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

Probit function technical support document

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substance name	CAS number
Bromine	**7726-95-6**

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 Bromine

1. Substance identification

CAS-number:	7726-95-6
IUPAC name:	Bromine
Synonyms:	NA
Molecular formula:	Br_2
Molecular weight:	159.8 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	59°C (at 101.3 kPa)
Vapour pressure:	23.3 kPa (at 20°C)
Saturated vapor conc:	233,000 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.150 ppm (at 20°C and 101.3 kPa)
	1 ppm = 6.647 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	Human 330, 314

2. Mechanism of action and toxicological effects following acute exposure

Acute effects: The main target organs and tissues for inhalation exposure to bromine are the eyes and respiratory epithelium, mainly in the middle and lower portions of the respiratory system. The health endpoints are edema, inflammation and epithelial damage in the affected portions of the respiratory system.
Symptoms of high exposure are cough, dyspnea, pain and bronchial secretions, and secondarily cardiac and central nervous symptoms effects as a result of the hypoxemia. Lethality results pulmonary edema and respiratory failure.
Effects of inhalation include initially: irritation of the eyes, nose and throat, followed by coughing and wheezing, dyspnea, sputum production and chest pain. Larger exposures may lead to acidosis; anoxia may lead to cardiac and/or respiratory arrest and pulmonary edema. Following chemical pneumonitis respiratory distress and chest pain generally subsides within 72 hours; cough may persist for up to 14 days, however in one case reduced airway flow and mild hypoxemia persisted for 14 months.
Long-term effects: Chronic exposure produces essentially the same effects as acute exposure. Irreversible lung damage, such as RADS has been described in humans and animals exposed to high concentrations of bromine.

3. Human toxicity data

No informative reports on human lethality following acute inhalation exposure were identified in which details about both health effects and the exposure have been documented in sufficient detail. One accident described by Morabia (1988) confirms the general picture of clinical signs and symptoms expected from animal studies: eye and upper respiratory irritation, cough, expectoration and headache.
Rupp and Henschler (1967) studied subjective symptoms in human test subjects following inhalation exposure to bromine. The maximum concentration used in the study, 6,7 mg/m^3 for 30 minutes, produced irritation in the nose and throat as well as headache, which was more severe than that experienced during chlorine exposure at the same concentration (in ppm).

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 bromine, 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 5 studies were identified -with 8 datasets for 5 species- with data on lethality following acute inhalation exposure. None of the datasets were assigned status A for deriving the human probit function, 1 dataset was assigned status B and 7 were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

No studies were identified in which sensory irritation was studied.

5. Probit functions from individual studies

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

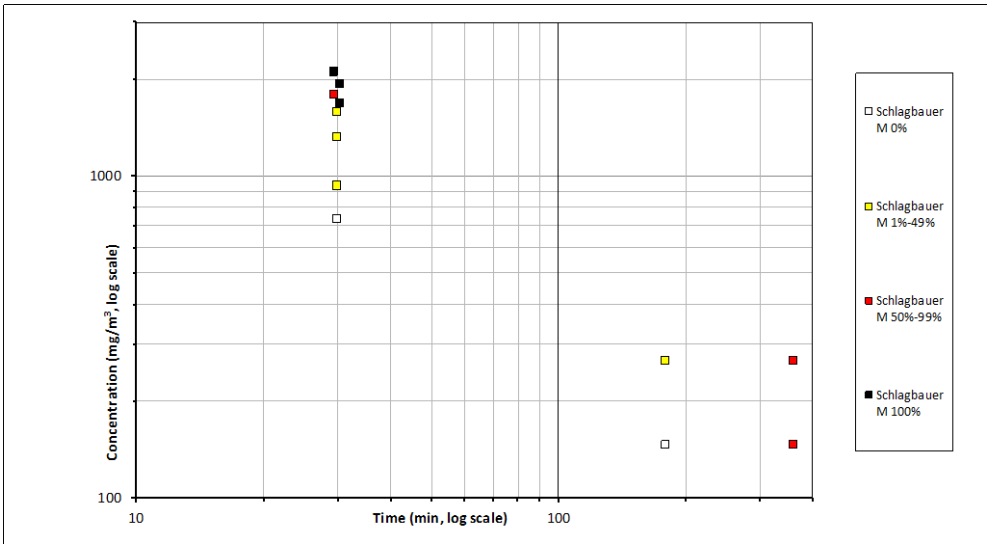


Figure 1 *All available acute lethality data for bromine.*

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

The only B-study (B2.1) was selected for initial analysis of the animal probit function for bromine. A probit function has been calculated and reported in Appendix 1 for study B2.1. The results of the calculations are presented in Table **1**.

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

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50}, 30 minutes (mg/m^3) 95% C.I.	n-value 95% C.I.
B2.1	Mouse	-26.89 + 3.290×lnC + 2.577×lnt	1128 (980 – 1264)	1.277 (1.113-1.420)

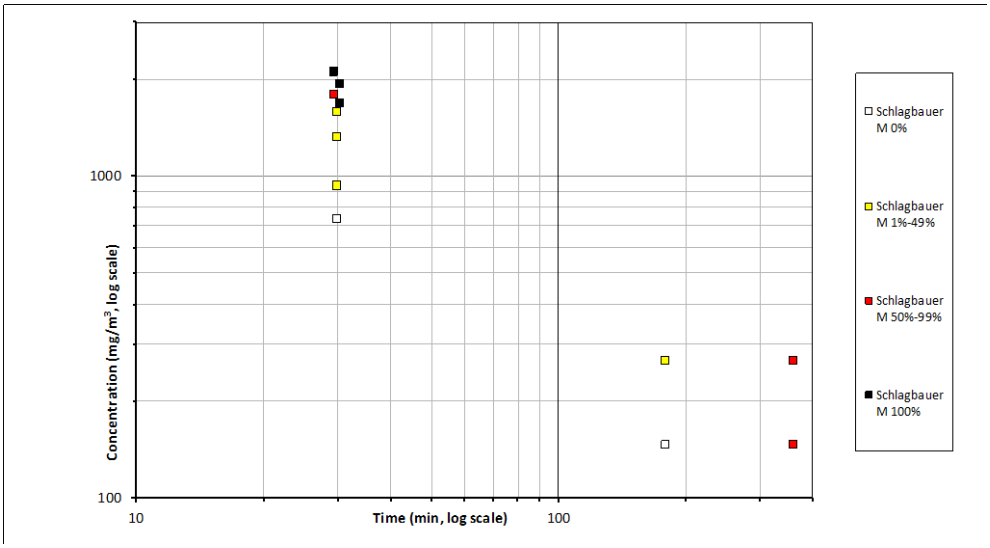


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of bromine (identical to figure 1).*

Based on criteria outlined in the guideline the data from study B2.1 were selected for the final dataset for the derivation of the animal probit function. Table 2 provides an overview of the LC_{50} value and the LC_{50}-time relationships for this study in the final analysis. The data that were selected for final analysis of the animal probit function are presented in Table 2 and Figure 3.

The final data eligible for calculating the animal probit function contains 4 datasets from 3 studies and includes data from 2 animal species.

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

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50}, 30 minutes (mg/m^3) 95% C.I.	n-value 95% C.I.
B2.1	Mouse	-26.89 + 3.290×lnC + 2.577×lnt	1128 (980 – 1264)	1.277 (1.113-1.420)

The data of the selected datasets are presented graphically below.

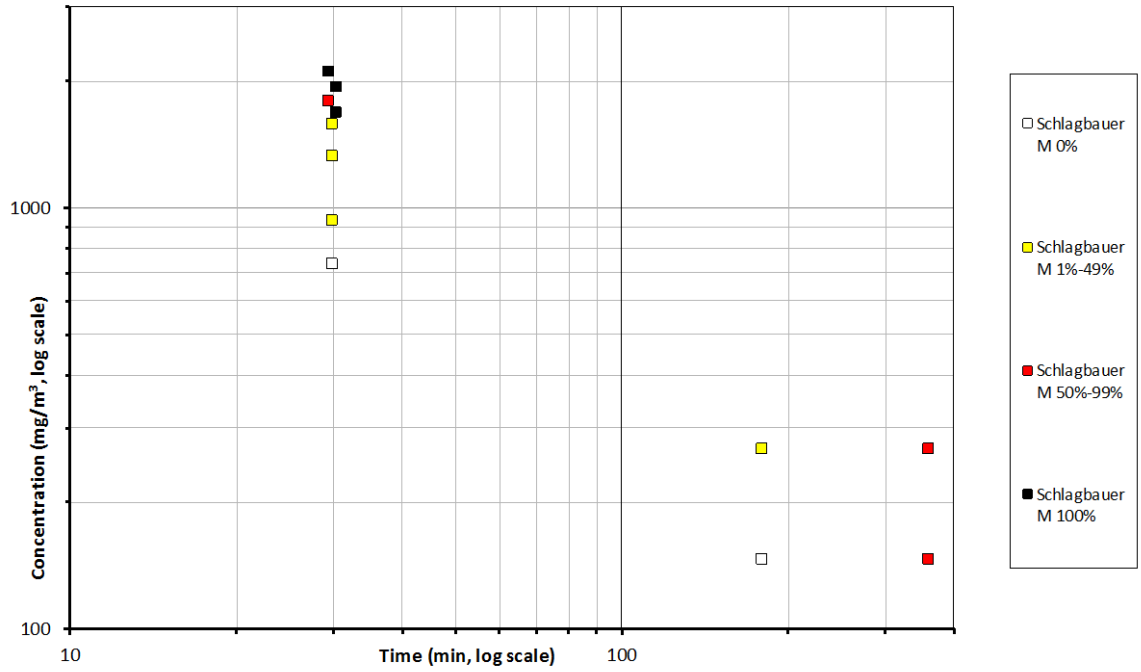


Figure 3 Final data selected for derivation of the animal probit function of bromine (identical to figure 1).

6. Derivation of the human probit function

To derive the human probit function the results from study B2.1 have been used to derive a point of departure as outlined above. All data from study B2.1 were included in the derivation of the probit function. This bromine dataset had 4 data points from exposure durations >30 minutes that showed a wide range (from 0-80%) in response rates. The observed <50% lethality for 180-min exposures and >50% lethality for 360-min exposures effectively fixate the slope of the concentration-time-lethality function and provide confidence in the internal validity of the n-value.

While study B2.1 does not produce the strongest database for a concentration × time relationship, the n-value of 1.277 is comparable to that of the sister compound chlorine (n=1.038). Alternatives to the application of the chemical-specific n-value would be to use the default n-value of 2 or the n-value derived for chlorine (1.038 for rats from the Zwart *et al* 1988 study). The expert panel considered the chemical-specific value to be the better choice.

The Point of Departure for the human probit function is a 30-minute mouse LC_{50} value of 1128 mg/m^3 and an n-value of 1.277.

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

Table 3 *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	No reason to deviate from the default value.
Nominal concentration	1	The maximum exposure level was well below 25% (actually ~1%) of the saturated vapor concentration.
Adequacy of database:	1	Not a very strong database, but the available B2 study was reasonably well conducted.

The estimated human equivalent 30-minute LC_{50} value is 1128 / 3 = **376 mg/m^3**.

The experimentally determined n-value was **1.277** (mouse data from study B2.1). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1.566**.
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): $5 = a + 1.566 \times \ln(376^{1.277} \times 30)$ resulting in the a-value of **–12.19**.

$Pr = -12.2 + 1.57 \times \ln(C^{1.28} \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically weak basis. The probit function is based on 1 study in the mouse with B2 quality, a total of 120 animals, with exposure durations ranging from 30-360 minutes and concentrations ranging from 146-2094 mg/m^3.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 46 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 67 mg/m^3.

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

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
0.1% lethality, this probit	79	46
1% lethality, this probit	115	67
AEGL-3[1] (2010, final)	80	57
ERPG-3[1] (2011)		33
LBW (2015)	250	130

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are approximately identical, with the exception of the recent Dutch intervention values which appear to be higher. The bromine probit can be verified by comparison with the chlorine probit which is derived from a more extensive database. The only reliable study that tested both bromine and chlorine (Schlagbauer, B2.1) produced a 30-min chlorine LC_{50} value of 127 ppm and a bromine LC_{50} value of 174 ppm. The 60-min LC_{01} values derived from the respective probit functions are 22.0 ppm for chlorine and 10.1 ppm for bromine, so the derived bromine probit function is considered to be conservative.

[1] 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: B2.1

Schlagbauer and Henschler 1967

Substance: Bromine
Species, strain, sex: Female NMRI mice
Number/sex/conc. group: 10 / concentration
Age and weight: 18-23 gram
Observation period: 10 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	*No issues reported or expected.*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body, individually housed during exposure in a 200-liter chamber. Animals were introduced quickly through a lock, without appreciable change of chamber concentration.*
Type of restrainer	*N/A*
Pressure distribution	*No information provided*
Homogeneity of test atmosphere in breathing zone of animals	*The air inside the chamber was continuously mixed with a 'powerful ventilator'.*
Number of air changes per hour	*12-15 Air Changes / Hour*
Equilibration time (t95)	*No information provided*
Start of exposure relative to equilibration	*The concentration in chamber had reached equilibrium before start of the exposure.*
Actual concentration measurement	*Potassium Iodide method. Sampling location uncertain.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A.*
Assessment of Reliability	***B2*** *Old study with a few weaknesses and uncertainty about some study details, but with concentration-time-response data.*

The last mortality occurred on day 10 after exposure.

Histopathological examination revealed severe tracheal damage including desquamation and (to a lesser extent) bronchi and bronchioles; lethal inhalations also produced alveolar edema.

The setup of the exposure system and analytical methods are incompletely described in the paper; the authors referred to earlier papers which again referred to earlier papers for details. Details were derived (pieced together) from Henschler and Laux

(1960), Henschler, Amend and Jüttner (1960), Henschler, Hahn and Assmann (1964), Rupp and Henschler (1966) and Henschler (1999, letter to NAC AEGL).

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Fatal	Exposed
Mouse	735		30	0	10
	929		30	3	10
	1321		30	6	10
	1569		30	9	10
	1676		30	10	10
	1779		30	9	10
	1930		30	10	10
	2094		30	10	10
	266		180	3	10
	146		180	0	10
	266		360	8	10
	146		360	7	10

Probit function

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

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

with C for concentration in mg/m^3 and t for time in minutes. Because most of the data were from the 30-min exposures, the probit functions and LC_{50}-values were calculated with all available data and with the 30-min data only.

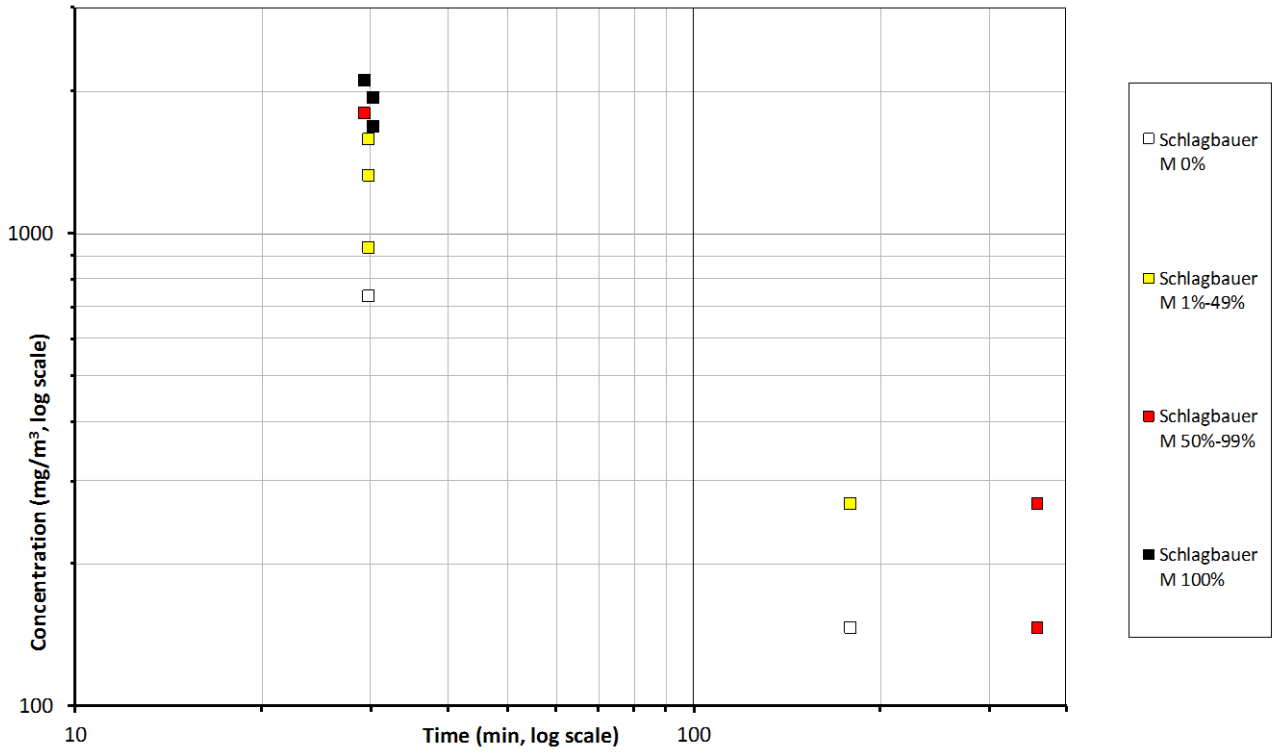
Probit function	Species	a	b	c	n-value
All data	Mouse	-26.89	3.290	2.577	1.277 (1.133 – 1.420)
30-min data only	Mouse	-23.51	4.045	N/A	N/A

The LC_{50}-values were as follows:

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Female, all data	LC_{50} (mg/m^3) 95%-C.I. Female, only 30 min data
10	2668 (2123 – 3211)	N/A
30	1128 (980 – 1264)	1151 (1002 – 1277)
60	656 (588 – 718)	N/A

The authors determined a 30-min LC_{50} value of 174 ppm (1157 mg/m^3).

All data for bromine were included in the derivation of the probit function. The bromine dataset had 4 data points from exposure durations > 30 minutes that showed a wide range (from 0-80%) in response rates. The observed <50% lethality for 180-min exposures and >50% lethality for 360-min exposures effectively fixate the slope of the concentration-time-lethality function and provide confidence in the internal validity of the n-value.


1000
100
Concentration (mg/m³, log scale)
10
Time (min, log scale)
100
Schlagbauer M 0%
Schlagbauer M 1%-49%
Schlagbauer M 50%-99%
Schlagbauer M 100%

1
2
3
4

Study ID: C.1

Bitron & Aharonson 1978

Substance: Bromine
Species, strain, sex: Mouse, male albino from local stock
Number/sex/conc. group: 5 / sex / concentration
Age and weight: 21 +/- 1 gram (age about 1 month)
Observation period: 30 days

Evaluation of study quality

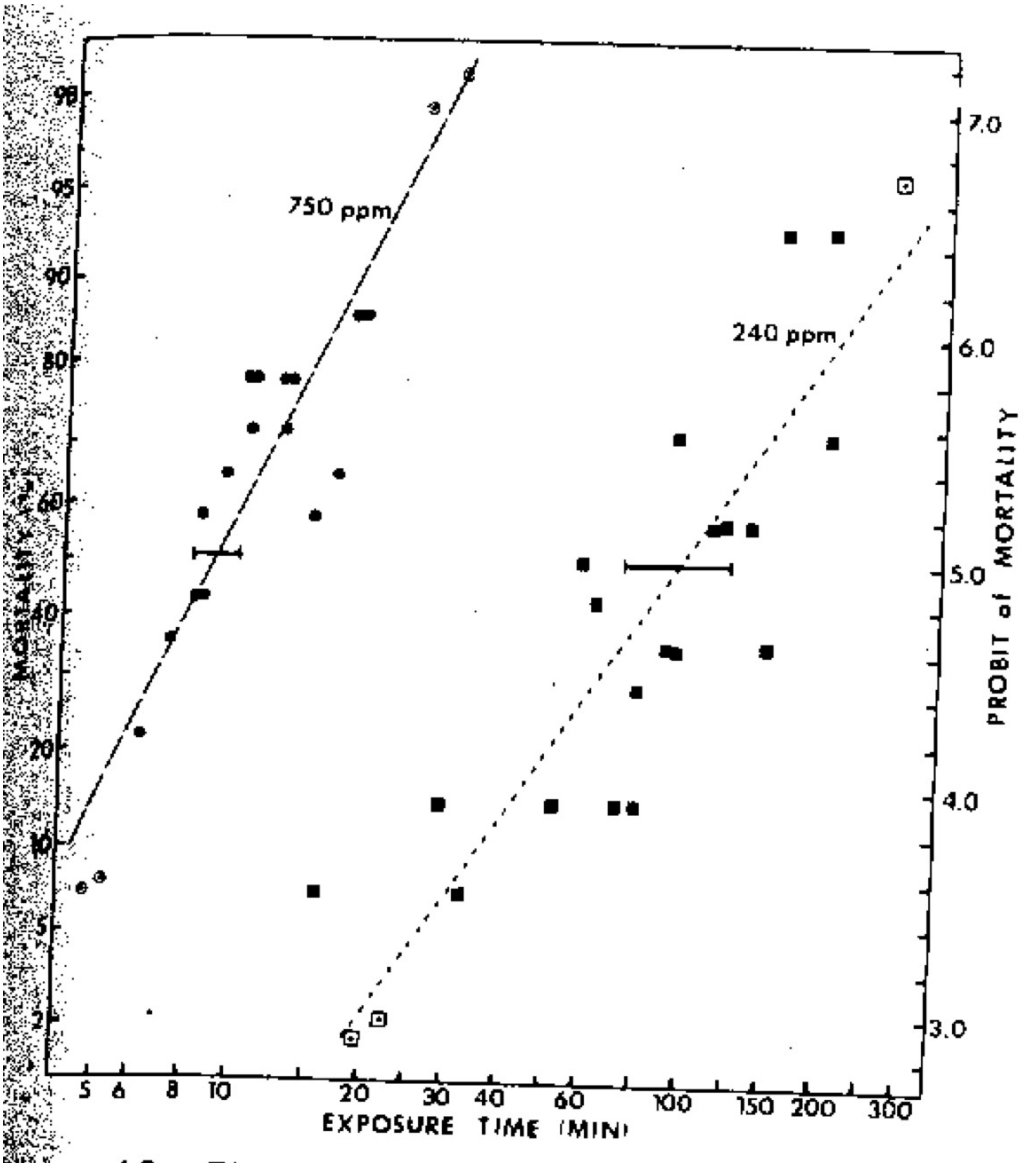
Criteria	***Comment***
Study carried out according to GLP	*GLP did not exist at the time*
Study carried out according to OECD 403 guideline(s)	*OECD guideline 403 did not exist at the time*
Stability of test compound in test atmosphere	*No issues reported*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose-only*
Type of restrainer	*A rather tight-fitting cylindrical glass cover, with the animal's nose turned towards the entrance of the air stream. Judging from a schematic drawing possibly completely closed, with animals' tails in the tube.*
Pressure distribution	*Each of the exposure tubes was individually fed with a gas stream from a manifold. A 10 mmHg negative pressure was maintained in the test tubes.*
Homogeneity of test atmosphere in breathing zone of animals	*Cl2 was fed from a pressure vessel prediluted 10-100 times with dry air.*
Number of air changes per hour	*1 l/min/animal*
Equilibration time (t95)	*Not specified and unable to calculate, but most likely short (15 sec if a tube volume of 50 ml is assumed)*
Start of exposure relative to equilibration	*Not specified, but probably at start of concentration build-up*
Actual concentration measurement	*Downstream of each exposure tube, with KI method.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***C*** *Study does not provide raw data to calculate LC_{50} values but only a graph, and there may be issues with hyperthermia due to the closed glass restrainers.*

The glass tubes, possibly completely closed, may have contributed to hyperthermia in the animals. It is unclear if and how this was balanced by hypothermia due to respiratory rate depression and to what extent the tube design may have affected the

outcome. This study was also characterized as C study because the authors appeared to have problems taking out animals simultaneously.

Results

This study was designed to expose animals to 2 concentrations (1550 and 4900 mg/m^3) for a range of exposure durations. The study did not provide the raw data other than on a log(time)-probability plot which is impossible to interpret quantitatively. Therefore, only the LT_{50} values as derived by the authors will be presented. The graph from the publication is presented below.



Probit function

Not calculated.

The Lt50 values as presented by the authors are:

Concentration (mg/m^3)	Lt_{50} (min.) 95%-C.I. Male
1595	100
4985	9

Study ID: other C studies

Based on an incomplete translation of a Russian study, Ivanov (1976) reported a rat LC_{50} of 2700 mg/m^3 and a mouse LC_{50} of 2900 mg/m^3 (exposure duration not specified).

Lehmann (1887, as cited in NAS 2010) reported that inhalation exposure of cats, rabbits and guinea pigs at 180 ppm (duration not reported) caused severe irritation and corneal clouding, the 7-h LC_{10} was 250 mg/m^3 for both the cat and guinea pig, and exposure at 538 mg/m^3 for 3 h caused deaths in rabbits and guinea pigs. Observations at the latter concentration-exposure time revealed pulmonary edema, deposits on the trachea and bronchi, and gastric hemorrhage.

Appendix 2 Reference list

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

Bitron MD and EF Aharonson. Delayed mortality of mice following inhalation of acute doses of CH_2O, SO_2, CL_2 and Br_2. Am Ind Hyg Assoc J 1978;39:129-138.

Chemiekaarten. Ed 32. Den Haag. TNO/SDU uitgevers, 2017.

Flury, F., and F. Zernik. Schädliche gase dämpfe, nebel, rauch- und staubarten. Brom. Berlin: Springer, 1931: pp 121-123.

Henschler D. Re: AEGLs for Bromine and Bromine. Letter to the NAC AEGL. 21 December 1999.

Ivanov, N.G., A.M. Kliachkina, and A.L. Germanova. Experimental data for hygienic standardization of bromine and hydrogen bromide content in the air of working areas [in Russian]. Gig. Tr. Prof. Zabol. 1976;20(3):36-39.

Lehmann, K.B. 1887. Arch. Hyg. 7:335 (as cited in Flury and Zernik 1931).

Morabia, A, C Selleger, JC Landry *et al*. Accidental bromine exposure in an urban population: An acute epidemiological assessment. Int. J. Epidemiol. 1988.;7:148-152

National Research Council. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Volume 9. Washington, DC. The National Academies Press, 2010.

RIVM 2016. Interventiewaarden gevaarlijke stoffen. http://www.rivm.nl/rvs/Normen/Rampen_en_incidenten/Interventiewaarden.

Ruijten M.W.M.M., J.H.E. Arts, P.J. Boogaard *et al*. Methods for the derivation of probit functions to predict acute lethality following inhalation of toxic substances. RIVM report 2015-0102. Bilthoven, RIVM, 2015.

Rupp H, D Henschler. Wirkungen geringer Chlor-und Bromkonzentrationen auf den Menschen. Int Arch Gewerbepath Gewerbehyg 1967;23:79-90.

Schlagbauer M, D. Henschler. Toxizität von Chlor und Brom bei einmaliger und wiederholter Inhalation. Int Archiv Gewerbepath. Gewerbehyg 1967;23:91-98.