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

Probit function technical support document

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Status: interim
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substance name	CAS number
Formaldehyde	50-00-0

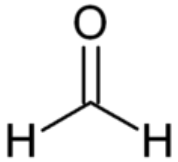
This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

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 Formaldehyde



1. Substance identification

CAS-number:	50-00-0
IUPAC name:	formaldehyde
Synonyms:	methanal, methyl aldehyde, methylene oxide, oxomethane, oxymethylene, formic aldehyde
Molecular formula:	CH_2O
Molecular weight:	30.0 g/mol
Physical state:	gas (at 20°C and 101.3 kPa)
Boiling point:	-19°C (at 101.3 kPa)
Vapour pressure:	440 kPa (at 20°C)
Saturated vapour conc:	N/A
Conversion factor:	1 mg/m^3 = 0.800 ppm (at 20°C and 101.3 kPa)
	1 ppm = 1.250 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H301, H311, H331, H314, H317, H341, H350

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

Acute effects: The main target organ and tissue for inhalation exposure to formaldehyde is the respiratory system. The substance is a highly cytotoxic eye, upper respiratory tract, and skin irritant. The exact mechanism of the cytotoxic action is unknown. Because formaldehyde is extremely water soluble, it is extensively scrubbed in the anterior nasal passages and low concentrations will not reach the lower respiratory tract. Acute lethality from high exposure to formaldehyde can occur as a result of lung oedema, hypovolemic shock or respiratory arrest. Dermal contact to formaldehyde may cause sensitization.

Long-term effects: Long term exposure to formaldehyde causes non-neoplastic and possibly neoplastic lesions in the nasal epithelium. Formaldehyde has a harmonized classification for carcinogenicity as Carc. 1B (H350: May cause cancer). In 2012, formaldehyde has been reevaluated by IARC and classified formaldehyde as human carcinogen (group 1). Tumor formation is thought to result from persistent tissue damage followed by sustained cell proliferation. A small proportion of the population may develop asthma as a result of occupational exposure to formaldehyde. In addition, allergic contact dermatitis may develop.

3. Human toxicity data

The AEGL TSD on formaldehyde reported a volunteer study where 12 healthy male subjects were exposed to 13.8 ppm (17.3 mg/m^3) formaldehyde for 30 minutes. Nasal and eye irritation with mild lacrimation were reported. No mortality was observed (Sim and Pattle, 1957; as cited in AEGL, 2008).

4. Animal acute toxicity data

Animal toxicity data describing lethality after acute inhalation exposure to formaldehyde are described in Appendix 1. A total of 8 studies were

[1] AEGL (2008)

identified -with 11 relevant datasets for 4 species- with data on lethality following acute inhalation exposure. No datasets have been assigned status A for deriving the human probit function, 1 dataset has been assigned status B and 10 have been assessed to be unfit (status C) for human probit function derivation.

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

1. AEGL interim TSD (2008), ERPG document and EU RAR and reference database for formaldehyde, 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:
   - Formaldehyde and synonyms
   - CAS number
   - lethal*
   - mortal*
   - fatal*
   - LC_{50}, LC
   - probit
3. Unpublished data were sought through networks of toxicological scientists.

Sensory irritation

A total of 4 studies were identified in which sensory irritation was studied (AEGL, 2008; Bos *et al*., 1991). In these studies the following RD_{50} values were observed:

Table 1 *Sensory irritation data for formaldehyde*

Species/strain	RD_{50} (mg/m^3)	Exposure duration (min)	Author/year
Rat/Crl-CD	17^F	15 minutes	Gardner *et al*., 1985
Rat/F-344	40^F	10 minutes	Chang *et al*., 1981
Mouse/B6C3F1	6.1^P	10 minutes	Chang *et al*., 1981
Mouse/Swiss-Webster	3.9^{NS}	10 minutes	Kane and Alarie, 1977

P: a plateau was reached, F: fading of response during exposure, NS: not specified if a plateau in response was reached.

5. Probit functions from individual studies

The available acute lethality data on formaldehyde are provided in Figure 1. These include data of one single study.

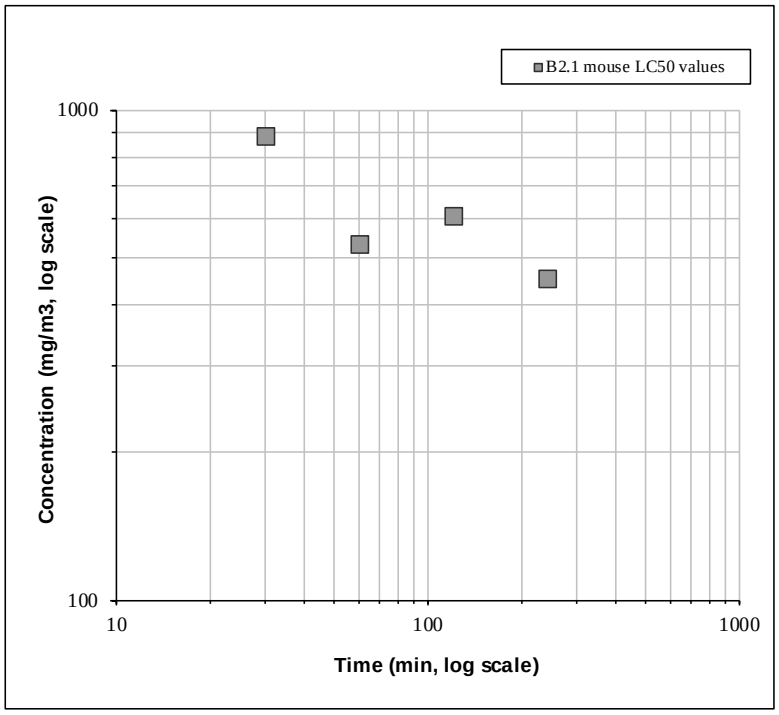


Figure 1 *All available acute lethality data for formaldehyde*

It was not possible to derive a probit function for formaldehyde based on studies with A quality. One study with B2 quality was available. The reported data of this study are listed in Appendix 1. However, no individual data on lethality were available for this study B2.1. LC_{50} values from study B2.1 were provided for different (> 2) exposure durations in the mouse and therefore an n-value could be calculated, as reported in Appendix 1. According to the study authors, the method of probit-analysis was used for LC_{50} calculation.
Below the results of the calculations can be found.

Study ID	Species	LC_{50}, 30 minutes (95% C.I.) (mg/m^3) (study data)	n-value (calculated)
B2.1	Mouse	886 (812-966)	3.70

6. Derivation of the human probit function

To derive the human probit function, the results from Safronov 1993 (study B2.1) have been used to derive a point of departure. The study of Safronov (1993) was the only B2-study and moreover the only study from which an n-value could be determined. Although the reported mouse LC_{50} values of the Safronov (1993) study could not be verified as the individual animal data were not available, results from the Safronov (1993) study, i.e. the 2h LC_{50} of 610 (530-703) mg/m^3, were supported by the mouse 2h LC_{50} value of 512 mg/m^3 in the Nagorny study (Nagorny *et al.*, 1979; C study) and the mouse Lt_{50} of 100 minutes at 400 mg/m^3 in Bitron and Aharonson (1978; C study).

As a point of departure for deriving the human probit function, the 30 min LC_{50} value of 886 mg/m^3 for the mouse from the B2.1 study of Safronov (1993) was taken as point of departure for deriving the human probit function.
The human equivalent LC_{50} was calculated by applying the following assessment factors:

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	Default.
Nominal concentration	1	Concentration "estimated using gas chromatography".
Adequacy of database:	2	One B2-study, supported by two C studies with mice.

The overall assessment factor to derive the human equivalent LC_{50} value would be 6. However, using an overall assessment factor of 6 would provide a lethality value, i.e. a 30-min $LC_{0.1}$ of 31 mg/m^3 and a 8-h $LC_{0.1}$ of 15 mg/m^3, which is in conflict with the human data. Volunteers exposed to 17.3 mg/m^3 for 30 minutes showing only nasal and eye irritation with mild lacrimation (Sim and Pattle, 1957; as cited in AEGL, 2008). Because of the availability of these human data a factor for the adequacy of the database was not considered necessary and an overall assessment factor of 3 was considered sufficient.

The estimated human equivalent 30-minute LC_{50} value is 886 / 3 = **295 mg/m^3**.

The experimentally determined n-value was **3.70** (calculated from the LC_{50} values determined in 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 = **0.541**.

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): $5 = a + 0.541 \times \ln (295^{3.70} \times 30)$ resulting in the a-value of **–8.22**.

$Pr = -8.22 + 0.54 \times \ln (C^{3.70} \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically acceptable sound basis as it is based on a single B2-quality study in mice, supported by two (C-quality) studies in mice and limited human data. The reported LC_{50} values in the C-studies were a 2h-LC_{50} of 512 mg/m^3 and an Lt_{50} of 100 minutes at 400 mg/m^3 exposure. The data available in the B2 study allowed the use of an experimentally derived n-value for probit calculations.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 76 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 52 mg/m^3.

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
1% lethality, this probit	92	76
0.1% lethality, this probit	63	52
AEGL-3 (2008, interim)	88	70
ERPG-3 (2015)	-	50
LBW (2015)	88	69

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

Appendix 1 Animal experimental research

Study ID: B2.1

Author, year: Safronov *et al*. 1993
Substance: formaldehyde
Species, strain, sex: albino mouse, strain not specified, male
Number/sex/concentration group: 10
Age and weight: 18-20 g
Observation period: 2 weeks

Evaluation of study quality

Criteria	Comment
Study carried out according to GLP	No GLP statement provided
Study carried out according to OECD guideline(s)	No statement of compliance with OECD guideline 403 provided
Stability of test compound in test atmosphere	Not specified
Use of vehicle (other than air)	Not specified
Whole body / nose-only (incl. head/nose-only) exposure	Whole body
Type of restrainer	N/A
Pressure distribution.	Not specified
Homogeneity of test atmosphere in breathing zone of animals	Not specified
Number of air changes per hour	Not specified
Equilibration time (t95)	No data available to calculate t95
Start of exposure relative to equilibration	Not specified
Actual concentration measurement	"Concentration in the chamber was estimated using the gas chromatography method"
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	N/A
Assessment of Reliability	B2 Multiple concentration levels and duration were tested. Few study details are available. LC_{50} values and confidence intervals are provided for 4 exposure durations. No individual animal data presented. The method of probit-analysis was used for LC_{50} calculation.

Results

Species	Exposure duration (min)	LC_{50} (mg/m^3) 95%-C.I. Male
Mouse	30	886 (812-966)
Mouse	60	534 (449-636)
Mouse	120	610 (530-703)
Mouse	240	454 (412-500)

The study authors calculated the LC_{50} values for each of the exposure durations with probit analysis.

Probit function

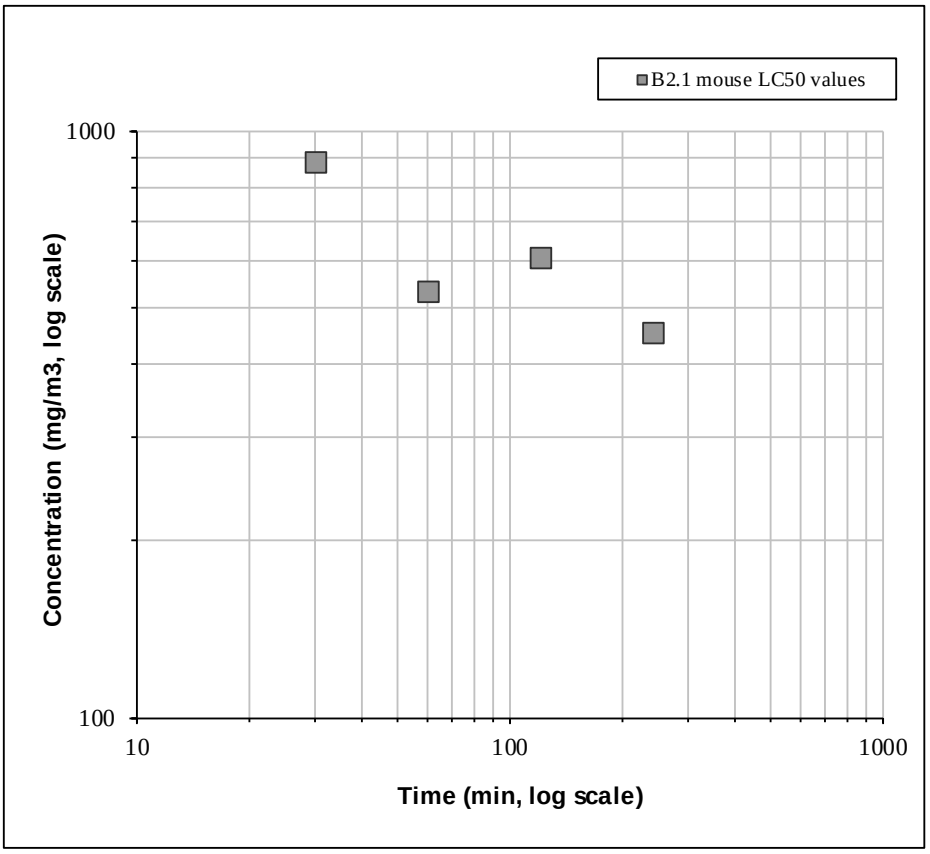
No C × t probit function could be calculated from these data alone. However, as LC_{50} values were provided for different exposure durations an n-value could be calculated using the following formula (AEGL SOP):

$$-n = \frac{N\sum(\log t)^2 - (\sum \log t)^2}{N\sum(\log t)(\log C) - (\sum \log t)(\sum \log C)}$$

The calculated n-value was 3.70.

This value was calculated taking into account all available LC_{50} values, even though data are somewhat inconsistent. Exclusion of the 30-minute LC_{50} results in an n-value of 8.5; exclusion of the 60-minute LC_{50} results in an n-value of 3.1; exclusion of the 120-minute LC_{50} results in an n-value of 3.4; exclusion of the 240-minute LC_{50} results in an n-value of 3.7.

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


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

1
2
3

Study ID: C studies

The AEGL TSD (AEGL 2008) references a number of acute inhalation studies with formaldehyde, which have been assigned the C status for the present purpose of deriving a probit function.

Swiss-Webster mice were exposed for 10 minutes to an unknown range of concentrations (Alarie, 1981 as cited in AEGL (2008)). The 10-min LC_{50} (95% confidence interval) reported was 2701 (2107-3460) mg/m^3. At 1249 mg/m^3 exposure for 10 minutes no deaths were observed. The animals were observed for only 3 hours after the exposure, which makes the results unreliable for the purpose of this evaluation.

Bitron and Aharonson (1978) exposed groups of 28-112 male albino mice to 400 mg/m^3 for exposure durations ranging from 40 to 370 min. No individual animal data on mortality were presented. The Lt_{50} was calculated (by the authors) to be 100 minutes. Exposure durations of 55, 90, 150, and 320 minutes resulted in mortalities of approximately 5, 44, 81, and 100%, respectively (data read from a graph).

A single 2-hour exposure to 900 ppm (1125 mg/m^3) resulted in 100% mortality from massive pulmonary haemorrhage and oedema in C3H mice (Horton *et al*. 1963).

Kamata *et al*. (1996) exposed 6 male Fischer rats per group to concentrations of 0, 160, and 370 mg/m^3 formaldehyde for 6 hours. No mortalities occurred during treatment, but one rat in the highest exposure group died just after completion of treatment. Animals were sacrificed immediately after exposure, therefore the true mortality incidence could not be ascertained.

In a study by Nagorny *et al*. (1979; manuscript written in Russian, information as provided by AEGL 2008) male rats and mice of both sexes were exposed to formaldehyde for 4 hrs and 2 hrs, respectively. Male rats were divided into 21 groups of 6 to 12 animals each. Male and female mice were divided into 14 groups of 6 to 8 animals each. Results are shown in the table below.

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Mortality
Rat	285-437	240	6-12	None
Rat	396-914	240	6-12	Some (data unclear)
Rat	597	240		LC_{50}
Rat	> 914	240	6-12	All
Mouse	80-122	120	6-8	None
Mouse	136-931	120	6-8	12.5%-83.3%
Mouse	512	120		LC_{50}
Mouse	932-1024	120	6-8	All

No full information on the study was available, therefore the study quality and the calculated LC_{50}'s could not be evaluated.

Salem and Cullumbine (1960) exposed groups of 50 mice, 20 guinea pigs, and 5 rabbits to 19 mg/m^3 formaldehyde vapour or 20 mg/m^3 formaldehyde aerosol. Further study details were lacking. Results are listed in the table below.

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Mortality
Mouse	19 (vapour)	Up to 600 minutes	50	17
Mouse	20 (aerosol)	Up to 600 minutes	50	48
Guinea Pig	19 (vapour)	Up to 600 minutes	20	8
Guinea pig	20 (aerosol)	Up to 600 minutes	20	1
Rabbit	19 (vapour)	Up to 600 minutes	5	3
Rabbit	20 (aerosol)	Up to 600 minutes	5	1

Skog (1950) exposed groups of 8 rats per group to concentrations ranging from 600 to 1700 mg/m^3 for 30 minutes. A 3 week observation period was included in the study. Few study details are available. The study was limited to one exposure duration. Individual animal data were not presented. A 30-min LC_{50} of 1000 mg/m^3 (no confidence interval presented) was derived by the study authors.

Appendix 2 Reference list

AEGL. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Interim TSD for formaldehyde. Washington, US EPA, 2008.

Bitron, M.D. and E.F. Aharonson. 1978. Delayed mortality of mice following inhalation of acute doses of CH2O, SO2, CL2, and Br2. Amer. Ind. Hyg. Assoc. J. 39:129-138.

Bos, P.M., A. Zwart, P.G. Reuzel, P.C. Bragt. 1991. Evaluation of the sensory irritation test for the assessment of occupational health risk. Crit Rev Toxicol. 21: 423-50.

Chang, J.C.F., W.H. Steinhagen, and C.S. Barrow. 1981. Effect of single or repeated formaldehyde exposure on minute volume of B6C3F1 mice and F-344 rats. Toxicol. Appl. Pharmacol. 61:451-459.

Gardner, R.J., Burgess, B.A., and Kennedy Jr., G.L. 1985. Sensory irritation potential of selected nasal tumorigens in the rat. Food Chem. Toxicol. 23: 87.

Horton, A.W., T. Tye, and K.L. Stemmer. 1963. Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. J. Natl. Cancer Inst. 30:31-40.

Kamata, E., M. Nakadate, Y. Ogawa, T. Kaneko, Y. Kurokawa, and M. Yukawa. 1996. Acute inhalation toxicity study of formaldehyde in rats: effect of vapor on the pulmonary surfactant. Pharmacometrics 51:33-37.

Kane, L.E. and Y. Alarie. 1977. Sensory irritation for formaldehyde and acrolein during single and repeated exposures in mice. Amer. Ind. Hyg. Assoc. J. 38:509-522.

Safronov, G.A., N.S. Nevmerzhitsky, L.A. Tiunov, and L.V. Tiunova. 1993. Comparative acute inhalation toxicity of aliphatic aldehydes and ketones according to exposure time. Curr. Toxicol. 1:47-51.

Salem, H. and H. Cullumbine. 1960. Inhalation toxicities of some aldehydes. Toxicol. Appl. Pharmacol. 2:183-187.

Skog, E. 1950. A toxicological investigation of lower aliphatic aldehydes. I. Toxicity of formaldehyde, acetaldehyde, propionaldehyde, and butyraldehyde, as well as of acrolein and crotonaldehyde. Acta Pharmacol. Toxicol. 6:299-318.