National Institute for Public Health
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Probit function technical support document

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substance name	CAS number
Sulfur dioxide	**7446-09-5**

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 Sulfur dioxide

1. Substance identification

CAS-number:	7446-09-5
IUPAC name:	(oxo-λ⁴-sulfanylidene)oxidane
Synonyms:	oxosulfane oxide, sulphur oxide, sulphur dioxide
Molecular formula:	SO_2
Molecular weight:	64 g/mol
Physical state:	gas (at 20°C and 101.3 kPa)
Boiling point:	-10°C (at 101.3 kPa)
Vapour pressure:	330 kPa (at 20°C)
Saturated vapor conc:	N/A (at 20°C)
Conversion factor:	1 mg/m^3 = 0.376 ppm (at 20°C and 101.3 kPa)
	1 ppm = 2.66 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H314-331

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

Acute effects:
The main target organs and tissues for airborne exposure to sulfur dioxide are the respiratory tract and the eyes. The substance is a direct acting irritant and may cause conjunctivitis, corneal burns. Corneal opacity may occur from direct contact with high concentrations of sulfur dioxide. Bronchitis, bronchopneumonia, and fibrosing bronchiolitis obliterans can occur at high exposures. Bronchoconstriction accompanied by increased pulmonary resistance may occur with high-pitched rales. Damage occurs to the upper respiratory tract. Lethality occurs from respiratory arrest. Apparently high concentrations of SO_2 may cause death via asphyxia secondary to pulmonary edema and irreversible airway obstruction.
Long-term effects: Chronic exposure produces similar effects as from acute exposures. It is noted that death can be significantly delayed (lethality after 17 days has been reported upon human accidental exposure), due to severe damage to the bronchioles.

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. There are four reports of accidents mentioned in the AEGL document on sulfur dioxide. The accidents showed either acute lethality within minutes, delayed lethality (17 days) due to severe damage in the bronchioles, or survival.

There are many controlled experiments performed with sulfur dioxide in healthy and asthmatic volunteers. Occupational and epidemiological data are available. Levels up to 25 ppm (66.5 mg/m^3) for 6 hours were irritating, induced mucous flow and lowered the FEV_1.

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 sulfur dioxide, covering references before and including 1995.

[1] AEGL 2010.

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 five studies were identified -with seven datasets for three species- with data on lethality following acute inhalation exposure. No datasets were assigned status A for deriving the human probit function, two datasets were assigned status B and five were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

A total of one study was identified in which sensory irritation was studied. In this study the following RD_{50} range was observed by interpretation of Figures 1 and 2 in the paper by Wakisaka 1975.

Table 1 *Sensory irritation data for sulfur dioxide*

Species/strain	RD_{50} (mg/m^3)	Exposure duration (min)	Author/year
Mice, dd strain	$340\text{-}665^{P,F}$	10	Wakisaka, 1975

P: a plateau was reached, F: slight fading of response during exposure

5. Probit functions from individual studies

All available acute lethality data on sulfur dioxide are displayed in Figure 1.

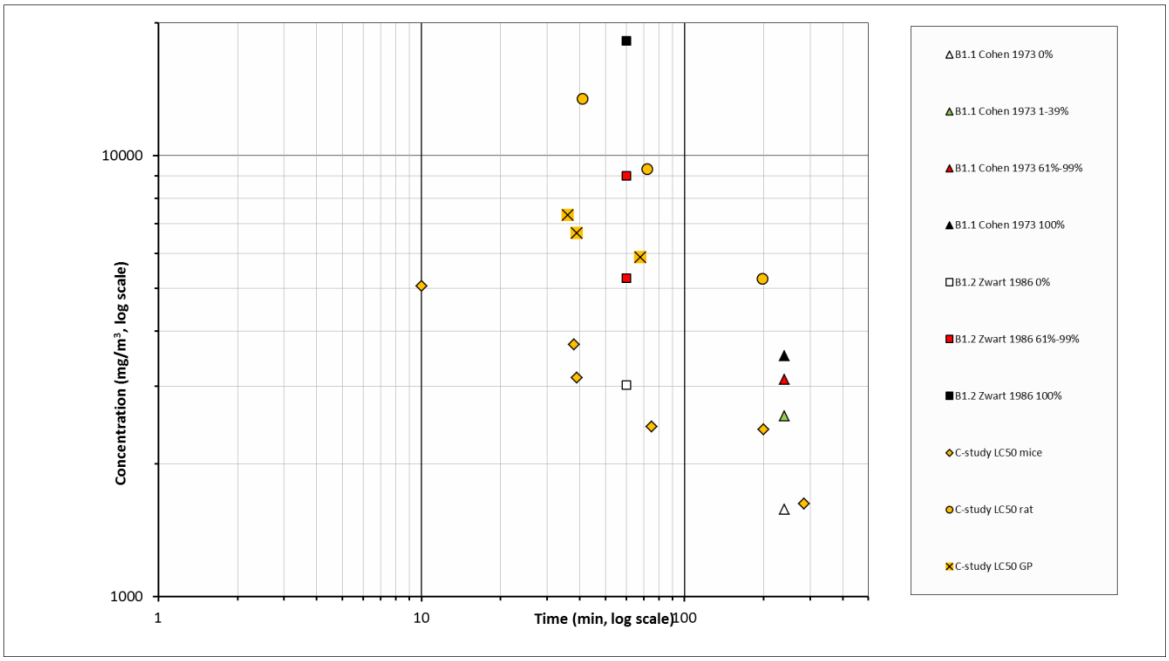

Figure 1 *All available acute lethality data for sulfur dioxide.*

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

All B1 studies were selected for derivation of the animal probit function for sulfur dioxide.

It was possible to derive a probit function for sulfur dioxide based on the available studies with B1 quality. Therefore, the probit function was derived using data from a study with B1 quality, which did not enable 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 Table 2.

Table 2 *Data selected for initial analysis of the animal probit function of sulfur dioxide*

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.
B1.1	Rat	*240-min LC_{50}*	2769 (2177 – 3058)	*7832*	N/A
B1.2	Rat	*60-min LC_{50}*	5139 (4033 – 6466)	*7268*	N/A

The data of the two B1 studies with rats are presented graphically below.

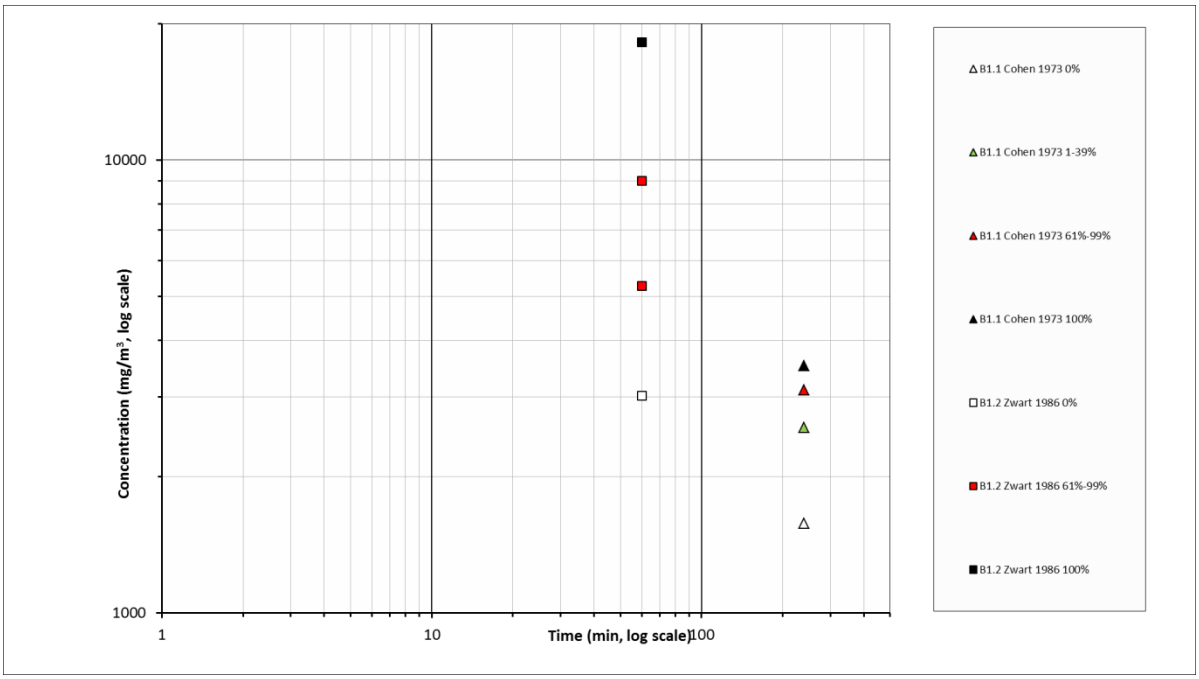

Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of sulfur dioxide*

Based on criteria outlined in the guideline the 60-min dataset from study B1.2 (Zwart, 1986) was selected for the final dataset for the derivation of the animal probit function. According to the methodology, studies with an exposure duration closer to the target of 30-60 min are preferred. An extrapolation from an exposure duration of

240 min to a 30-60 min value, which, in absence of a substance-specific n-value, should in this case be applied with the default n-value of 2, is believed to be too uncertain. Therefore, preference was given to the 60-min dataset of rat study B1.3.

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

Table 3 *Data selected for the derivation of the animal probit function of sulfur dioxide*

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.
B1.2	Rat	*60-min LC_{50}*	5139 (4033 – 6466)	*7268*	N/A

The data of the selected dataset are presented graphically below.

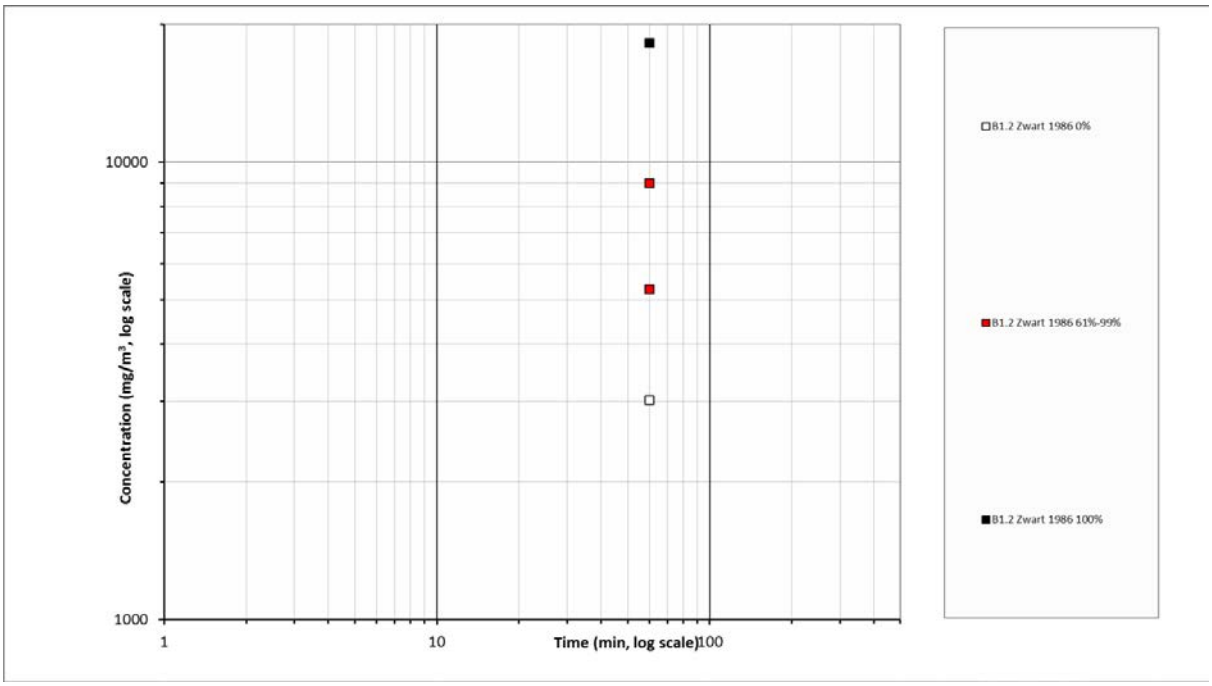

Figure 3 Final data selected for derivation of the animal probit function of sulfur dioxide.

6. Derivation of the human probit function

To derive the human probit function the results from B1.2 (Zwart, 1986) have been used to derive a point of departure as outlined above.

The Point of Departure for the human probit function is a 60-minute animal LC_{50} value of 5139 mg/m^3.

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 value
Nominal concentration	1	Actual concentrations were determined. Study description allowed to calculate the adjusted concentrations as a result of concentration build-up.
Adequacy of database:	2	Two B1 studies available, however only one could be used for probit function derivation because of uncertainty extrapolating a 240-min LC_{50} value to 30 minutes with the default n-value. The database is relatively small. Additional uncertainty includes weight loss of survivors in the 2nd (last) observation week in the only remaining study.

The estimated human equivalent 60-minute LC_{50} value is 5139 / 6 = **857 mg/m^3**.

No reliable experimentally determined n-value was available, so the default n-value of **2.0** 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 = **1.0**.

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): $5 = a + 1 \times \ln (857^2 \times 60)$ resulting in the a-value of **-12.60**.

$Pr = -12.6 + 1 \times \ln (C^2 \times t)$ 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 one study in the rat with B1 quality, including 40 animals.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 183 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 267 mg/m^3.

Table 5 *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	258	183
1% lethality, this probit	378	267
AEGL-3[2] (2010, final)	79.8	79.8
ERPG-3[2] (2011)		66.5
LBW (2016)	310	240

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are similar to the LBW but higher than the AEGL and ERPG.

[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: B1.1

Author, year: ***Cohen et al., 1973***
Substance: sulfur dioxide
Species, strain, sex: Rats, Charles River CD outbred, males
Number/sex/conc. group: 8 males per concentration
Age and weight: about 150 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*
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*
Homogeneity of test atmosphere in breathing zone of animals	*Atmospheres of SO_2 were maintained by metering directly into the incoming air and monitored at frequent intervals.*
Number of air changes per hour	*No information*
Equilibration time (t95)	*Cannot be derived*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*The air was monitored at frequent intervals by an iodometric procedure. Once the desired concentration was achieved it varied less than 5% during exposure.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Well performed study with one exposure duration included. Limited information is available on the exposure design.*

Besides a 4hr lethality study, also the survival times of three concentrations were determined.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Rat	596		240	0/8	
	1577		240	0/8	
	2567		240	3/8	
	3107		240	5/8	
	3509		240	8/8	

Probit function

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

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

with C for concentration in mg/m^3.

Only the data from the 4hr acute toxicity study were used in the analysis.

Probit function	Species	a	b	n-value
	Rat	-44.5	6.25	N/A

The derived 240-min LC_{50} value was as follows:

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Male
240	2769 (2177 – 3058)

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

In addition to the regular 240-min inhalation lethality study described above, the authors also performed a study to determine the mean survival time (MST) at three concentrations. The MST was found to be 176 ± 9 min at 6251 mg/m^3, 12.5 ± 0.9 hrs at 2461 mg/m^3 and about 32 hrs (determined from a graph) at 1569 mg/m^3 exposure group; the 1569 mg/m^3 exposure was discontinued after 65 hrs with 1/8 surviving animals.

Study ID: B1.2

Author, year: ***Zwart, 1986***
Substance: sulfur dioxide
Species, strain, sex: Rat, SPF-reared (Bor:WISW=Cpb:WU), males and females
Number/sex/conc. group: 5/sex/group
Age and weight: at study onset 158 g (males) and 130 g (females)
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 information*
Stability of test compound in test atmosphere	*The stability of the lowest concentrations has been checked by infrared monitoring. Stable.*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body, exposure glass chamber capacity about 0.015 m^3*
Type of restrainer	*N/A*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*Sulfur dioxide test atmosphere was generated by mixing an adjustable flow of the substance with the airflow before entering the chamber.*
Number of air changes per hour (ACH)	*Ventilation with 1.2 m^3/h corresponds to 80 ACH.*
Equilibration time (t95)	*2.25 minutes*
Start of exposure relative to equilibration	*Probably at the start of concentration build-up.*
Actual concentration measurement	*Ports at entry and exit allowed sampling. Test atmosphere was drawn through a glass fritted washing bubbler filled with 3% hydrogen peroxide to oxidize sulfur dioxide. By acid-base titration the accumulated SO_3 was determined as H_2SO_4.* *The actual concentration of the test atmosphere was determined at regular intervals during each exposure period.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Well performed study; one exposure duration was tested.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Combined*	
Rat	3.05×10^3		60	0/10	
	5.34×10^3		60	7/10	
	9.01×10^3		60	9/10	
	18.43×10^3		60	10/10	

* The author did not provide mortality data by sex.

The study author further noted that: “A considerable decrease in body weights of these groups was seen in the last week of the observation period. It is therefore very likely that a prolonged observation period would have resulted in a higher mortality in these groups. This would have resulted in a slightly lower LC_{50} value”.

Probit function

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

$Pr = a + b \times lnC$

with C for concentration in mg/m^3.

Probit function	Species	a	b	n-value
	Rat	-21.15	3.06	N/A

The derived 60-min LC_{50} value was as follows:

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. combined
60	5139 (4033 – 6466)

The LC_{50} value and 90% confidence interval reported by the study author are 5140 (2350 – 10000) mg/m^3.

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

Study ID: C studies

Mice

Hilado and Machado (1977) exposed groups of four Swiss-albino mice to nominal SO_2 concentrations (no analytical data were presented) of 1190 to 14,286 ppm (3165 to 38001 mg/m^3) and monitored time to the first sign of incapacitation, time to convulsions and time to death. Animals were exposed in a 4.2 liter, polymethyl methacrylate chamber. The SO_2 was injected with a 60 ml syringe which had been filled from a gas supply cylinder. Time to first sign of incapacitation was under 3 min for 3500 to 14,300 ppm (9310 to 38038 mg/m^3) SO_2 and increased to 6 min as SO_2 concentration was decreased to 1100 ppm (2926 mg/m^3). Average time to staggering increased from 1 to 6 min and average time to convulsions increased from 2 to 8 min as SO_2 concentration decreased from 14,300 to 3500 ppm. Average time to death increased from 3 to 8 min as SO_2 concentration decreased from 14,300 to 4800 ppm (12768 mg/m^3). There were no deaths in animals exposed to 1190 ppm SO_2 for 30 min. This study does not provide useful concentration-time-lethality information for the derivation of a probit function because all lethality occurred during exposure.

Bitron and Aharonson (1978) exposed groups of 14 male albino mice (21±1 g, 1 month old) to 900 ppm (2394 mg/m^3) SO_2 for 25-640 min (9 exposure groups), 1400 ppm (3724 mg/m^3) SO_2 for 15 to 180 min (13 exposure groups), or 1900 ppm (5054 mg/m^3) SO_2 for 10 to 75 min (9 exposure groups). Median lethal exposure time (Lt50) for each concentration was calculated to be 200 min, 38 min, and 10 min for the 900, 1400, and 1900 ppm SO_2 concentrations, respectively. The authors did not report concentration-time-lethality data, so the information could not be used to derive a probit function.

Leong et al. (1961) exposed 12 male mice (25-30 g) to concentrations of 610, 913, and 1178 ppm (1623, 2429, and 3133 mg/m^3) SO_2. The exposures were carried out in a 1 m^3 gassing chamber with an air flow rate of 500 liters per minute. Concentrations were analytically determined by titration with sodium hydroxide. The mean survival times were 283.6 (172.8-472.3), 74.5 (42.2-131.3), and 38.7 (23.6-63.4) minutes (limits of SD) for the low, medium and high dose group, respectively. This study does not provide useful concentration-time-lethality information for the derivation of a probit function because all lethality occurred during exposure.

Rats

Leong et al. (1961) exposed 12 male albino Sprague Dawley rats (200-215 g) to concentrations of 1975, 3498, and 5052 ppm (5254, 9305, and 13438 mg/m^3) SO_2. The exposures were carried out in a 1 m^3 gassing chamber with an air flow rate of 500 liters per minute. Concentrations were analytically determined by titration with sodium hydroxide. The mean survival times were 197.6 (148.0-263.9), 71.7 (63.0-81.6), and 41.0 (29.6-56.9) minutes (limits of SD) for the low, medium and high dose group, respectively. This study does not provide useful concentration-time-lethality information for the derivation of a probit function because all lethality occurred during exposure.

Guinea pigs

Leong et al. (1961) exposed 12 male guinea pigs (300-350 g) to concentrations of 2207, 2508, and 2750 ppm (5871, 6671, and 7315 mg/m^3) SO_2. The exposures were carried out in a 1 m^3 gassing chamber with an air flow rate of 500 liters per minute. Concentrations were analytically determined by titration with sodium hydroxide. The mean survival times were 68.2 (17.1-270.7), 38.7 (9.2-163.5), and 35.5 (10.9-115.5) minutes (limits of SD) for the low, medium and high dose group, respectively. This study does not provide useful concentration-time-lethality information for the derivation of a probit function because all lethality occurred during exposure.

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