven were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

No studies on sensory information were found.

5. Probit functions from individual studies

All available acute lethality data on sulfuric acid are displayed in Figure 1.

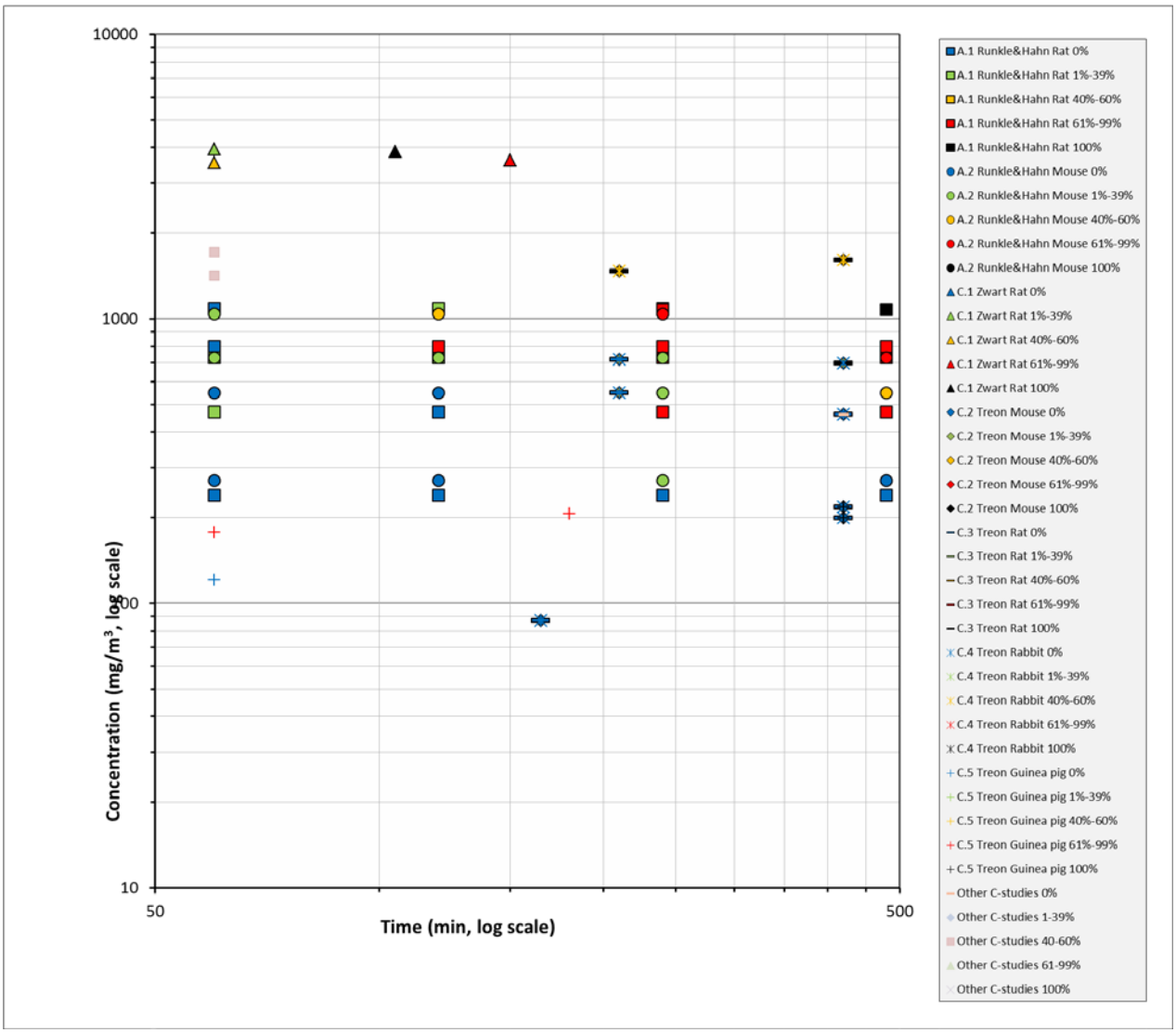


Figure 1 *All available acute lethality data for sulfuric acid.*

The data that were selected for initial analysis of the animal probit function are presented in Table 1 and Figure 2.

Both A studies were selected for derivation of the animal probit function for sulfuric acid. 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 1.

Table 1 *Data selected for initial analysis of the animal probit function of sulfuric acid.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} (60 min.) (mg/m^3) 95% C.I.	LC_{50}, 30 minutes (mg/m^3) 95% C.I.	n-value 95% C.I.
A.1	Rat	-12.46 + 1.63×lnC + 1.28×lnt	1789 (1304-2965)	3083 (1927 – 6599)	1.27 (0.81-1.73)
A.2	Mouse	-13.35 + 2.16×lnC + 0.72×lnt	1249 (1017-1705)	1573 (1165 – 2441)	3.00 (1.51-4.50)

The data of the two A studies with rats and mice are presented graphically below.

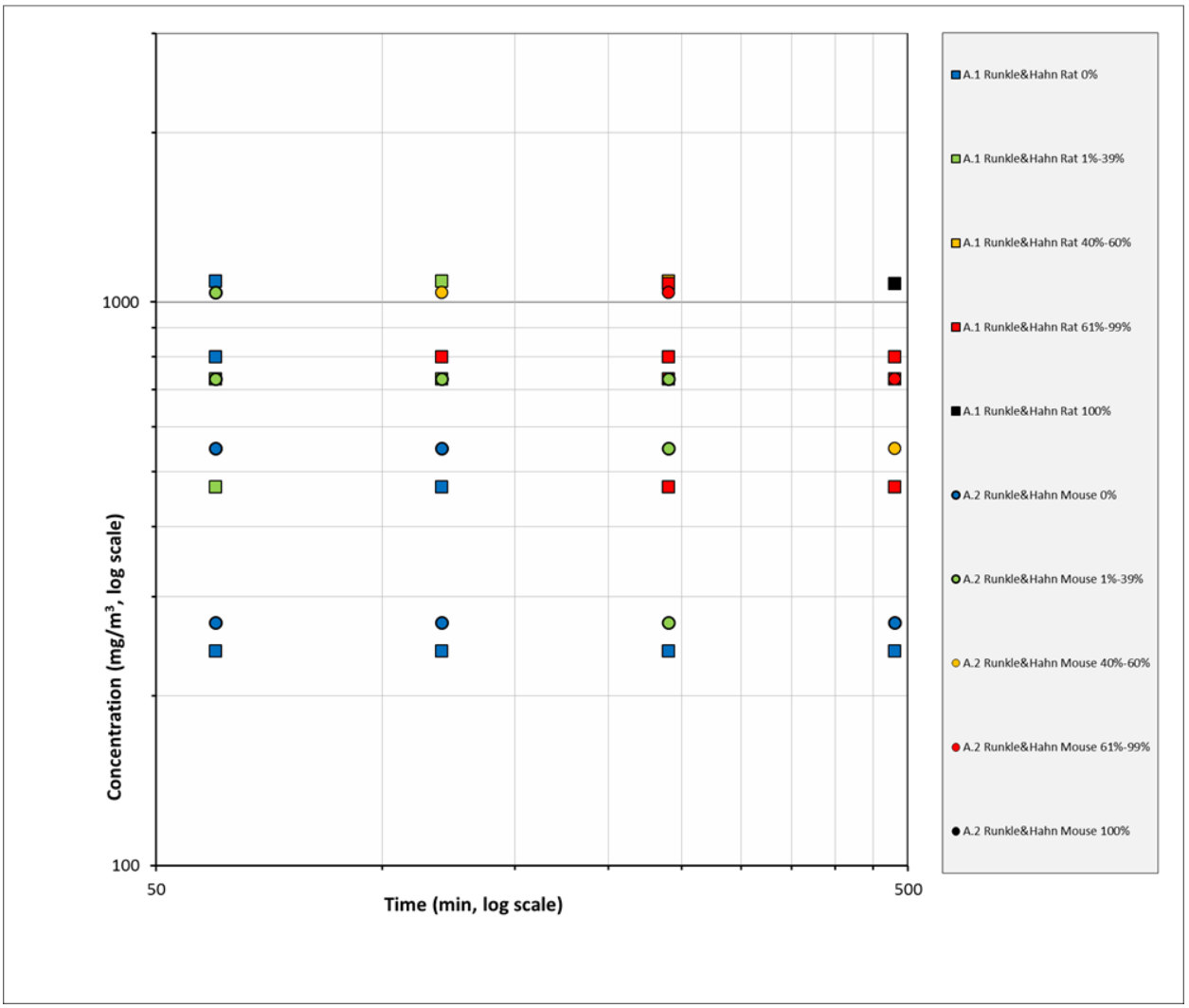


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of sulfuric acid.*

Based on criteria outlined in the guideline the data from rat study A.1 and mouse study A.2 were selected for the final dataset for the derivation of the animal probit function. 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 2 and Figure 4.

The final data eligible for calculating the animal probit function contains two datasets from one study and includes data from two animal species.

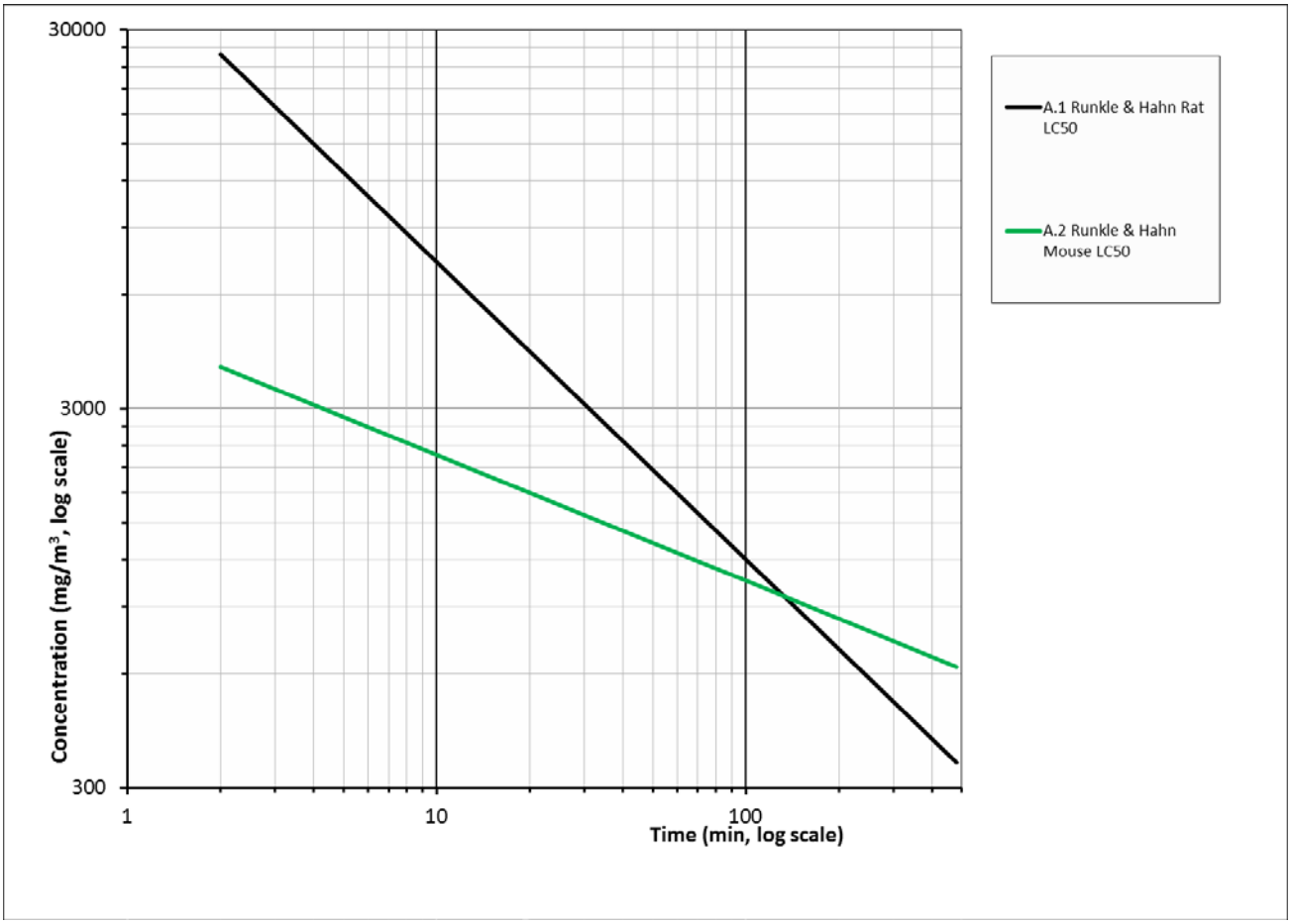


Figure 3 *LC_{50} values of A datasets for sulfuric acid, over time where available.*

Table 2 *Data selected for the derivation of the animal probit function of sulfuric acid (identical to table 1).*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} (60 min.) (mg/m^3) 95% C.I.	LC_{50}, 30 minutes (mg/m^3) 95% C.I.	n-value 95% C.I.
A.1	Rat	-12.46 + 1.63×lnC + 1.28×lnt	1789 (1304-2965)	3083 (1927 – 6599)	1.27 (0.81-1.73)
A.2	Mouse	-13.35 + 2.16×lnC + 0.72×lnt	1249 (1017-1705)	1573 (1165 – 2441)	3.00 (1.51-4.50)

The data of the selected datasets are presented graphically below.

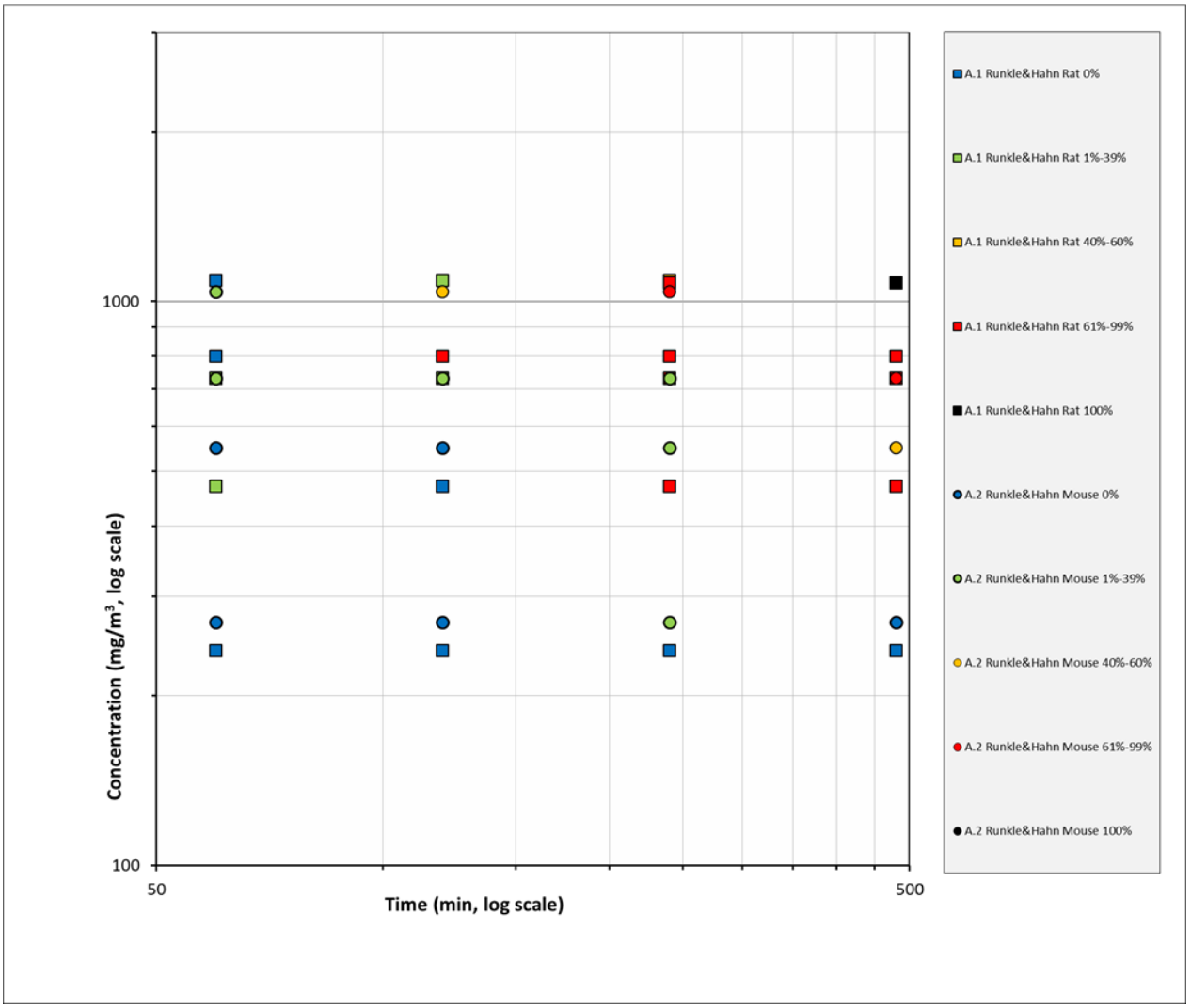


Figure 4 *Final data selected for derivation of the animal probit function of sulfuric acid (identical to figure 2).*

6. Derivation of the human probit function

To derive the human probit function the results from rat and mouse study A.1 and A.2 have been used to derive a point of departure as outlined above. The study of Runkle and Hahn (1976) was the only available A study and the only study from which a reliable n-value could be determined and included exposure durations of 60-480 minutes.

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

The species-specific n-value was 1.27 for the rat and 3.00 for the mouse. The arithmetic mean n-value across species is the simple arithmetic mean of the species-specific mean n-values, without weight and was calculated to be 2.14.

Finally, a geometric mean overall LC_{50}-value was calculated from the available LC_{50} values (1 per species). The formula for the geometric mean of (time-scaled) LC_{50}-values between species is as follows:

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

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

This resulted in an overall 60-min LC_{50} value of 1495 mg/m^3.

The Point of Departure for the human probit function is a 60-minute geometric mean animal LC_{50} value of 1495 mg/m^3 and an arithmetic mean n-value of 2.14.

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	Default.
Nominal concentration	1	Analytically determined concentrations.
Adequacy of database:	1	Database consists of two well conducted A datasets.

The estimated human equivalent 60-minute LC_{50} value is 1495 / 3 = **498 mg/m^3**.

The experimentally determined n-value was **2.14** (A.1 and A.2; Runkle and Hahn (1976)). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = 0.94.

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 + 0.94 × ln (498$^{2.14}$× 60) resulting in the a-value of **-11.30**.

Pr = -11.3 + 0.94 × ln (C $^{2.14}$× 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 one study in the rat and one study in mouse with A quality. The data of the rat study includes 168 animals and 21 C x t combinations, including durations from 60 to 480 minutes and lethality in the range of 0-100%. The data of the mouse study includes 160 animals and 15 C x t combinations, including durations from 60 to 480 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 108 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 158 mg/m^3.

Table 4 *LC-values 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	150	108
1% lethality, this probit	219	158
AEGL-3[2] (2008, interim)	200	160
ERPG-3[2] (2016)	-	120
LBW (2017)	200	160

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

[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: Runkle and Hahn, 1976
Substance: sulfuric acid
Species, strain, sex: male and female Fischer F344 rats
Number/sex/conc. group: 4 animals/sex/group
Age and weight: 6-7 weeks old, weight unspecified
Observation period: 21 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 (prior to 1981). Insufficient details to assess compliance with OECD guideline 403*
Stability of test compound in test atmosphere	*Aerosol formation present*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*No information provided*
Homogeneity of test atmosphere in breathing zone of animals	*SO_3 gas was mixed with humid air to produce H_2SO_4 droplets*
Number of air changes per hour	*Air flow was 10 cubic feet/min (17 m^3/h) into a 27-inch Rochester chamber (approx. volume 410 ℓ), which equals 41 air changes/h*
Equilibration time (t95)	*t95 is 4.3 minutes*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Actual concentrations appear to be measured since the concentrations reported deviate from the target concentration. In two elaborate studies* describing the atmosphere generation system and chamber airflow distribution (resulting in modifications to the chambers to achieve a uniform concentration distribution), actual concentrations were determined by titration for SO_4 with barium chloranilate as well as conductivity measurements after measurement of concentration of aerosol in a Mercer cascade impactor*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Approximately 1.1-1.4 µm MMAD (GSD 1.6-2.2)*

Assessment of Reliability	***A*** *Relatively well performed study, including multiple C x t combinations*

* Carpenter (1975-1976), Beethe and Carpenter (1975-1976)

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		Male+female
				Dead/tested
Rat	240	N/A	60	0/8
Rat	240	N/A	120	0/8
Rat	240	N/A	240	0/8
Rat	240	N/A	480	0/8
Rat	470	N/A	60	1/8
Rat	470	N/A	120	0/8
Rat	470	N/A	240	5/8
Rat	470	N/A	480	7/8
Rat	730	N/A	60	1/8
Rat	730	N/A	120	3/8
Rat	730	N/A	240	5/8
Rat	730	N/A	480	7/8
Rat	800	N/A	60	0/8
Rat	800	N/A	120	5/8
Rat	800	N/A	240	6/8
Rat	800	N/A	480	7/8
Rat	1080	N/A	240	7/8
Rat	1080	N/A	480	8/8
Rat	1090	N/A	60	0/8
Rat	1090	N/A	120	3/8
Rat	1090	N/A	240	5/8

Probit function

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

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

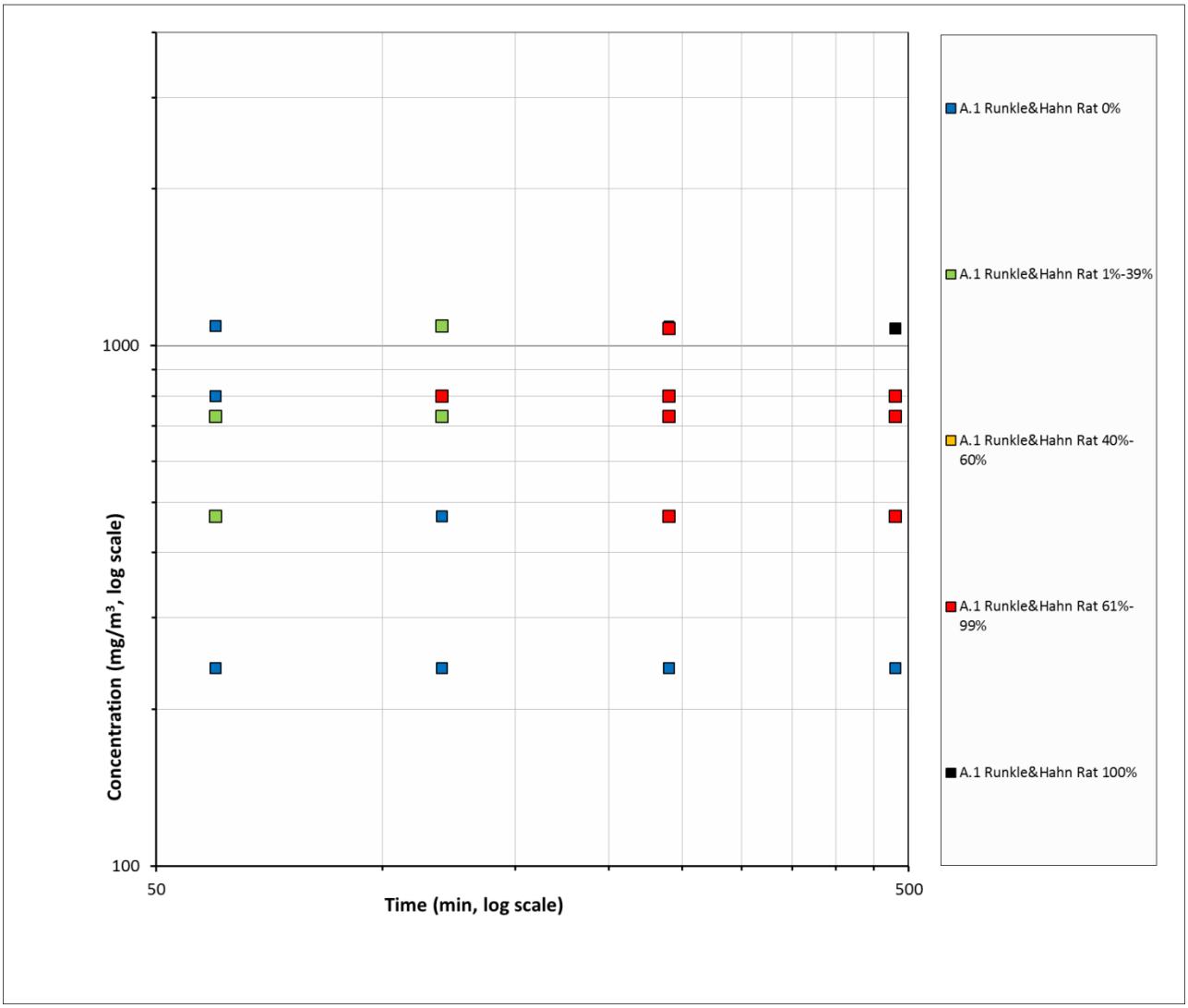
with C for concentration in mg/m^3, t for time in minutes.

Probit function	Species	a	b	c	n-value
Sexes combined	Rat	-12.46	1.63	1.28	1.27 (0.81-1.73)

As results have been provided for males and females combined, there is no information on sex differences.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combined
10	7303 (3543-23690)
30	3083 (1927-6599)
60	1789 (1304-2965)

A graphical overview of the data is presented below. Each concentration-time combination (with 4 male and 4 female animals) represents one point in the plot.


1000
100
50
500
Concentration (mg/m³, log scale)
Time (min, log scale)
A.1 Runkle&Hahn Rat 0%
A.1 Runkle&Hahn Rat 1%-39%
A.1 Runkle&Hahn Rat 40%-60%
A.1 Runkle&Hahn Rat 61%-99%
A.1 Runkle&Hahn Rat 100%

Study ID: A.2

Author, year: Runkle and Hahn, 1976
Substance: sulfuric acid
Species, strain, sex: male and female CD-1 mice
Number/sex/conc. group: 5-7 animals/sex/group
Age and weight: 6-7 weeks old, weight unspecified
Observation period: 21 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 (prior to 1981). Insufficient details to assess compliance with OECD guideline 403*
Stability of test compound in test atmosphere	*Aerosol formation present*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*No information provided*
Homogeneity of test atmosphere in breathing zone of animals	*SO_3 gas was mixed with humid air to produce H_2SO_4 droplets*
Number of air changes per hour	*Air flow was 10 cubic feet/min (17 m^3/h) into a 27-inch Rochester chamber (approx volume 410 ℓ), which equals 41 air changes/h*
Equilibration time (t95)	*t95 is 4.3 minutes*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Actual concentrations appear to be measured in the toxicology study since the concentrations reported deviate from the target concentration.* *In two elaborate studies* describing the atmosphere generation system and chamber airflow distribution (resulting in modifications to the chambers to achieve a uniform concentration distribution), actual concentrations were determined by titration for SO_4 with barium chloranilate as well as conductivity measurements after measurement of the concentration of aerosol in a Mercer cascade impactor*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Approximately 0.85-1.2 µm MMAD (GSD 1.6-2.0*
Assessment of Reliability	***A*** *Relatively well performed study, including multiple C x t combinations*

* Carpenter (1975-1976), Beethe and Carpenter (1975-1976)

Most of the fatalities occurred during exposure or within 1-2 days afterwards.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		Male+female
				Dead/tested
Mouse	270	N/A	60	0/10
Mouse	270	N/A	120	0/10
Mouse	270	N/A	240	1/10
Mouse	270	N/A	480	0/10
Mouse	550	N/A	60	0/10
Mouse	550	N/A	120	0/10
Mouse	550	N/A	240	2/10
Mouse	550	N/A	480	4/10
Mouse	730	N/A	60	3/10
Mouse	730	N/A	120	1/10
Mouse	730	N/A	240	3/10
Mouse	730	N/A	480	7/10
Mouse	1040	N/A	60	4/12
Mouse	1040	N/A	120	8/14
Mouse	1040	N/A	240	11/14

Probit function

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

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

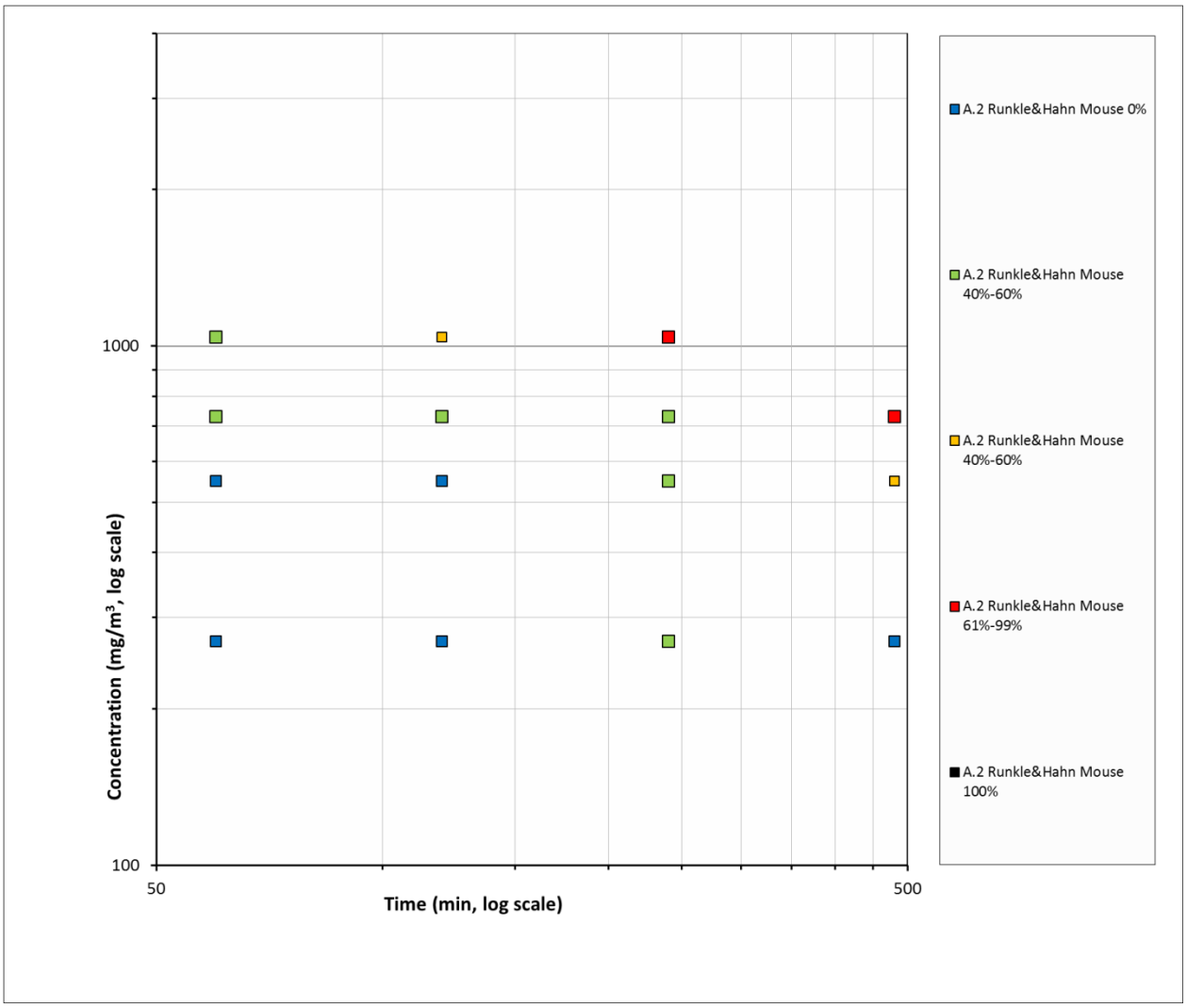
with C for concentration in mg/m^3, t for time in minutes.

Probit function	Species	a	b	c	n-value
Sexes combined	Mouse	-13.35	2.16	0.72	3.00 (1.51-4.50)

As results have been provided for males and females combined, there is no information on sex differences in the response.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combined
10	2268 (1428-4363)
30	1573 (1165-2441)
60	1249 (1017-1705)

A graphical overview of the data is presented below. Each concentration-time combination (with 5-7 male and 5-7 female animals) represents one point in the plot.


1000
100
Concentration (mg/m³, log scale)
50
500
Time (min, log scale)
A.2 Runkle&Hahn Mouse 0%
A.2 Runkle&Hahn Mouse 40%-60%
A.2 Runkle&Hahn Mouse 40%-60%
A.2 Runkle&Hahn Mouse 61%-99%
A.2 Runkle&Hahn Mouse 100%

Study ID: C.1

Author, year: Zwart, 1984
Substance: Sulfuric acid mist
Species, strain, sex: Male Wistar rats
Number/sex/conc. group: 10 animals/group
Age and weight: age unspecified, average weight 172 grams
Observation period: 14 days post exposure

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*GLP statement provided*
Study carried out according to OECD 403 guideline(s)	*No statement of compliance with OECD guideline 403 provided. Many conditions prescribed in the guideline appear to be met*
Stability of test compound in test atmosphere	*Aerosol formation*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*No information on pressure distribution provided*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere was generated by passing air, saturated with water, through a nebulizer fed with 97% H_2SO_4. Large particles were removed with a cyclone*
Number of air changes per hour	*Animals were exposed in 700 ml glass tubes with an air flow of 2 l/min from a centrally generated sulphuric acid mist (170 air changes/hour)*
Equilibration time (t95)	*t95 is 1.05 minutes*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*5-11 samples per exposure period were taken. The exact sampling location was not specified. The total amount H_2SO_4 was determined by titration*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Particle size was not determined*
Assessment of Reliability	***C*** *Insufficient number of concentration-time combinations to calculate a reliable probit function, particle size not determined*

Results

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

Rat	3.54 ×10^3	N/A	60	5/10
Rat	3.61×10^3	N/A	150	7/10
Rat	3.87×10^3	N/A	105	10/10
Rat	3.94 ×10^3	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×lnC + c×lnt

with C for concentration in mg/m^3 and t for time in minutes.

Probit function	Species	a	b	c	n-value
	Rat	-82.37	10.47	0.44	23.86 (-1494.38-1542.11)

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combined
10	3830 (a C.I. could not be determined)
30	3657 (a C.I. could not be determined)
60	3553 (a C.I. could not be determined)

The study author calculated a 60-min LC50 value of 3.6×10^3 mg/m^3 (70%-C.I.: 3.1×10^3 - 3.7×10^3 mg/m^3).

Study ID: C.2

Author, year: Treon et al., 1950
Substance: sulfuric acid mist
Species, strain, sex: mouse, sex and strain unspecified
Number/sex/conc. group: 5/group
Age and weight: 6-7 weeks old, weight unspecified
Observation period: unspecified; publication mentions time of death at 5 days post exposure, as well as sacrifice 48 hours after the last period of exposure (may apply only for repeated exposure regimens)

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 (prior to 1981). Insufficient details to assess compliance with OECD guideline 403*
Stability of test compound in test atmosphere	*Aerosol formation present*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body. It appears that 5 mice were exposed simultaneously*
Type of restrainer	*N/A*
Pressure distribution	*No information provided*
Homogeneity of test atmosphere in breathing zone of animals	*Aerosols were generated by spraying compressed air through an orifice fed with an aqueous solution (with different H_2SO_4 concentrations (10-60%)) through a capillary. A 'large fan' circulated the test atmosphere in the exposure chamber*
Number of air changes per hour	*Air flow was 3.2-34.5 L/min in a 223 L chamber, which equals 0.86-9.3 air changes/h*
Equilibration time (t95)	19-209 min
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Sampling location unspecified. Six samples for 7 hour periods, 3-4 samples for 1-3 hour periods. Material was collected in a 2-stage absorption train and analysed photometrically (after chemical reaction with BaCl). Acidity was determined by back titration in the absorption bottles with a standard solution of hydrochloric acid, the titration being carried out in an ice bath, with methylorange as the indicator*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Diameter of 93-99% of the particles was < 2 µm as determined with thermal precipitation*

Assessment of Reliability	***C*** *Difference in H_2SO_4 concentration of test material which results in different particle sizes, uncertain homogeneity and low exchange rate of test atmosphere. Duration of observation period not clear*

Most of the fatalities occurred during exposure or within 1-2 days afterwards.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		
				Dead/tested
Mouse	1.61×10^3	N/A	420	3/5
Mouse	1.47×10^3	N/A	210	2/5
Mouse	0.699×10^3	N/A	420	2/5
Mouse	0.718×10^3	N/A	210	3/5
Mouse	0.549×10^3	N/A	210	2/5
Mouse	0.461×10^3	N/A	420	0/5
Mouse	0.218×10^3	N/A	420	0/5
Mouse	0.199×10^3	N/A	420	0/5
Mouse	0.087×10^3	N/A	165	0/5

Probit function

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

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

with C for concentration in mg/m^3, t for time in minutes.

Probit function	Species	a	b	c	n-value
	Mouse	1.25	1.02	-0.60	-1.69 (-5.47-2.09)

As sex was not specified, there is no information on sex differences.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combined
10	154 (0.097-19220)
30	295 (2.35-8410)
60	444 (17.27-5085)

The calculated probit function for mice was meaningless, with non-physiological results (such as negative n-values) and large confidence intervals.

To calculate mouse LC_{50} values in the 10-60 minute range the data had to be extrapolated far beyond the range of the actual data (exposure durations of 165-420 min), rendering these LC_{50} values almost meaningless.

Study ID: C.3

Author, year: Treon et al., 1950
Substance: sulfuric acid mist
Species, strain, sex: rat, sex and strain unspecified
Number/sex/conc. group: 2/group
Age and weight: 6-7 weeks old, weight unspecified
Observation period: unspecified; publication mentions time of death at 5 days post exposure, as well as sacrifice 48 hours after the last period of exposure (may apply only for repeated exposure regimens)

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 (prior to 1981). Insufficient details to assess compliance with OECD guideline 403*
Stability of test compound in test atmosphere	*Aerosol formation present*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body. It appears that 2 rats were exposed simultaneously*
Type of restrainer	*N/A*
Pressure distribution	*No information provided*
Homogeneity of test atmosphere in breathing zone of animals	*Aerosols were generated by spraying compressed air through an orifice fed with an aqueous solution (with different H_2SO_4 concentrations (10-60%)) through a capillary. A 'large fan' circulated the test atmosphere in the exposure chamber*
Number of air changes per hour	*Air flow was 3.2-34.5 L/min in a 223 L chamber, which equals 0.86-9.3 air changes/h*
Equilibration time (t95)	19-209 min
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Sampling location unspecified. Six samples for 7 hour periods, 3-4 samples for 1-3 hour periods. Material was collected in a 2-stage absorption train and analysed photometrically (after chemical reaction with BaCl). Acidity was determined by back titration in the absorption bottles with a standard solution of hydrochloric acid, the titration being carried out in an ice bath, with methylorange as the indicator*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Diameter of 93-99% of the particles was < 2 µm as determined with thermal precipitation*

Assessment of Reliability	***C*** *Difference in H_2SO_4 concentration of test material which results in different particle sizes, uncertain homogeneity and low exchange rate of test atmosphere. Duration of observation period not clear. No information on data between 0 and 100% lethality*

Most of the fatalities occurred during exposure or within 1-2 days afterwards.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		
				Dead/tested
Rat	1.61×10^3	N/A	420	2/2
Rat	1.47×10^3	N/A	210	2/2
Rat	0.699×10^3	N/A	420	2/2
Rat	0.718×10^3	N/A	210	0/2
Rat	0.549×10^3	N/A	210	0/2
Rat	0.461×10^3	N/A	420	0/2
Rat	0.218×10^3	N/A	420	0/2
Rat	0.199×10^3	N/A	420	0/2
Rat	0.087×10^3	N/A	165	0/2

Probit function

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

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

with C for concentration in mg/m^3, t for time in minutes.

Probit function	Species	a	b	c	n-value
	Rat	-110.11	10.39	8.15	1.28 (-0.45-3.00)

As sex was not specified, there is no information on sex differences.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combined
10	10620 ($17.59-1.98x10^{15}$)
30	4487 ($50.87-3.97x10^{11}$)
60	2606 ($99.05-1.85x10^{9}$)

To calculate rat LC_{50} values in the 10-60 minute range the data had to be extrapolated far beyond the range of the actual data (exposure duration of 165-420 min), rendering these LC_{50} values almost meaningless.

Study ID: C.4

Author, year: Treon et al., 1950
Substance: sulfuric acid mist
Species, strain, sex: rabbit, sex and strain unspecified
Number/sex/conc. group: 2/group
Age and weight: 6-7 weeks old, weight unspecified
Observation period: unspecified; publication mentions time of death at 5 days post exposure, as well as sacrifice 48 hours after the last period of exposure (may apply only for repeated exposure regimens)

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 (prior to 1981). Insufficient details to assess compliance with OECD guideline 403*
Stability of test compound in test atmosphere	*Aerosol formation present*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body. It appears that 2 rabbits were exposed simultaneously*
Type of restrainer	*N/A*
Pressure distribution	*No information provided*
Homogeneity of test atmosphere in breathing zone of animals	*Aerosols were generated by spraying compressed air through an orifice fed with an aqueous solution (with different H_2SO_4 concentrations (10-60%)) through a capillary. A 'large fan' circulated the test atmosphere in the exposure chamber*
Number of air changes per hour	*Air flow was 3.2-34.5 L/min in a 223 L chamber, which equals 0.86-9.3 air changes/h*
Equilibration time (t95)	19-209 min
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Sampling location unspecified. Six samples for 7 hour periods, 3-4 samples for 1-3 hour periods. Material was collected in a 2-stage absorption train and analysed photometrically (after chemical reaction with BaCl). Acidity was determined by back titration in the absorption bottles with a standard solution of hydrochloric acid, the titration being carried out in an ice bath, with methylorange as the indicator*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Diameter of 93-99% of the particles was < 2 µm as determined with thermal precipitation*

Assessment of Reliability	***C*** *Difference in H_2SO_4 concentration of test material which results in different particle sizes, uncertain homogeneity and low exchange rate of test atmosphere. Duration of observation period not clear. No information on data between 0 and 100% lethality*

Most of the fatalities occurred during exposure or within 1-2 days afterwards.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		
				Dead/tested
Rabbit	1.61×10^3	N/A	420	1/2
Rabbit	1.47×10^3	N/A	210	1/2
Rabbit	0.699×10^3	N/A	420	0/2
Rabbit	0.718×10^3	N/A	210	0/2
Rabbit	0.549×10^3	N/A	210	0/2
Rabbit	0.461×10^3	N/A	420	0/2
Rabbit	0.218×10^3	N/A	420	0/2
Rabbit	0.199×10^3	N/A	420	0/2
Rabbit	0.087×10^3	N/A	165	0/2

Probit function

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

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

with C for concentration in mg/m^3, t for time in minutes.

Probit function	Species	a	b	c	n-value
	Rabbit	-581.96	89.05	-11.69	-7.62 (-9.01 - -6.23)

As sex was not specified, there is no information on sex differences.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combined
10	986 (C.I. could not be calculated)
30	1139 (C.I. could not be calculated)
60	1247 (C.I. could not be calculated)

The probit function for rabbit was meaningless, with non-physiological results (such as negative n-values) and a large variance that did not allow calculating large confidence intervals.

To calculate rabbit LC_{50} values in the 10-60 minute range the data had to be extrapolated far beyond the range of the actual data, rendering these LC_{50} values meaningless.

Study ID: C.5

Author, year: Treon et al., 1950
Substance: sulfuric acid mist
Species, strain, sex: guinea pig, sex and strain unspecified
Number/sex/conc. group: 2-3/ group
Age and weight: 6-7 weeks old, weight unspecified
Observation period: unspecified; publication mentions time of death at 5 days post exposure, as well as sacrifice 48 hours after the last period of exposure (may apply only for repeated exposure regimens)

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 (prior to 1981). Insufficient details to assess compliance with OECD guideline 403*
Stability of test compound in test atmosphere	*Aerosol formation present*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body. It appears that 2-3 guinea pigs were exposed simultaneously*
Type of restrainer	*N/A*
Pressure distribution	*No information provided*
Homogeneity of test atmosphere in breathing zone of animals	*Aerosols were generated by spraying compressed air through an orifice fed with an aqueous solution (with different H_2SO_4 concentrations (10-60%)) through a capillary. A 'large fan' circulated the test atmosphere in the exposure chamber*
Number of air changes per hour	*Air flow was 3.2-34.5 L/min in a 223 L chamber, which equals 0.86-9.3 air changes/h*
Equilibration time (t95)	19-209 min
Start of exposure relative to equilibration	*In experiments which did not exceed 60 minutes in duration, the desired concentration of mist was built up in the chamber before the animals were introduced through a door in its side. For experiments of >60 minutes exposure duration, no information on the start of exposure relative to equilibration is available*

Actual concentration measurement	*Sampling location unspecified. Six samples for 7 hour periods, 3-4 samples for 1-3 hour periods and 2 samples for 30 minute periods. Material was collected in a 2-stage absorption train and analysed photometrically (after chemical reaction with BaCl).Acidity was determined by back titration in the absorption bottles with a standard solution of hydrochloric acid, the titration being carried out in an ice bath, with methylorange as the indicator*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Diameter of 93-99% of the particles was < 2 µm as determined with thermal precipitation*
Assessment of Reliability	***C*** *Difference in H_2SO_4 concentration of test material which results in different particle sizes, uncertain homogeneity and low exchange rate of test atmosphere. Insufficient number of tested exposure durations. Duration of observation period not clear*

Most of the fatalities occurred during exposure or within 1-2 days afterwards.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		
				Dead/tested
Guinea pig	0.218×10^3	N/A	420	2/2
Guinea pig	0.199×10^3	N/A	420	3/3
Guinea pig	0.206×10^3	N/A	180	2/3
Guinea pig	0.178×10^3	N/A	60	2/3
Guinea pig	0.178×10^3	N/A	30	1/2
Guinea pig	0.165×10^3	N/A	15	1/2
Guinea pig	0.121×10^3	N/A	60	0/3
Guinea pig	0.120×10^3	N/A	30	0/3
Guinea pig	0.116×10^3	N/A	15	0/3
Guinea pig	0.087×10^3	N/A	165	3/3

Probit function

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

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

with C for concentration in mg/m^3, t for time in minutes.

Probit function	Species	a	b	c	n-value
	Guinea pig	-2.82	0.84	0.87	0.97 (-1.47-3.40)

As sex was not specified, there is no information on sex differences.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combined
10	1020 (C.I. could not be calculated)
30	327 (C.I. could not be calculated)
60	160 (C.I. could not be calculated)

The probit function for guinea pigs was meaningless, with non-physiological results and a large variance that did not allow calculating reliable confidence intervals.

The guinea pig has been frequently used as an animal model to study the effects of sulfuric acid inhalation. This animal species appeared to be far more sensitive than other animal species. For example, the 8 hour LC_{50} values as determined by Runkle and Hahn (1976) in rats and mice of 425 and 600 mg/m^3, respectively, are much higher than the 8-hour value of 31 mg/m^3 for guinea pigs as stated in the same reference. These findings are confirmed by the results of Treon (1950). Schwartz *et al*. (1977) found that guinea pigs are far more sensitive to pulmonary damage by sulfuric acid inhalation than other species. For the reasons stated above, the guinea pig is considered as no suitable animal model to predict the acute health effects of sulfuric acid inhalation in humans. No reliable data can be obtained by using guinea pigs as test species.

Study ID: Other C studies

In a publication with toxicological data on many chemicals Vernot (1977) reported 1-hour LC_{50} values for sulphuric acid of 420 ppm (1714 mg/m^3) for male rats and 347 ppm (1416 mg/m^3) for female rats. Many relevant details of the study design were not provided. The SO_3 content of the fuming sulfuric acid was not given. It is not clear what exactly was measured (SO_3 or H_2SO_4 or both) and what methods were used to generate, control and monitor the exposure. Results are expressed in ppm. No information is provided on the particle size of the mist or on the follow-up period.

One cat (strain and sex not given) was exposed (whole body) for 7 h to an actual concentration of 461 mg/m^3 sulfuric acid mist and survived (Treon et al. 1950). The cat showed salivation, a striking response of cats to irritant materials in air. Postmortem examination (48 hours after exposure) of the lungs showed numerous partially atelectatic alveoli, a few areas of definite emphysema, numerous small hemorrhages and severe congestion of the lungs. Shreds of desquamated epithelium were seen in the lumen of the bronchioles and mucous degeneration of the cells lining the bronchi occurred. The trachea showed marked infiltration of polymorphonuclear granulocytes and there was edema of the vocal folds infiltrated with leukocytes.

Appendix 2 Reference list

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