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

Probit function technical support document

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Author: Drs. W ter Burg, RIVM.

substance name	CAS number
Trimethylamine	75-50-3

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

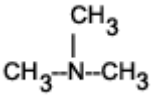
This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have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

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

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

Technical support document Trimethylamine

1. Substance identification



CAS-number:	75-50-3
IUPAC name:	Trimethylamine
Synonyms:	N,N-dimethylmethanamine, TMA, (Dutch: trimethylamine)
Molecular formula:	$(CH_3)_3N$
Molecular weight:	59.1 g/mol
Physical state:	gas (at 20°C and 101.3 kPa)
Boiling point:	3°C (at 101.3 kPa)
Vapour pressure:	220 kPa (at 20°C)
Saturated vapour conc:	NA (at 20°C)
Conversion factor:	1 mg/m^3 = 0.406 ppm (at 20°C and 101.3 kPa)
	1 ppm = 2.46 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H315-318-332-335 (gaseous)
	H302-314-332 (40% aqueous solution)

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

Acute effects: The substance is a basic tertiary aliphatic amine. Trimethylamine causes irritation of the eyes, skin and respiratory tract manifested as lacrimation and lesions in the nasal mucosa. Severe lesions in the nasal area and lungs are reported and occasionally also in the liver, kidney and spleen. Trimethylamine also caused neurotoxicity in animals, of which the aetiology is unclear. Compared to the other methylamines (dimethylamine and monomethylamine) the neurotoxic potential of trimethylamine is much higher. Mortality probably is the result of central nervous system effects in contrast to the other methylamines, where pulmonary damage is generally considered the cause of death.

Long-term effects: There is no information available about the long-term effects following acute exposure. Non-lethal toxicity studies reveal that tissue repair occurs. This does not, however, rule out the possibility of irreversible effects. Chronic studies reveal similar toxicity signs as indicated under acute effects. Systemic effects, such as liver and kidney toxicity are more often seen in chronic studies.

3. Human toxicity data

No informative reports on the 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.

4. Animal acute toxicity data

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

[1] Source: AEGL document (interim 2008) and Chemiekaart (2016).

1. AEGL interim (2008) TSD, ERPG document, ECHA database and EU RAR and reference database for trimethylamine, covering references before and including 1995.
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, IUCLID, RTECS, IRIS and ToxNet with the following search terms:
   - Substance name and synonyms
   - CAS number
   - lethal*
   - mortal*
   - fatal*
   - LC_{50}, LC
   - probit
3. Unpublished data were sought through networks of toxicological scientists.

Animal lethal toxicity data considering acute exposure are described in Appendix 1. In total four studies were identified. One study has been assigned with status A, no studies were assigned the B status, and three have been 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} values were observed:

Table 1 *Sensory irritation data for trimethylamine*

Species/strain	*RD_{50} (mg/m^3)*	*Exposure duration (min)*	*Author/year*
Mouse / Swiss-OF_1	150*	15	Gagnaire et al. 1989

* No information on whether a plateau was reached.

5. Probit functions from individual studies

All available acute lethality data on trimethylamine are provided in Figure 1.

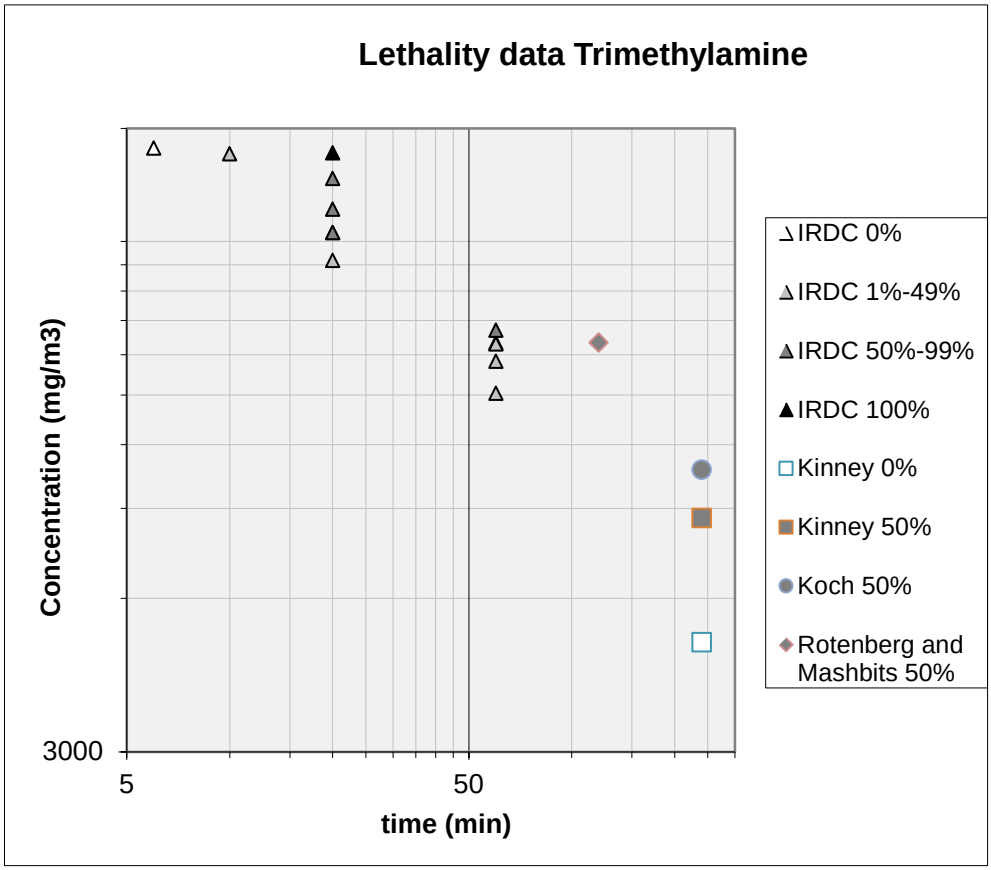


Figure 1 *All available acute lethality data for trimethylamine*

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

The available A study was selected for derivation of the animal probit function for trimethylamine.

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

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

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50}, 30 minutes (mg/m^3) 95% C.I.
A.1	Rat	-68.6 + 6.23×lnC + 3.00×lnt	26,240 (24,420-28,500)

The data of the one A study with rats are presented graphically below.

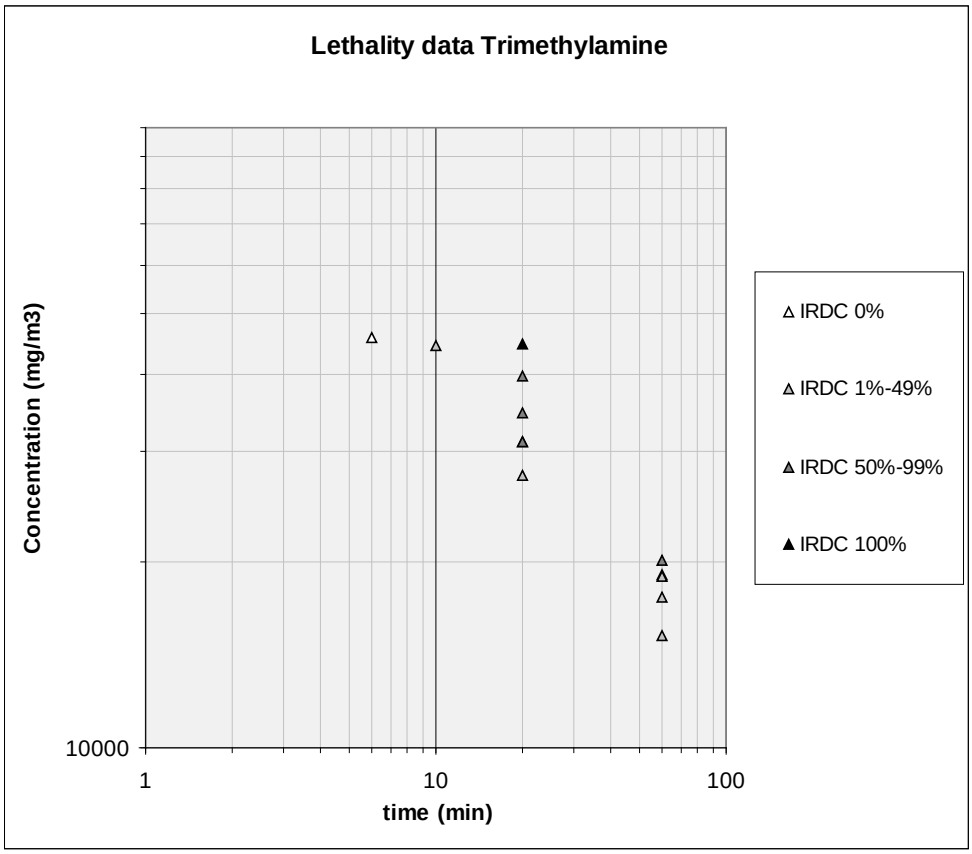


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of trimethylamine*

The final data for calculating the animal probit function contains one dataset from one study and includes data from one animal species (see Figure 2 and Table 2).

6. Derivation of the human probit function

To derive the human probit function the results from IRDC (1992; A.1) have been used to derive a point of departure. The study included four exposure durations and provided proper experimental descriptions, making the derivation of a probit function for trimethylamine possible. Three additional studies (Kinney et al. 1990; Koch et al., 1980 and Rotenberg and Mashbits, 1967) only tested at one exposure duration. Furthermore, the available descriptions of these studies are too poor to judge their quality and these studies were therefore given the C status.

As point of departure for deriving the human probit function the 30 min LC_{50} value of 26,240 mg/m^3 for the rat from the IRDC 1992 study was taken. The human equivalent LC_{50} was calculated by applying the following assessment factors:

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	Default value. In addition, sensory irritation as defined by the RD_{50} value is well below the calculated LC_{50} values, indicating an additional protection mechanism in the test species in comparison to humans.
Nominal concentration	1	Actual concentration was measured.
Adequacy of database:	1	One study was found of sufficient quality including three (excl. 6 minute data) exposure durations.

The estimated human equivalent 30-minute LC_{50} value is 26240 / 3 = **8747 mg/m^3**.

The experimentally determined n-value was **2.08** (IRDC 1992). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **0.962**.

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): $5 = a + 0.96 \times \ln (8703^{2.08} \times 30)$ resulting in the a-value of **-16.42**.

$Pr = -16.4 + 0.96 \times \ln (C^{2.08} \times t)$ 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, where ten animals per concentration group (total of 13 concentration groups) were exposed to trimethylamine concentrations ranging from 15,000 to 46,000 mg/m^3 for 6, 10, 20 or 60 minutes resulting in mortality ranging from 0 to 100%.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 1962 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 1341 mg/m^3.

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
1% lethality, this probit	2738	1962
0.1% lethality, this probit	1871	1341
AEGL-3 (2008, interim)	1205	935
ERPG-3 (2015)		1230
LBW (2015)	1200	940

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

Appendix 1 Animal experimental research

Study ID: A.1

Author, year: IRDC, 1992
Substance: Trimethylamine
Species, strain, sex: Rat, CD Sprague-Dawley, male and female
Number/sex/concentration group: 5/sex/concentration
Age and weight: 49-82 days old, weights ranged 213-293g (males) and 156-222g (females) on day of exposure.
Observation period: 14 days

Evaluation of study quality

Criteria	Comment
Study carried out according to GLP	Author states that the test was performed in accordance with US EPA GLP standards effective 1989.
Study carried out according to OECD 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)	None
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	NA
Pressure distribution.	No information
Homogeneity of test atmosphere in breathing zone of animals	Test atmosphere was generated by introducing a metered amount of test material and air into the chamber inlet. Actual concentrations were measured every two minutes, which did not deviate much from target concentration.
Number of air changes per hour	Ranged from 50-52 l/min in a 150 l chamber resulted in approximately 20 air changes per hour.
Equilibration time (t95)	t95 is approximately 9 minutes
Start of exposure relative to equilibration	No information is available to determine the start of exposure relative to equilibrium. A transfer chamber is used to rapidly put in and out the test animals in the larger glass chamber of 150 l and thus is seems that exposure started after equilibration. Actual concentrations have been determined every 2 minutes.
Actual concentration measurement	Gas-phase IR spectrometry,with 20 meter variable-pathlength gas cell, which was calibrated prior to exposure. Measurements were continuous and reported every two minutes.
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	NA

Assessment of Reliability	A; Well conducted study.

Results

Species	Concentration (mg/m^3)	Exposure duration (min)	Lethality	
			Exposed	Responded
Rat	45,756	6	10	0
	44,526	10	10	2
	27,552	20	10	2
	31,242	20	10	6
	39,852	20	10	8
	34,686	20	10	9
	31,242	20	10	9
	44,772	20	10	10
	15,129	60	10	1
	17,466	60	10	3
	18,991	60	10	3
	18,893	60	10	4
	20,098	60	10	7

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, 2015) as

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

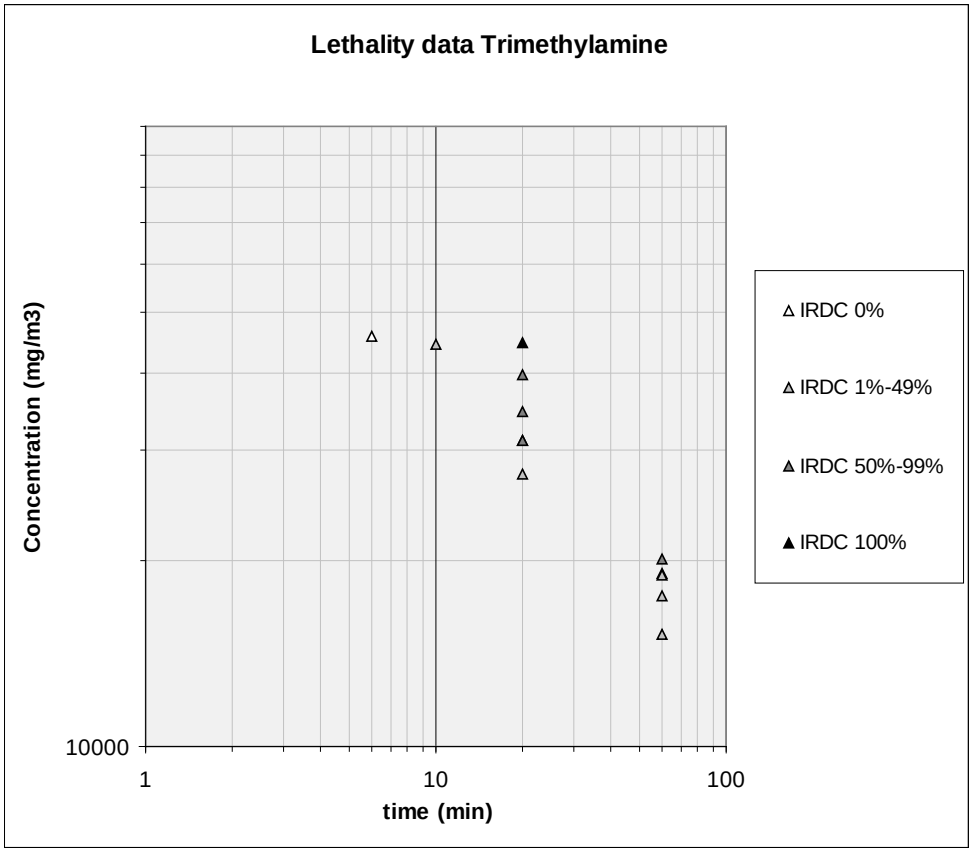
with C for concentration in mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male).

As short term exposures in acute inhalation studies may sometimes give deviating results due to difficulties in achieving the targeted test atmosphere and breath-holding of the test animals, the data were analysed with and without the six minute data. As the study has been well performed there is no reason the exclude the 6 minute data. The analyses also do not support exclusion of the data.

Probit function	*Species*	*a*	*b*	*c*	*n-value*
Sexes combined, Excl. 6 minutes	*Rat*	*-60.0*	*5.54*	*2.54*	*2.18 (1.61-2.76)*
Sexes combined, All data		*-68.6*	*6.23*	*3.00*	*2.08 (1.70-2.45)*

Duration (minutes)	*LC_{50} (mg/m^3) 95%-C.I.* *Sexes combined* Excl. 6 minutes	*LC_{50} (mg/m^3) 95%-C.I.* *Sexes combined* All data
10	43,190 (32,410-50,920)	44,540 (39,340-51,600)
30	26,110 (23,400-28,810)	26,240 (24,420-28,500)
60	19,000 (16,740-22,900)	18,800 (16,850-21,020)

1
2



3
4

Study ID: C.1
Author, year: Kinney et al., 1990.
Substance: Trimethylamine
Species, strain, sex: Rat, Crl:CD(SD)BR rats, male
Number/sex/concentration group: 6/group, 2 groups.
Age and weight: 7 to 8 weeks old weighing 234 to 293 g.
Observation period: 14 days

Criteria	*Comment*
Study carried out according to GLP	No information
Study carried out according to OECD guideline(s)	No information
Stability of test compound in test atmosphere	No information
Use of vehicle (other than air)	Dilution with air
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	NA
Pressure distribution.	No information
Homogeneity of test atmosphere at breathing zone of animals	No information
Number of air changes per hour	No information
Equilibration time (t95)	No information
Start of exposure relative to equilibration	No information
Actual concentration measurement	The authors performed analytical measurements in the repeated exposure protocol, which did not show large deviations from the nominal concentrations. Measurements were performed using the Miran model 1A IR spectrometry at a flow rate of 5 l/min. Actual measurement every 30 minutes. However, it is not known whether analytical measurements were performed during the single exposures.
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	NA
Assessment of Reliability	C
	This study was assigned the C-status, because only two exposure concentrations for one exposure duration were tested.

Results

Species	Concentration (mg/m^3)	Exposure duration (min)	Lethality
			Male
Rat	4920	240	0/6
	8610	240	3/6

Probit function

No probit function can be derived because of too limited data.

Study ID: C.2
Author, year: Koch et al., 1980.
Substance: Trimethylamine
Species, strain, sex: Rat, Wistar, female
Number/sex/concentration group: 10/group, 44 groups.
Age and weight: 8 weeks old. Weights not specified.
Observation period: 14 days

Criteria	*Comment*
Study carried out according to GLP	No information
Study carried out according to OECD guideline(s)	No information
Stability of test compound in test atmosphere	No information
Use of vehicle (other than air)	No information
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	NA
Pressure distribution.	No information
Homogeneity of test atmosphere at breathing zone of animals	No information
Number of air changes per hour	No information
Equilibration time (t95)	No information
Start of exposure relative to equilibration	No information
Actual concentration measurement	Gas chromatography.
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	NA
Assessment of Reliability	C
	The exposure-response data were not provided by the authors. The study investigated the influence of temperature on the toxicity of the test compound.

Results

Species	Concentration (mg/m^3)	Exposure duration (min)	Lethality Female
Rat	10,701	240, T = 21.8°C	LC_{50}
	9,619	240, T = 25.7°C	LC_{50}
	9,446	240, T = 27.0°C	LC_{50}
	8,315	240, T = 29.0°C	LC_{50}

Probit function
Data are unfit for deriving an animal probit function including both concentration and time.

Study ID: C.3
Author, year: Rotenberg and Mashbits, 1967
Original is in Russian, abstract in English. Study details are based on the summary in the AEGL TSD.
Substance: Trimethylamine
Species, strain, sex: Mouse, white, sex not specified.
Number/sex/concentration group: A total of 114 animals were used.
Age and weight: Age and weights not specified.
Observation period: 14 days

Criteria	*Comment*
Study carried out according to GLP	No information
Study carried out according to OECD guideline(s)	No information
Stability of test compound in test atmosphere	No information
Use of vehicle (other than air)	No information
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	NA
Pressure distribution.	No information
Homogeneity of test atmosphere at breathing zone of animals	No information
Number of air changes per hour	No information
Equilibration time (t95)	No information
Start of exposure relative to equilibration	No information
Actual concentration measurement	A colorimetric assay using ortho-nitrophenol was used for chemical and analytical measurements
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	NA
Assessment of Reliability	C
	The reliability of the study cannot be assessed due to lack of details.

Results

Species	Concentration (mg/m^3) (C.I.)	Exposure duration (min)	Lethality
Rat	19,000 (17,900 – 22,200)	120	LC_{50}
	14,300	120	LC_{16} (reported)
	24,800	120	LC_{84} (reported)

Probit function
Data are unfit for deriving a probit function.

Appendix 2 Reference list

AEGL 2008, NAS/COT subcommittee for Acute Exposure Guideline Levels, Trimethylamine (CAS Reg. No. 75-50-3), Interim 06/2008.

Chemiekaarten. Trimethylamine (drukhouder), 31e editie. 2016

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Koch F., G. Mehlhorn, R.Kliche, and R. Lang. 1980. [Untersuchungen zur aerogenen Intoxication bei Ratten durch Methylamine]. Wiss Z. Karl-Marx-Univ. Leipzig. Naturwiiss. R. 29: 463-474.

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