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
Oxalonitrile	**460-19-5**

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.

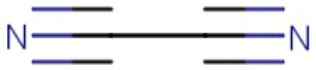
This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have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

1. Substance identification



CAS-number:	460-19-5
IUPAC name:	oxalonitrile
Synonyms:	cyanogen, dicyanogen
Molecular formula:	C_2N_2
Molecular weight:	52.04 g/mol
Physical state:	gas (at 20°C and 101.3 kPa)
Boiling point:	-21.2°C (at 101.3 kPa)
Vapour pressure:	- (gas)
Saturated vapor conc:	5170000 ppm = 11192 g/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.462 ppm (at 20°C and 101.3 kPa)
	1 ppm = 2.165 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H331

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

Acute effects: The acute toxicity is dominated by local irritation as well as effects of cyanide poisoning. The irritation appears to be localized in the upper respiratory tract, particularly the nasal region. The main target organs and tissues for inhalation exposure to *cyanide* are the respiratory system, the central nervous system and the cardiovascular system. Cyanide inhibits cellular respiration, which is especially detrimental in the brain and may result in neurological symptoms, loss of consciousness and hyperpnea. In addition, exposure to hydrogen cyanide may result in weakness, paralysis, and cardiac irregularities. The cyanide poisoning is the most likely cause of death following exposure to oxalonitrile. According to the Dutch Health Council (2003) oxalonitrile causes in general a similar type of toxicity and mode of action as HCN, but oxalonitrile appears to exhibit a higher irritating potential (see section 6).

Long-term effects: Usually death occurs rapidly or there is prompt recovery. Survivors of severe *hydrogen cyanide* exposure may suffer brain damage due to a direct effect of the toxin on nerve cells, to a lack of oxygen, or due to insufficient blood circulation. Examples of long-term neurological effects include personality changes, memory loss, and disturbances in movement. Some damage may be permanent (NIOSH, 2011).

3. Human toxicity data

McNerny and Schrenk (1960) exposed human subjects either to oxalonitrile in a sealed room or by breathing the gas through a tube attached to the small chamber to test the odor detection. The humans in the sealed room were exposed to 8 ppm (17 mg/m^3) for six minutes or 16 ppm (35 mg/m^3) for six or eight minutes. The human subjects who attempted to detect any odor from the sample tube were exposed to 50 (108 mg/m^3), 100 (216 mg/m^3) or 250 ppm (541 mg/m^3). The study showed that two human subjects experienced nasal and/or eye irritation at concentrations of 16 ppm (35 mg/m^3) oxalonitrile. None of the subjects detected the odor by smelling the gas sampled from the chamber.

[1] AEGL (2014)

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 oxalonitrile, 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. Two studies were identified -with four datasets for four species- with data on lethality following acute inhalation exposure. All four datasets 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

The available acute lethality data on oxalonitrile are displayed in Figure 1. Because only C-studies were available, it was not possible to derive a probit function for oxalonitrile from these data.

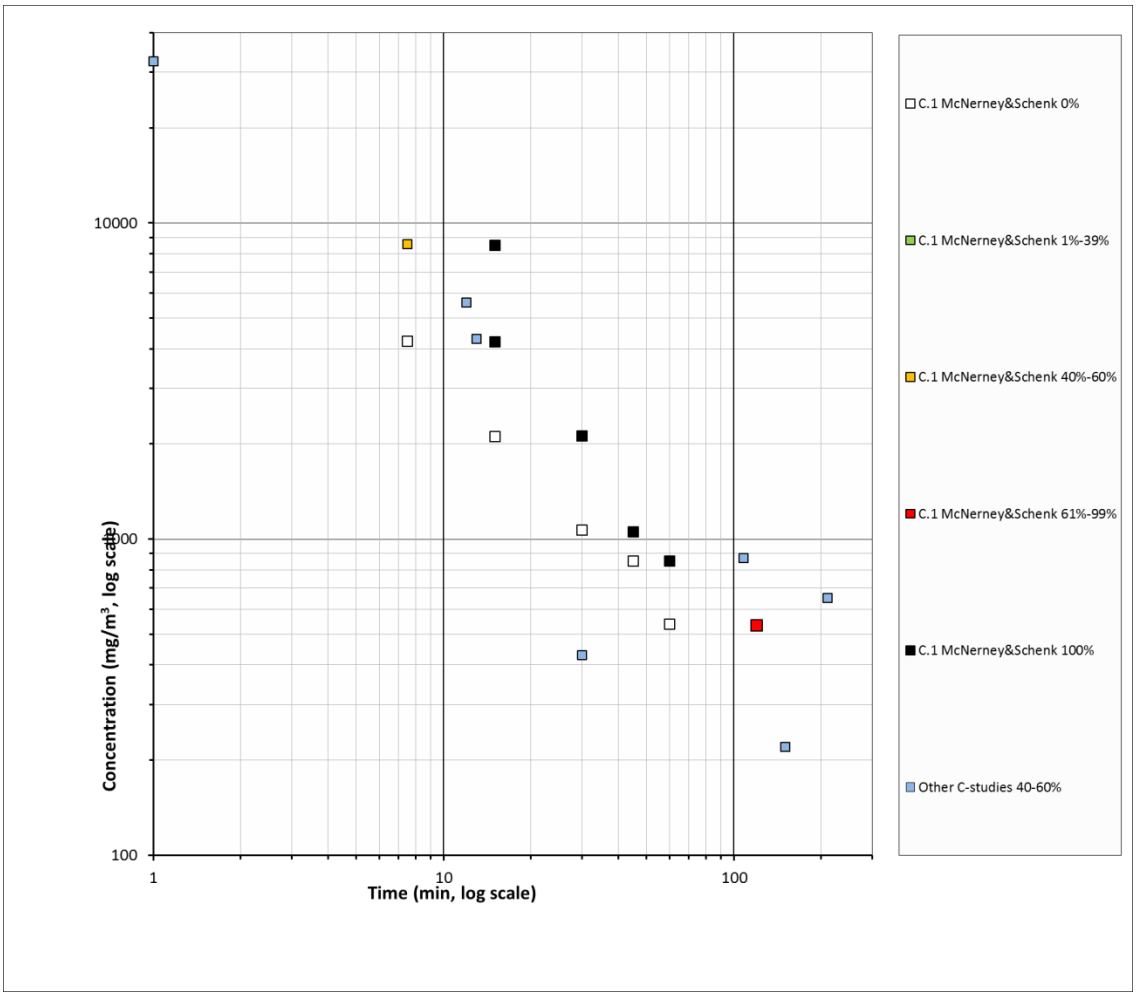


Figure 1 *All available acute lethality data for* oxalonitrile*.*

6. Derivation of the human probit function

The available acute lethality data on oxalonitrile are not suitable for probit function derivation.

Considering the weakness of the chemical specific database, an alternative approach is used to derive the probit function of oxalonitrile. It was verified whether the toxicity data on HCN data (Appendix 3; RIVM, 2017) could be used instead to derive a probit function for oxalonitrile. Oxalonitrile hydrolyses to one molecule of hydrogen cyanide and one of cyanate, so systemic effects may arise which are due to the cyanide formed (AEGL, 2014; Dutch Health council, 2003). The cause of lethality seems to be related to cyanide formation.

AEGL (2014) noted that at relatively low concentrations, oxalonitrile appears to be much more irritating than hydrogen cyanide. In human subjects exposed to oxalonitrile at 16 ppm for 6 or 8 min, ocular irritation was noted immediately. The ocular irritation was perceived simultaneously with or slightly before the occurrence of nasal irritation (McNerney and Schrenk 1960). In contrast, although signs of systemic cyanide poisoning (headache, vertigo, weakness, and numbness) were noted in humans occupationally exposed to hydrogen cyanide at concentrations of 11-162 mg/m^3 (5-75 ppm), no irritation was reported (NRC 2002).

Calculated rat 30-min LC_{50}s of HCN ranged from 108-292 mg/m^3 (96-260 ppm) and calculated 2h LC_{50}s ranged from 37-108 (32-96 ppm) (Lapin, 1981; Sweeny, 2014;2015). The study by McNerny & Schrenk (1960) showed that exposure to 541

mg/m^3 (250 ppm) for 2 hours and to 1082 mg/m^3 (500 ppm) oxalonitrile for 30 minutes caused no mortality in rats. In addition, rat 1h LC_{50}s of oxalonitrile ranged from 788-819 mg/m^3 (364-378 ppm) (McNerny and Schrenk, 1960). Calculated rat 1h LC_{50}s of HCN ranged from 64-175 mg/m^3 (56-156 ppm) (Lapin, 1981; Sweeny, 2014;2015). Compared to the 1h LC_{50}s of oxalonitrile HCN 1h LC_{50}s are lower. This could indicate that oxalonitrile is less toxic than HCN, meaning that by using HCN data the derived probit function for oxalonitrile will be a conservative approach.

Table 1 *Overview of mortality data of oxalonitrile and HCN.*

Time (minutes)	Oxalonitrile in ppm (mg/m^3)	HCN in ppm (mg/m^3)
30	No mortality: 500 (1082)	LC_{50}: 96-260 (108-292)
60	LC_{50}: 364-378 (788-819)	LC_{50}: 56-156 (64-175)
120	No mortality: 250 (541)	LC_{50}: 32-96 (37-108)

The 30-minute rat LC_{50} of HCN that was used as a point of departure for derivation of the HCN probit function (RIVM, 2017), which was 181 mg/m^3 (161 ppm; geometric mean LC_{50} based on three datasets: Lapin (1981; restrained+unrestrained) and Sweeney (2014/2015)).
Assuming a similar mode of action for oxalonitrile, the n-value of HCN was also used for derivation of the human probit of oxalonitrile.

One mole of oxalonitrile will produce one mole of cyanide. Following this approach the point of departure for the human probit function is an estimated 30-minute geometric mean animal LC_{50} value of 349 mg/m^3 (161 ppm) and an arithmetic mean n-value of 1.71, which is conservative in comparison to the 30-minute rat LC_{50} of HCN that was used as a point of departure for derivation of the HCN probit function (RIVM, 2017), which was 181 mg/m^3 (161 ppm)

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

Table 2 *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation	1	Standard value of 3 was reduced to 1 because of available monkey data (see TSD HCN)
Nominal concentration	1	Lapin and Sweeny reported analytically determined HCN concentrations.
Adequacy of database	1	The database of oxalonitrile is weak. However, the database of HCN consists of 3 A-studies, two B2-studies and two C-studies, with in total a large number of C x t combinations. In addition, the limited comparison of oxalonitrile and HCN LC_{50} values indicate that using HCN data provides a conservative probit function for oxalonitrile.

The estimated human equivalent 30-minute LC_{50} value is 349 / 1 = **349 mg/m^3**.

The experimentally determined n-value for HCN was **1.71** (Lapin (1981) and Sweeny (2014&2015)). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1.17.**

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): $5 = a + 1.17 \times \ln (349^{1.71} \times 30)$ resulting in the a-value of **-10.69**

$Pr = -10.7 + 1.17 \times \ln (C^{1.71} \times t)$ with C in mg/m³ and t in min.

The derived human probit function of HCN has a scientifically strong basis. However, the probit function for oxalonitrile is based on an analogy with HCN which is the main metabolite and considered responsible for the mortality.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 50 mg/m³ and the calculated human 60 min LC_1 (Pr = 2.67) is 73 mg/m³.

Table 3 *LC-values calculated with the derived probit function compared with existing acute inhalation exposure guidelines.*

Estimated level	30 min (mg/m³)	60 min (mg/m³)
0.1% lethality, this probit	75	50
1% lethality, this probit	109	73
AEGL-3[2] (2014, final)	108	54
ERPG-3 (-)	-	-
LBW (2018)	110	54

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 values were converted from ppm to mg/m³ with the conversion factor calculated in section 1. Therefore, the AEGL values in mg/m³ can deviate slightly from those reported in the AEGL TSDs.

Appendix 1 Animal experimental research

Study ID: C.1

Author, year: ***McNerny and Schrenk, 1960***
Substance: Oxalonitrile
Species, strain, sex: Male albino rats
Number/sex/conc. group: 6 per concentration/time combination
Age and weight: Average weight 135g, age unspecified
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	*The compound is a gas, no information on possible aerosol formation following hydrolysis*
Use of vehicle (other than air)	*Static chamber conditions with room air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body Mesh wire cage in a 2-foot cube (227L) inhalation chamber*
Type of restrainer	*Not applicable*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*No information*
Number of air changes per hour	*No information*
Equilibration time (t95)	*Insufficient data available to calculate t95*
Start of exposure relative to equilibration	*The build-up of concentration was claimed to expend up to 3 minutes for the highest (target) concentration*
Actual concentration measurement	*No concentration measurement was made*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N.A.*
Assessment of Reliability	***C*** *Static exposure conditions, no concentration measurement, most of the responses are either 0% or 100%*

All fatalities except for 2 at the 250 ppm (476 mg/m^3) level were during exposure. The 2 delayed deaths occurred after 7 hours and 7 days, respectively.

Results

Species	Nominal concentrations in ppm (mg/m^3 (ppm))*	Exposure duration (min)	Lethality
			Dead/tested
Rat	8571 (4000)	7.5	3/6
Rat	8508 (4000)	15	6/6

Rat	4223 (2000)	7.5	0/6
Rat	4207 (2000)	15	6/6
Rat	2111 (1000)	15	0/6
Rat	2115 (1000)	30	6/6
Rat	1066 (500)	30	0/6
Rat	1054 (500)	45	6/6
Rat	851 (400)	45	0/6
Rat	851 (400)	60	6/6
Rat	537 (250)	60	0/6
Rat	533 (250)	120	4/6

*: ppm to mg/m^3 conversion differences caused by difference in average temperature of the test atmosphere.

Probit function

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

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

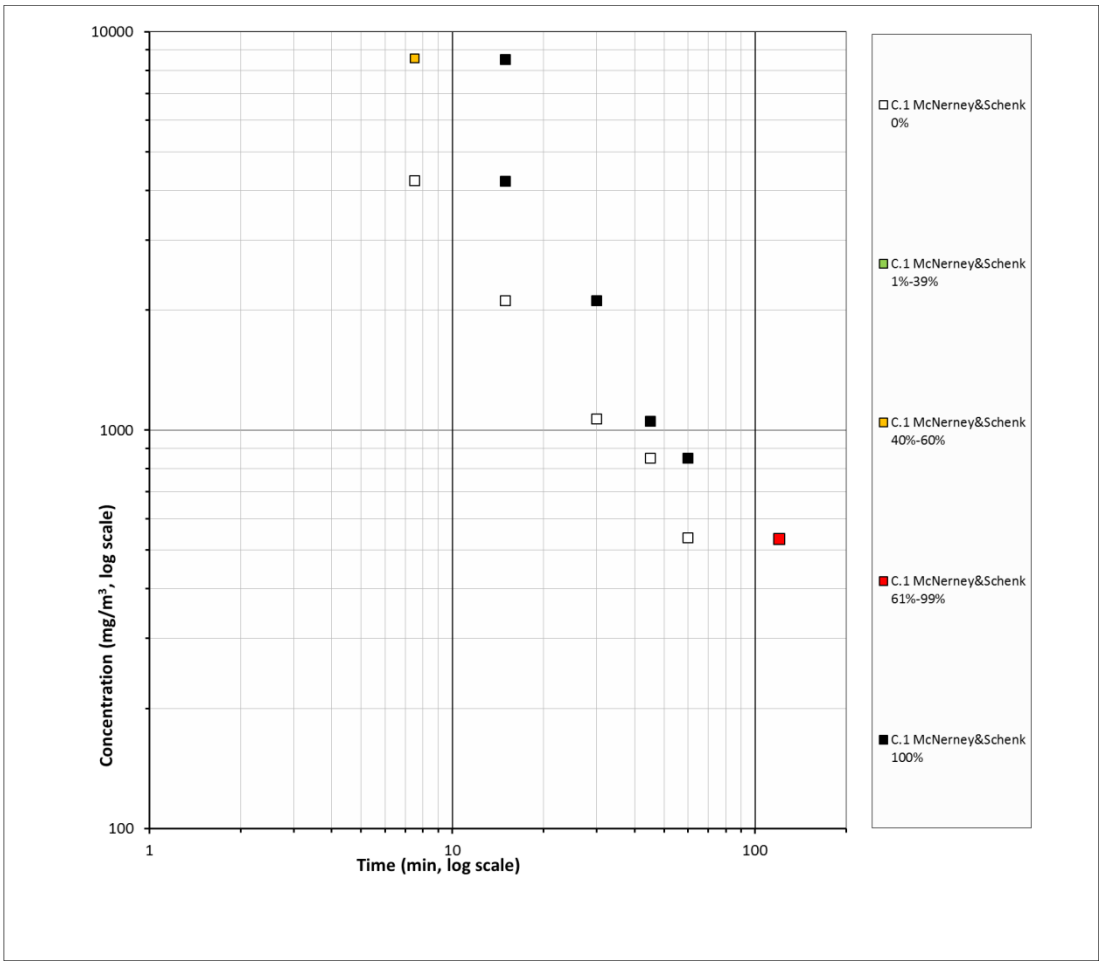
with C for concentration in mg/m^3 and t for time in minutes.

Probit function	Species	a	b	c	n-value
Including 7.5 minute data	Rat	-45.8	4.61	4.90	0.94 (0.76 – 1.12)
Excluding 7.5 minute data	Rat	-125	13.4	9.86	1.36 (1.22-1.50)

The outcome of the 7.5 minute data is questionable since the build-up of the test concentration was reported to be 3 (for ~8500 mg/m^3) and 1.5 minutes (for ~4200 mg/m^3).

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. (male) – including 7.5 minute data	LC_{50} (mg/m^3) 95%-C.I. (male) – excluding 7.5 minute data
10	5307 (3293-7627)	3061 (2757-3770)
30	1648 (1279-2024)	1364 (1301-1476)
60	788 (590-1046)	819 (775-854)

A graphical overview of the data is presented below. Each concentration-time combination (with 6 male animals) represents one point in the plot.


10000
1000
100
1
10
100
Concentration (mg/m³, log scale)
Time (min, log scale)
C.1 McNerney&Schenk 0%
C.1 McNerney&Schenk 1%-39%
C.1 McNerney&Schenk 40%-60%
C.1 McNerney&Schenk 61%-99%
C.1 McNerney&Schenk 100%

1
2
3

Study ID: Other C studies

A secondary source cited in the AEGL TSD (Flury and Zernik 1931, as cited in Kopras 2012) reported the following lethality information:
Lethality was reported in mice exposed to oxalonitrile at 2600 ppm (5600 mg/m^3) for 12 minutes or 15000 ppm (32500 mg/m^3) for 1 minute.
Lethality was reported in cats exposed to oxalonitrile at 100 ppm (220 mg/m^3) for 2-3 hours, at 200 ppm (430 mg/m^3) for 0.5 hour, or 2000 ppm (4300 mg/m^3) for 13 minutes.
Lethality was reported in rabbits exposed to 300 ppm (650 mg/m^3) oxalonitrile for 3.5 hours or 400 ppm (870 mg/m^3) for 1.8 hours.
The actual lethality incidence was not specified and no additional details were available for any of the three species.

Appendix 2 Reference list

Dutch Health Council. Health-based Reassessment of Administrative Occupational Exposure Limits; oxalonitrile. No. 2000/15OSH/063, The Hague, 3 March 2003.

Flury and Zernik. Schadliche Gase. Berlin: Springer, 1931 (as cited in Kopras 2012).

Health Council of the Netherlands: Committee on Updating of Occupational Exposure Limits. Oxalonitrile; Health-based Reassessment of Administrative Occupational Exposure Limits. The Hague: Health Council of the Netherlands, 2003; 2000/15OSH/063.

Kopras, E.J. Cyanides and nitriles. Patty's Toxicology. New York: John Wiley & Sons. 2012; 1-52.

McNerney, J. M. & Schrenk, H.H. The Acute Toxicity of Cyanogen. American Industrial Hygiene Association Journal, 1960; 2(21):121-124

NAC/AEGL. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Final TSD for cyanogen. Washington, US EPA, 2014.

NIOSH, CDC. Page last reviewed may 12, 2011. https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750038.html

NRC (National Research Council). Exposure Guideline Levels for Selected Airborne Chemicals, Vol. 2, hydrogen cyanide. Pp. 211-276 in Acute. Washington, DC: The National Academies Press (2002).

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

RIVM, 2017. Probit waterstofcyanide (interim). https://www.rivm.nl/waterstofcyanide

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.

Appendix 3 TSD Hydrogen Cyanide

Probit function technical support document

Date: 11 May, 2017
Comments before: Day, Month, 2017
Document id: 20170511-hydrogen cyanide-PROPOSED
Status: proposed
Author: Drs. W ter Burg, RIVM
E-mail response to: omgevingsveiligheid@rivm.nl

substance name	CAS number
Hydrogen Cyanide	**74-90-8**

This draft 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 for Public Health and the Environment (RIVM) and has been assigned the status "proposed". The scientific expert panel on probit functions has approved this document for public discussion and comments. Interested parties are invited to submit comments and suggestions concerning this document within 6 weeks after the issue date to the email address mentioned above.

If the proposed probit function is approved by the expert panel on scientific grounds, after review and revisions following of public comments, the status of the document and probit function will be raised to "interim".

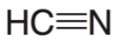
Subsequently, the Ministry of Infrastructure and the Environment will decide whether the probit function will be formally implemented. The decision on actual implementation will primarily be based on the results of a consequence analysis.

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 Hydrogen cyanide

1. Substance identification

CAS-number:	74-90-8
IUPAC name:	hydrogen cyanide
Synonyms:	formonitrile, hydrocyanic acid, prussic acid
Molecular formula:	HCN
Molecular weight:	27.03 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	26°C (at 101.3 kPa)
Vapour pressure:	83 kPa (at 20°C)
Saturated vapour conc:	830,000 ppm = 930 g/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.889 ppm (at 20°C and 101.3 kPa)
	1 ppm = 1.124 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H300-310-330



2. Mechanism of action and toxicological effects following acute exposure[3]

Acute effects: The main target organs and tissues for inhalation exposure to hydrogen cyanide are the respiratory system, the central nervous system and the cardiovascular system. Hydrogen cyanide inhibits cellular respiration. This is especially detrimental in tissues and organs with high energy demand, such as the brain. Exposure to hydrogen cyanide may cause neurological symptoms such as loss of consciousness and inhibition of the respiratory system. In addition, exposure to hydrogen cyanide may result in weakness, paralysis, and cardiac irregularities. Lethality caused by exposure to hydrogen cyanide is due to respiratory arrest.
Long-term effects: Although some neurological symptoms have been related to chronic exposure of workers to hydrogen cyanide, in none of the reports concomitant exposure to other chemicals could be ruled out. Reported symptoms, of which some increased with increasing number of years of work, included headache, fatigue, nausea, weakness, tremors and changes in taste and smell. Besides, chronic exposure to hydrogen cyanide has been associated with hypothyroidism. Information concerning possible long-term effects of acute exposure to toxic concentrations of hydrogen cyanide is limited, but shows that recovery may be uneventful without any permanent adverse health effects.

3. Human toxicity data

No informative reports on 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.

Cases of inhalation toxicity have been reported, however in most case reports the exposure assessments were inadequate and therefore are not considered sufficiently reliable for probit derivation. ATSDR (2006) provided an overview of fatal concentrations reported in or derived for humans. They estimated that exposure to airborne concentrations of HCN at 180 to 270 ppm (202 to 303 mg/m^3) would be fatal, usually within several minutes, and a concentration of 135 ppm (152 mg/m^3) would be fatal after an exposure duration of 30 min.

During therapeutic use of sodium nitroprusside (SNP, $Na_2[Fe(CN)_5NO]$, 262 g/mol (anhydrous)) to reduce blood pressure by peripheral vasodilation in patients, cyanide is released causing toxic effects that are comparable to the effects following inhalation

[3] AEGL final 2002.

exposure to HCN. Cases have been reported where patients purportedly died from cyanide toxicity due to intravenous administration of SNP, although the contribution to the mortality of confounding factors such as the patients' illness are difficult to assess. Schulz et al. (1982) derived a threshold of 400 nmol CN^- /mL erythrocytes[4], above which fatalities may occur. The threshold for clinical symptoms was reported to be 200 nmol CN^- /mL erythrocytes. The threshold for lethality of 400 nmol CN^- /mL erythrocytes was based on a number of articles where lethal doses and concentrations in blood were reported; however, the authors fail to indicate how the lethal threshold of 400 nmol CN^-/mL erythrocytes was actually derived. The oral lethal doses that were referred to by Schulz et al. were 3 mg CN^- in blood/kg body weight (Davies et al. 1975), 2.5-3 mg/L cyanide in blood (Naughton 1974), and >3mg/L cyanide in blood (Graham et al. 1977).

Schulz et al. predicted the accumulation of cyanide after a single intravenous dose of SNP from the maximum detoxification rate and the volume of distribution. Based on their model, assuming maximal detoxification (in absence of i.v. administered thiosulfate) they estimated that a dose rate of 20 µg/kg bw/min SNP for 90 minutes would lead to the threshold concentration of 400 nmol CN^- /mL erythrocytes. The NVIC background document (NVIC, 2011) refers to the abovementioned life threatening value of 400 nmol CN^- /mL erythrocytes as >10,4 mg/L CN^- in erythrocytes. The NVIC document further notes that a serum CN^- concentration greater than 2 mg/L CN^- is considered a severe intoxication, however it is unknown which airborne concentration would lead to this blood CN^- level (see also appendix 2).

Monitoring studies indicate that workers were routinely exposed at ≤10 ppm (11.2 mg/m^3), where studies showed 8-hour time-weighted (TWA) averages of 4-8 ppm (4.5 to 9 mg/m^3). One study reported a mean 15 minute concentration of 10 ppm (11.2 mg/m^3); it is unknown if the 15 minute time weighted average (TWA) reflects a peak exposure or if it is representative for exposure during the entire workshift. In one study a plant was closed down due to a HCN induced worker fatality. NIOSH concluded that the 8h-TWA must have been >15 ppm (16.8 mg/m^3) since this was the measured concentration at the plant the day after the accident. Occupational HCN exposures at 1-10 ppm were acceptable at the time of these surveys as 10 ppm was the maximum allowable concentration (MAC) for workers (AEGL, 2002).

The available case studies do not provide information with sufficient detail and reliability such that they can be used as a quantitative point of departure for a human probit function. Further, the lethal levels of cyanide in humans referred to in literature are inadequately supported by experimental data but seem to rely on incompletely reported clinical observations only. It was therefore decided not to use human data as basis for a probit function but they will be used as supportive data.

4. Animal acute toxicity data

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

1. AEGL final TSD (2002), ERPG document and EU RAR and reference database for hydrogen cyanide, 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:
    - hydrogen cyanide and synonyms
    - CAS number
    - lethal*

4 Both cyanide concentrations in blood and in erythrocytes have been reported in literature. The relation between those two units is unclear and therefore the concentrations and units are given as reported in literature.

- mortal*
- fatal*
- LC50, LC
- probit

3. Unpublished data were sought through networks of toxicological scientists.

Animal lethal toxicity data considering acute exposure to hydrogen cyanide are described in Appendix 1. A total of 9 datasets were identified in seven studies. Three datasets have been assigned status A for deriving the human probit function, 2 datasets were assigned status B2 and 4 datasets have been assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

Two studies were identified in which sensory irritation was studied. In these studies the following RD_{50} values were observed:

Table 1 *Sensory irritation data for <substance name>*

Species/strain	RD_{50} (mg/m^3)	Exposure duration (min)	Author/year
Rat	140^{NS}	5-60	Lapin, 1981
Mouse	71^{NS}	30	Matijak-Schaper and Alarie, 1982

NS: not specified if a plateau in response was reached.

5. Probit functions from individual studies

All available acute lethality data on hydrogen cyanide are displayed in Figure 1. Please note that the C studies described in the TSD are grouped in the graph below, thus including several C studies.

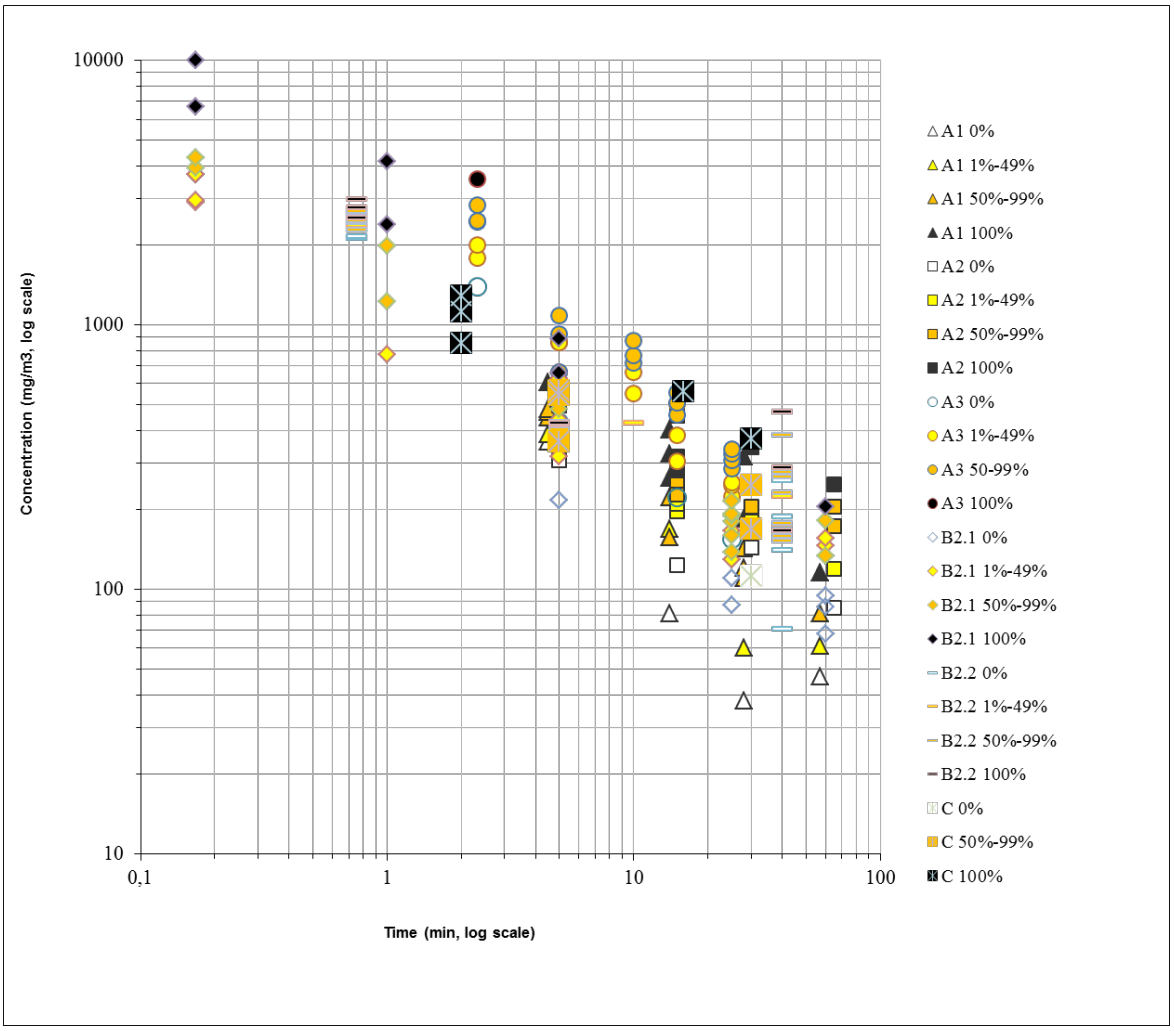


Figure 2 *All available acute lethality data for hydrogen cyanide*

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

All A studies were selected for derivation of the animal probit function for hydrogen cyanide.

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 hydrogen cyanide*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50}, 30 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
A.1	Rat, restrained	-18.6 + 3.22 × ln(C) + 2.49 × ln(t)	109 (92.3-125)	1.29 (1.10-1.49)

A.2	Rat, unrestrained	-32.2 + 5.63 × ln(C) + 2.27 × ln(t)	189 (177-202)	2.48 (2.12-2.83)
A.3	Rat	-13.6 + 2.28 × ln(C) + 1.67 × ln(t)	288 (247-345)	1.37 (1.17-1.56)

The data of the three A studies with rats are presented graphically below.

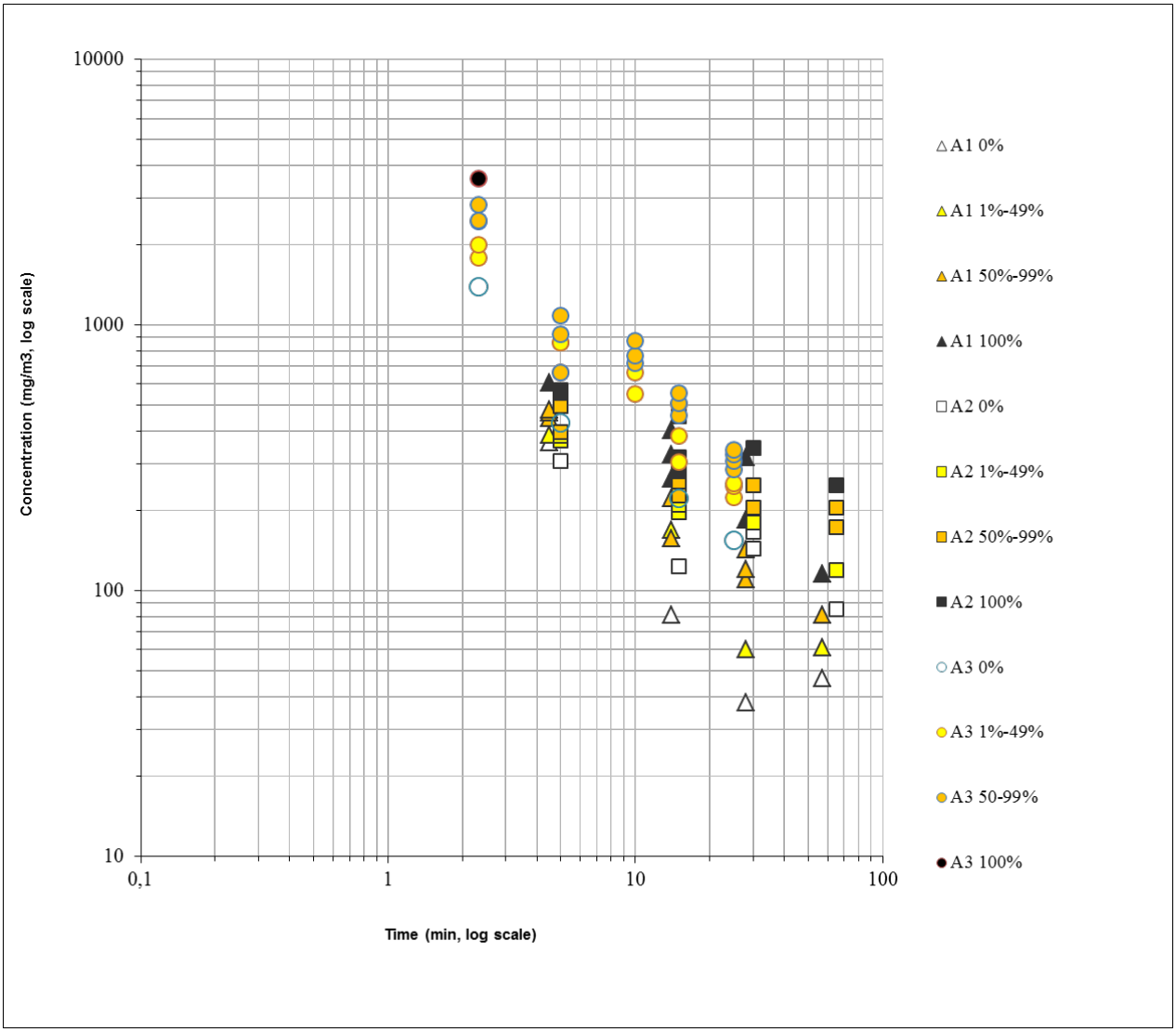


Figure 3 *Data selected for the initial analysis for the derivation of the animal probit function of hydrogen cyanide*

Based on criteria outlined in the guideline the data from studies A.1, A.2 and A.3 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 3 and Figure 4.

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

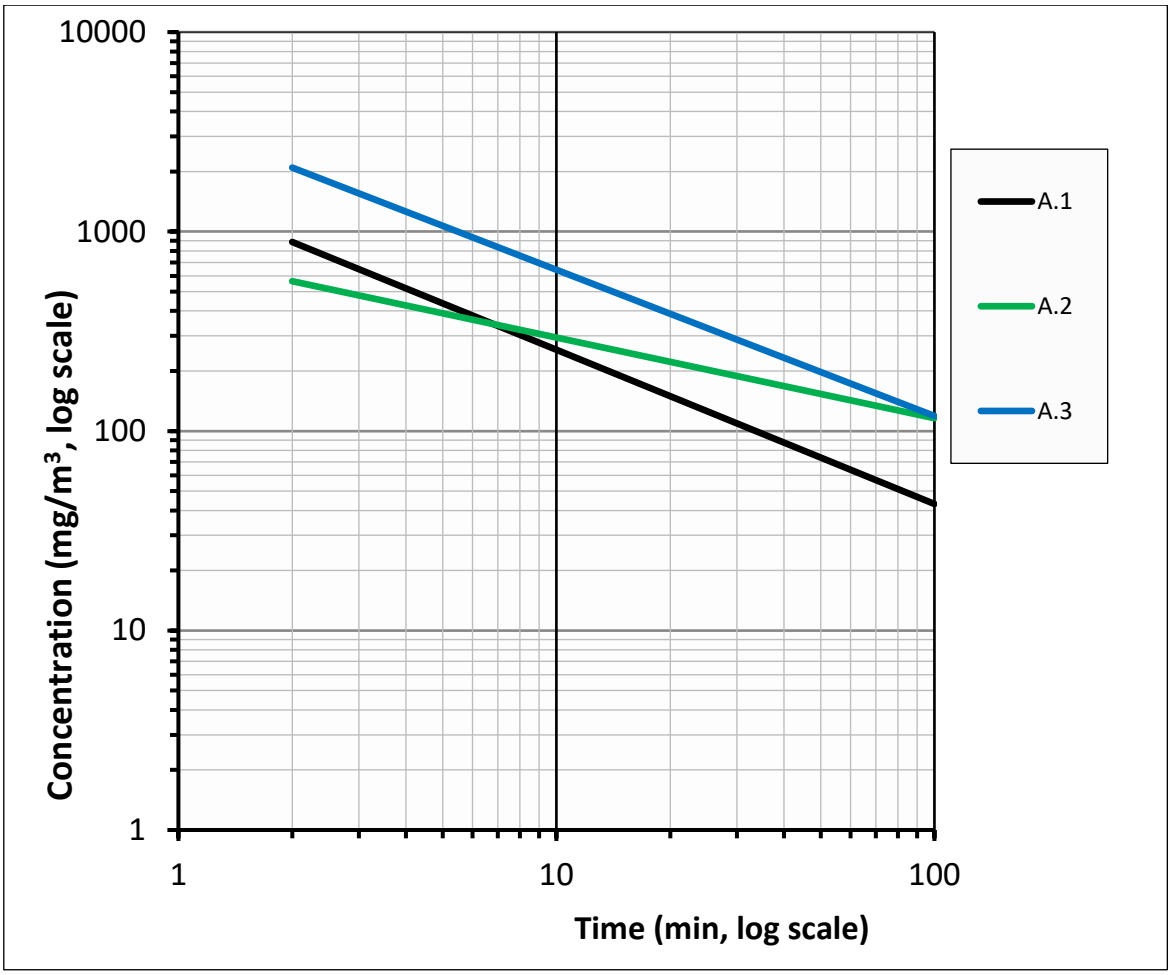


Figure 4 *LC_{50} values of A study datasets for hydrogen cyanide, over time where available.*

Table 3 *Data selected for the derivation of the animal probit function of hydrogen cyanide.*

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.
A.1	Rat, restrained	-18.6 + 3.22 × ln(C) + 2.49 × ln(t)	109 (92.3-125)	1.29 (1.10-1.49)
A.2	Rat, unrestrained	-32.2 + 5.63 × ln(C) + 2.27 × ln(t)	189 (177-202)	2.48 (2.12-2.83)
A.3	Rat	-13.6 + 2.28 × ln(C) + 1.67 × ln(t)	288 (247-345)	1.37 (1.17-1.56)

The data of the selected datasets are presented graphically below.

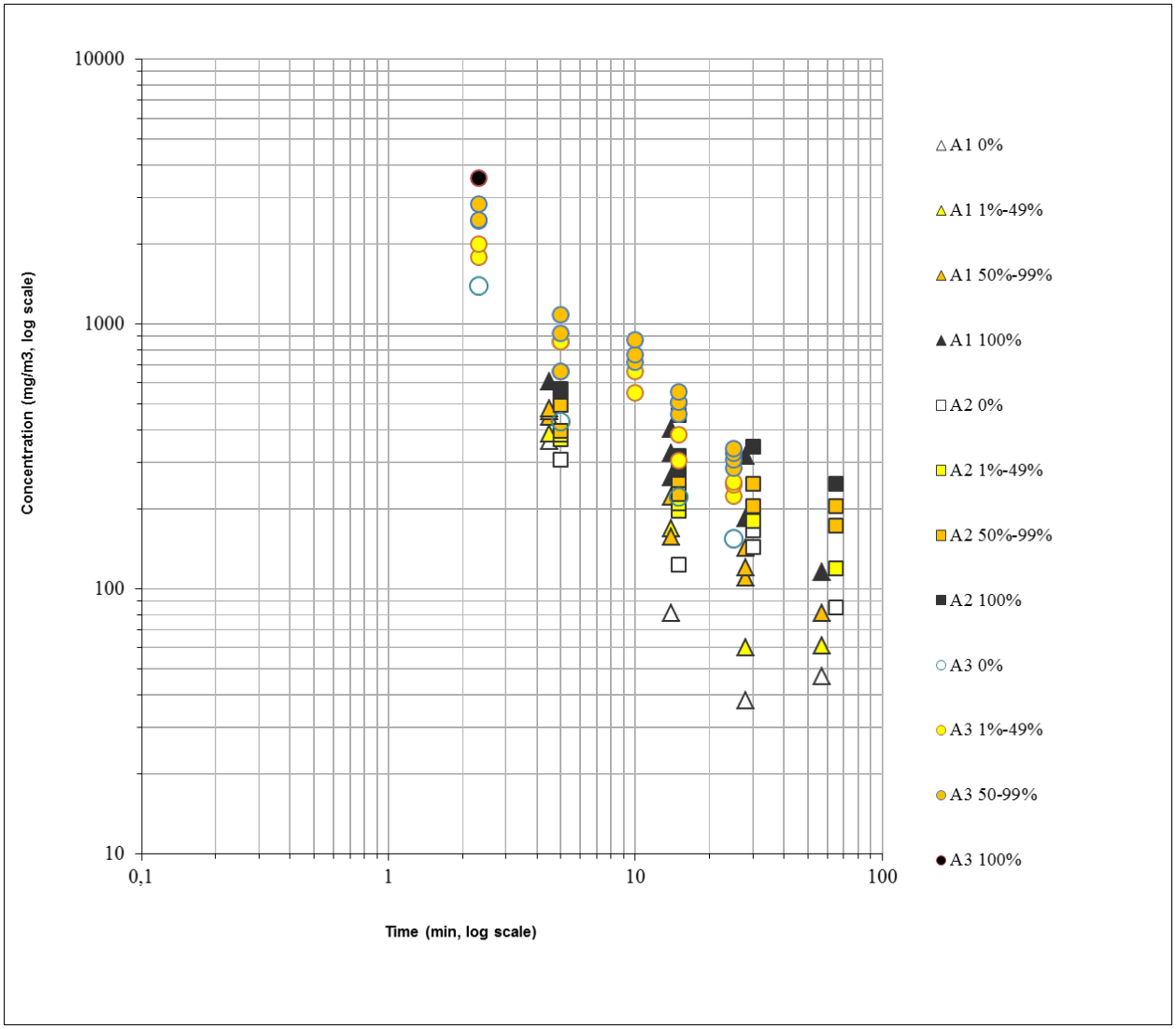


Figure 5 Final data selected for derivation of the animal probit function of hydrogen cyanide (identical to figure 2).

6. Derivation of the human probit function

To derive the human probit function, the results from A.1, A.2, and A.3 studies have been used to derive a point of departure.

First, the arithmetic mean n-value was calculated from studies A.1, A.2, A.3. The arithmetic mean species-specific (species) n-value was calculated to be 1.71.

Second, the LC_{50}-values of all applicable A-studies were calculated for a common exposure duration of 30 minutes.

Finally, the geometric mean LC_{50}-values were calculated from all available (time-scaled) LC_{50} values of studies A.1, A.2, A.3, all with rats. The LC_{50}-value was 181 mg/m^3 for the rat derived from the general formula for the geometric mean of time-scaled LC_{50}-values below:

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

With $\overline{LC_{50}}$ = geometric mean LC_{50}-value
$LC_{50,i}$ = LC_{50}-value of study i.
m = number of observations on LC_{50}-values within a species (i=1…m).
s = number of species for which LC_{50}-values are pooled (j= 1).

The Point of Departure for the human probit function is a 30-minute geometric mean animal LC_{50} value of 181 mg/m^3 and an arithmetic mean n-value of 1.71.

Application of an overall assessment factor of 3 (determined by an interspecies factor of 3) would result in a 60-min LC_{01} of 13 mg/m^3 (data not shown), which is in conflict with human data. For instance, workers routinely exposed to TWA exposures up to 11.2 mg/m^3 did not display overt signs of toxicity. In addition, the Schulz et al. (1982; see also appendix 2) model suggests that exposure to approximately 12.5 mg/m^3 for 90 minutes (approximately a factor 2 lower than the threshold for lethality, see section 3) leads to clinically recognizable symptoms, but not to lethality in subjects with no limitation of the detoxification of cyanide. This suggests that an overall assessment factor of 3, and thus an interspecies factor of 3, is too high.

Considering the animal data on lethality, the derived 30-min LC_{50} values presented below are in a close range for 3 different species:
Rat 30-min LC_{50} (Lapin, A.1): 109 mg/m^3 (restrained)
Rat 30-min LC_{50} (Lapin, A.2): 189 mg/m^3 (unrestrained)
Rat 30-min LC_{50} (Sweeney, A.3): 288 mg/m^3
Rat 30-min LC_{50} (Ballantyne, B2.1): 198 mg/m^3
Rabbit 30-min LC_{50} (Ballantyne B2.2): 184 mg/m^3
Mouse 30-min LC_{50} (Matijak-Schaper C.1): 187 mg/m^3
This comparison suggests that interspecies differences with respect to lethality following inhalation exposure to HCN are expected to be small.

In addition, the study by Barcroft (1931; study ID C.2) suggests that of a number of species the monkey was the least susceptible towards HCN lethality. The relative susceptibility was in decreasing order: dog > mouse = cat = rabbit > rat = guinea pig > goat > monkey. A possible explanation for this observation is that the detoxification of HCN is determined by the enzyme activity of rhodanese and the availability of substrate (sulphur donors) in the body (Schulz et al. 1982; AEGL, 2002). The amount of rhodanese is relatively low in humans, but still abundant with respect to the sulphur donors. It is therefore concluded that the limiting factor in detoxification of HCN is the presence of sulphur donors (predominantly thiosulphate) and transfer of sulphur to rhodanese in the mitochondria in the body, rather than rhodanese activity (SCOEL, 2010). As presented in Health Council of the Netherlands (2002; also reported in SCOEL 2005; 2010): "The activity of rhodanese in serum of 31 healthy humans ranges from 11.4 to 36.1 U/L in males and from <7.6 to 47.5 U/L in females with an overall mean of 20.9 U/L." According to SCOEL on HCN, KCN and NaCN the detoxification in humans is significantly slower than in experimental animals (SCOEL, 2005). However, SCOEL does not discuss the relevance of depletion of sulphur donors and possible differences between species (at high exposure levels). Moreover, in the later version by SCOEL (2010) the statement related to differences of detoxification between humans and animals was removed. Rhodanese and more importantly sulphur donors are present everywhere in the body and thus are related to body size and weight, which seems to be in favour of humans compared to rodents.

Hence, it is reasonable to assume that humans are unlikely to be more susceptible to HCN toxicity than rodents. Based on the small interspecies differences, a larger amount of sulphur donors in humans and the relatively high occupational exposure levels that were apparently well tolerated by the workers involved (see section 3), an overall assessment factor of 1 was considered appropriate for HCN.

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:	1	Standard value of 3 was reduced to 1, see text above.
Nominal concentration	1	A-studies with analytically determined concentrations.
Adequacy of database:	1	Three A-studies and one B2.1 study with in total a large number of C x t combinations

The estimated human equivalent 30-minute LC_{50} value is 181 / 1 = **181 mg/m³**.

The experimentally determined n-value was **1.71** (arithmetic mean of the 3 n-values). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1.17**.

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): $5 = a + 1.17 \times \ln (181^{1.71} \times 30)$ resulting in the a-value of **-9.367.**

$Pr = -9.37 + 1.17 \times \ln (C^{1.71} \times t)$ with C in mg/m³ and t in min.

The derived human probit function has a scientifically sound basis. The probit function is based on three studies in the rat with A quality, where in total 84 C x t combinations are included, including durations ranging from 2.5 to 60 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 26 mg/m³ and the calculated human 60 min LC_1 (Pr = 2.67) is 37 mg/m³.

Table 5 *LC-vales calculated with the derived probit function compared with existing acute inhalation exposure guidelines.*

Estimated level	30 min (mg/m³)	60 min (mg/m³)
0.1% lethality, this probit	38	26
1% lethality, this probit	56	37
AEGL-3[5] (2002)	24	17
ERPG-3[3] (2006)	-	28
LBW (2015)	51	31

[5] AEGL and ERPG values were converted from ppm to mg/m³ with the conversion factor calculated in section 1. Therefore, the AEGL and ERPG values in mg/m³ can deviate slightly from those reported in the AEGL and ERPG TSDs.

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

Appendix 1 Animal experimental research

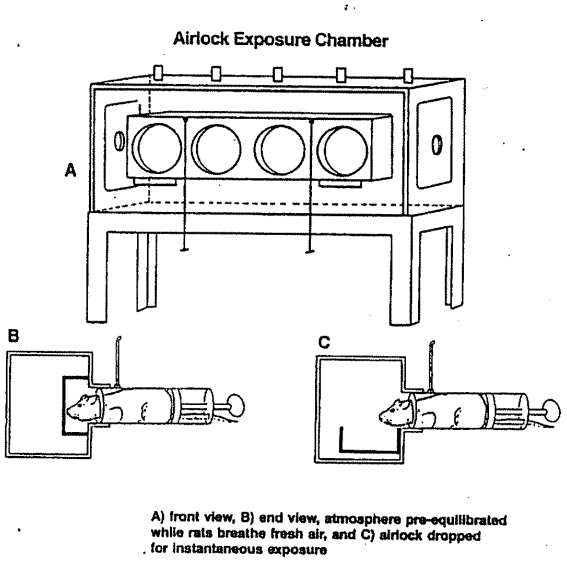
Study ID: A.1(restrained) & A.2 (unrestrained)
Author, year: Lapin, 1981
Substance: Hydrogen cyanide
Species, strain, sex: Rat, Crl:CD, male
Number/sex/concentration group: 6 (restrained), 10 (unrestrained)
Age and weight: weights 250 ± 25 gram, age not specified.
Observation period: at least 7 days

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 guideline(s)	*OECD guideline 403 did not exist at the time*
Stability of test compound in test atmosphere	*No information*
Use of vehicle (other than air)	*No*
Whole body / nose-only (incl. head/nose-only) exposure	*Restrained: Head only* *Unrestrained: whole body*
Type of restrainer	*Rats were restrained in whole body holders inside the chamber (175 l) used (in some cases simultaneously) for unrestrained animals. By using a switch a hinged box was swung down to start an exposure (see figure below) after the required concentration was reached.*
Pressure distribution.	*Not specified*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere was generated by dilution of bottled gas and passed into the chamber by flow-through.*
Number of air changes per hour	*Unknown*
Equilibration time (t95)	*Information on chamber flow is missing, t95 cannot be derived.*
Start of exposure relative to equilibration	*Whole body: insufficient information.* *Nose-only (in chamber): The hinges were dropped once the required concentration (steady state) was reached.*
Actual concentration measurement	*Continuous measurements with infrared spectrophotometry. This method was validated by gas chromatography. No details were presented.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*

Assessment of Reliability	***A*** *Study data were suitable to derive a probit function. Multiple concentration levels and durations were tested, resulting in a good concentration response relation with mortality in the range of 0-100%.*

1



A) front view, B) end view, atmosphere pre-equilibrated while rats breathe fresh air, and C) airlock dropped for instantaneous exposure

2
3 Figure showing how the restrained animals were exposed. Figures B and C show
4 respectively a closed and opened hinged box.
5
6
7

Results (restrained animals)

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Responded
Rat	363	5	6	0
Rat	386	5	6	1
Rat	447	5	6	3
Rat	468	5	6	3
Rat	478	5	6	5
Rat	605	5	6	6
Rat	81	15	6	0
Rat	158	15	6	3
Rat	168	15	6	1
Rat	222	15	6	4
Rat	264	15	6	6
Rat	325	15	6	6
Rat	402	15	6	6
Rat	38	30	6	0
Rat	60	30	6	2
Rat	110	30	6	3
Rat	121	30	6	4
Rat	143	30	6	4
Rat	185	30	6	6
Rat	319	30	6	6
Rat	47	60	6	0
Rat	61	60	6	2
Rat	81	60	6	4
Rat	116	60	6	6

Results (unrestrained animals)

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Responded
Rat	307	5	10	0
Rat	368	5	10	1
Rat	382	5	10	2
Rat	396	5	10	5
Rat	495	5	10	9
Rat	554	5	10	10
Rat	570	5	10	10
Rat	124	15	10	0
Rat	197	15	10	2
Rat	211	15	10	4
Rat	229	15	10	7
Rat	258	15	10	7
Rat	282	15	10	10
Rat	318	15	10	10
Rat	453	15	10	10
Rat	144	30	10	0
Rat	167	30	10	0
Rat	180	30	10	4
Rat	206	30	10	8
Rat	249	30	10	9
Rat	344	30	10	10
Rat	85	60	10	0
Rat	120	60	10	1
Rat	173	60	10	7
Rat	206	60	10	9
Rat	249	60	10	10

All non-surviving animals died during exposure or within 24 hours after exposure. The author reported the following 5, 15, 30, and 60 minute LC_{50} values for the restrained and unrestrained animals.

Duration (minutes)	LC_{50} (mg/m^3) **Male (restrained)** Reported by study authors	LC_{50} (mg/m^3) **Male (unrestrained)** Reported by study authors
5	447	414
10	183	220
30	95	194
60	71	156

Probit function

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

$Pr = a + b \times \ln(C) + c \times \ln(t) + dxS$

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

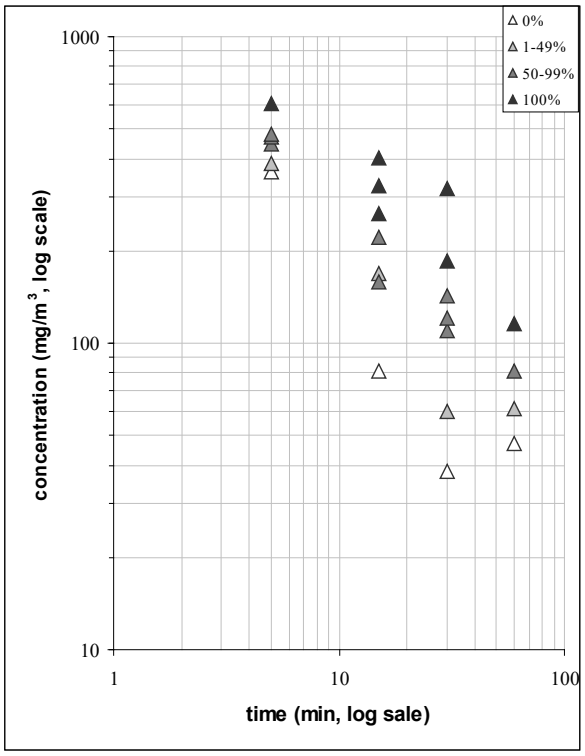
As only male rats were used in the study, sex could not be included as covariate in the probit function. The method of exposing the animals was included as covariate in the analysis of the combined data. It appeared that the results for the restrained animals and unrestrained animals were significantly different. This conclusion was based on the comparison of the ln(likelihood) of the fitted data. The ln(likelihood) for the data combined with method as covariate was -52.16, whereas the ln(likelihood)s of the separately analysed data was approximately -17 for each of the studies separately. As a general rule, a model is considered a better fit when the additional use of parameters (in this case by fitting the data separately) lead to a significant increase in the ln(likelihood). Since the ln(likelihood)s indicate that analysing the data separately is significantly better (-52 versus -34), it is not appropriate to combine the data from restrained and unrestrained animals, and the data will be treated as 2 different datasets.

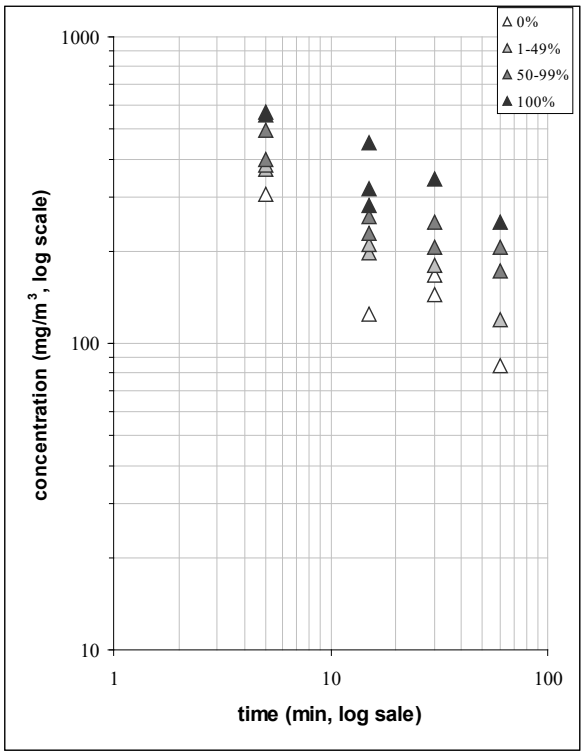
Probit function	Species	a	b	c	n-value
Restrained	Rat	-18.6	3.22	2.49	1.29 (1.10-1.49)
Restrained, excl 5 min.	Rat	-14.4	2.80	1.87	1.50 (1.05-1.94)
Unrestrained	Rat	-32.2	5.63	2.27	2.48 (2.12-2.83)
Unrestrained, excl 5 min.	Rat	-33.5	6.39	1.46	4.38 (2.87-5.88)

Duration (minutes)	LC_{50} (mg/m^3) 95%-C.I. Male (restrained)	LC_{50} (mg/m^3) 95%-C.I. Male (restrained, excl 5 min.)	LC_{50} (mg/m^3) 95%-C.I. Male (unrestrained)	LC_{50} (mg/m^3) 95%-C.I. Male (unrestrained, excl 5 min.)
10	256 (221-289)	224 (168-283)	294 (278-311)	244 (224-266)
30	109 (92.3-125)	108 (93.7-120)	189 (177-202)	190 (181-199)
60	64 (50.5-78.0)	67.6 (55.1-82.3)	143 (130-158)	162 (149-176)

For further analyses of the data, the results using all data were used.

A graphical overview of the data is presented below. Each concentration-time combination represents one point in the plot.





Top: data of restrained animals, bottom: data of unrestrained animals.

Study ID: A.3
Author, year: Sweeney, 2014 and 2015
Substance: Hydrogen cyanide
Species, strain, sex: Rat Crl:CD (SD) BR Sprague-Dawley, males
Number/sex/concentration group: 10 males/group
Age and weight: weights 213-325.1 g, 5-6 weeks old.
Observation period: 24hrs after exposure

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Not stated*
Study carried out according to OECD guideline(s)	*No, however follows similar protocol, where the observation period was shorter in the study than stated in the OECD guideline. Study was performed for research of the toxic load model in acute inhalation toxicology, including intermittent exposure profiles (on-off-on exposure). Only continuous exposure profiles were considered for probit function derivation.*
Stability of test compound in test atmosphere	*No information*
Use of vehicle (other than air)	*21% oxygen, balanced nitrogen mixed with breathing air*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose-only*
Type of restrainer	*In house constructed 12-position Cannon style exposure unit. No specific information on the tubes themselves.*
Pressure distribution.	*Static pressure in the range of -0.05 to -0.10 inches of water*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere was generated by prefilled cylinders of HCN/oxygen/nitrogen (certified by gravimetric method by supplier). The atmosphere flowed through ports to a central inner plenum and out through the delivery nozzles directed to the breathing zone. The outer plenum carried the exhaled air from the animals.*
Number of air changes per hour	*Flow rate per port: 0.5 l/min. Total 6 l/min target supply rate for the exposure unit.*
Equilibration time (t95)	*Cannot be determined as information on volume of exposure unit is lacking. Although the data do not allow quantification of equilibrium time, graphs show that concentration built up in the chambers were within several seconds.*
Start of exposure relative to equilibration	*Not specified*

Actual concentration measurement	*Continuous measurements with Fourier Transform infrared spectrophotometry. The FT-IR sampled at 500 ml/min from the intake tube leading to the nose-only exposure unit. This was validated previously by sampling at the intake, exhaust, and at a port on the exposure unit. Uniformity of the atmosphere at various ports was determined by sampling at 5 different ports.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*
Assessment of Reliability	***A*** *Study data were suitable to derive a probit function. Multiple concentration levels and durations were tested, resulting in a good concentration response relation with mortality in the range of 0-100%. The observation period of 24 hours is too short, however, in view of the mechanism of action of HCN where one would expect to have rapid mortality, and the fact that animals either died or recovered completely within 24 hours in this study, it did not affect the results. A similar observation was made in the Lapin study (A1 and A2 dataset) where all fatalities occurred during exposure or within 24 hours after exposure.*

1

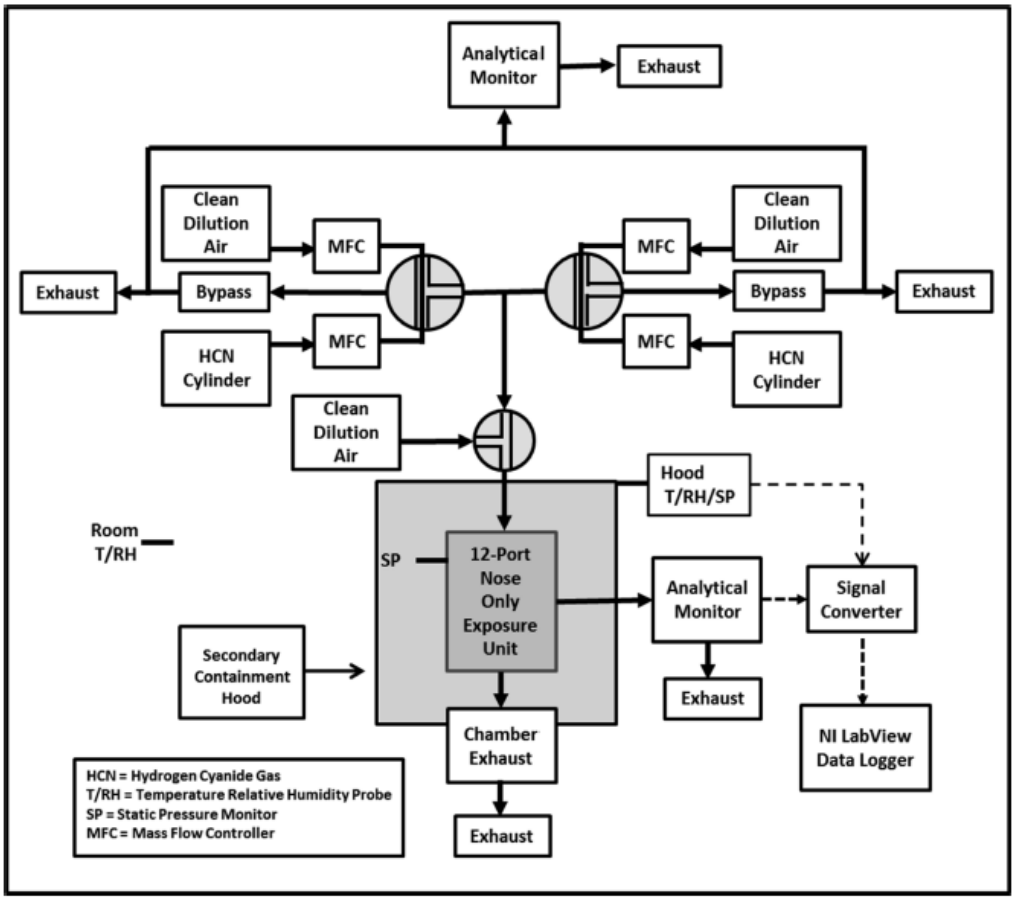


FIG. 1. Representation of the hydrogen cyanide exposure system.

Figure representing the exposure system in this study (copied from Sweeney et al. 2014).

Results

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Responded
Rat	1391	2.33	10	0
Rat	1794	2.33	10	1
Rat	2014	2.33	10	4
Rat	2455	2.33	10	9
Rat	2473	2.33	10	7
Rat	2839	2.33	10	5
Rat	3566	2.33	10	10
Rat	430	5	10	0
Rat	663	5	10	2
Rat	666	5	10	6
Rat	857	5	10	4
Rat	859	5	10	4
Rat	923	5	10	7

Rat	1085	5	10	8
Rat	549	10	10	2
Rat	663	10	10	4
Rat	722	10	10	5
Rat	765	10	10	8
Rat	874	10	10	9
Rat	222	15	10	0
Rat	306	15	10	2
Rat	382	15	10	3
Rat	458	15	10	5
Rat	506	15	10	4
Rat	510	15	10	5
Rat	554	15	10	8
Rat	156	30	10	0
Rat	225	30	10	1
Rat	247	30	10	2
Rat	254	30	10	1
Rat	286	30	10	6
Rat	307	30	10	6
Rat	326	30	10	7
Rat	340	30	10	8

The author reported 2.33, 5, 10, 15 and 30 minute LCt_{50} values for the restrained and unrestrained animals.

Duration (minutes)	LCt_{50} (ppm x min) **Male**
2.33	4460
5	3871
10	6049
15	6340
30	7841 (Sweeney et al. 2014) 7851 (Sweeney et al. 2015)

Probit function

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

$Pr = a + b \times \ln(C) + c \times \ln(t) + dxS$

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

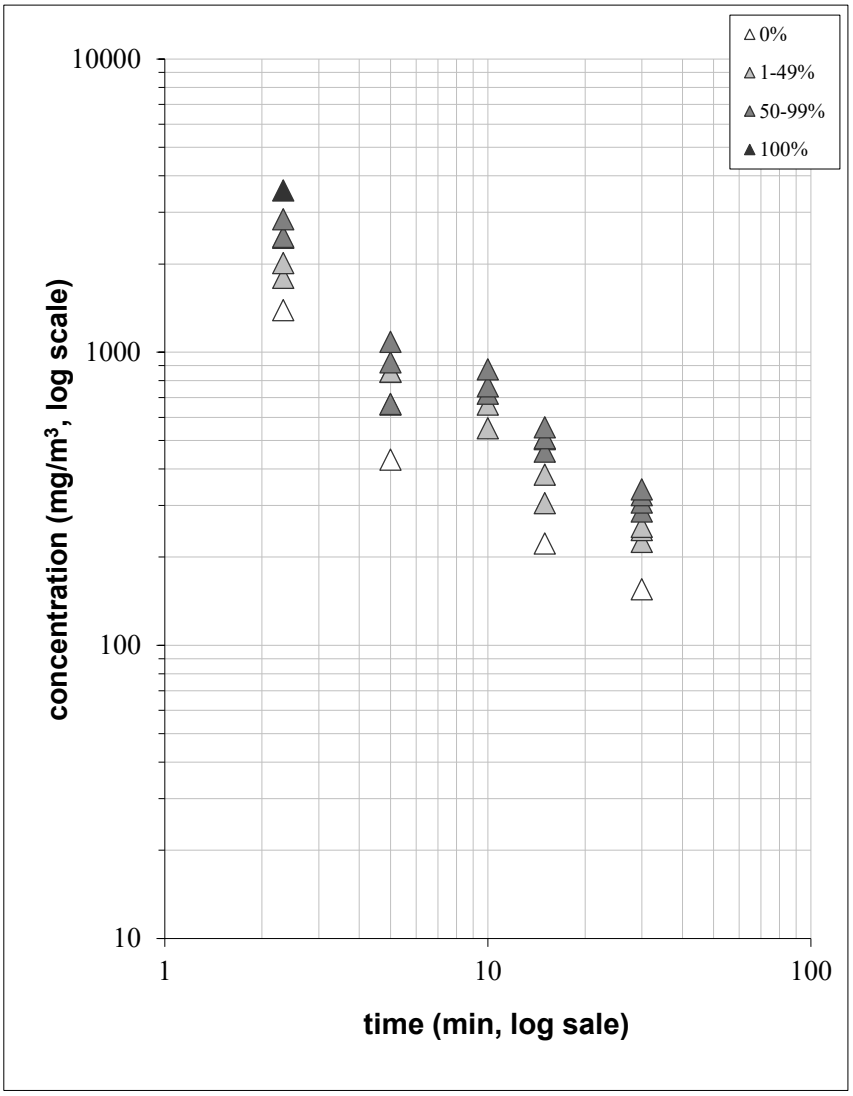
As only male rats were used in the study, sex could not be included as covariate in the probit function.

Probit function	Species	a	b	c	n-value
All data	Rat	-13.6	2.28	1.67	1.37 (1.17-1.56)
Excl. 2.33 and 5 min data	Rat	-24.6	3.62	2.66	1.36 (1.14-1.58)

Duration (minutes)	LC_{50} (mg/m^3) 95%-C.I. Male	LC_{50} (mg/m^3) 95%-C.I. Male – excl. 2.33 and 5 min data
10	644 (585-718)	654 (594-714)
30	288 (247-345)	292 (269-321)
60	173 (140-223)	175 (150-210)

For further analyses of the data, the results of all data were used.

A graphical overview of the data is presented below. Each concentration-time combination represents one point in the plot.


△ 0%
△ 1-49%
▲ 50-99%
▲ 100%
10000
1000
100
10
concentration (mg/m³, log scale)
1
10
100
time (min, log sale)

1

2

3

Study ID: B2.1
Author, year: Ballantyne, 1994
Substance: Hydrogen cyanide
Species, strain, sex: Rat, Porton, male
Number/sex/concentration group: 10 (1 group of 20)
Age and weight: 176-221 g
Observation period: not specified

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)	*No*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body, 1 m^3 chamber.*
Type of restrainer	*N/A*
Pressure distribution.	*Not specified*
Homogeneity of test atmosphere at breathing zone of animals	*HCN vapour was generated by mixing solutions of NaCN and HCl, and vaporizing the HCN so produced by an air stream. This led to a premixing chamber were various concentrations were produced by adjusting the rate of pumping of NaCN and HCl.*
Number of air changes per hour	*Unknown*
Equilibration time (t95)	*Cannot be derived due to lack of chamber flow.*
Start of exposure relative to equilibration	*No information.*
Actual concentration measurement	*Chamber atmosphere concentrations were monitored by aspirating chamber air through bubblers containing 5 ml 1.0 N NaOH at a flow rate of 1 L min-1. Concentrations of cyanide in the alkaline extract were determined using a cyanide-specific electrode.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*
Assessment of Reliability	***B2*** *The observation period was not stated and therefore the study was not given the A status.*

The publication states that 'within a few minutes of the end of exposure, blood was collected by cardiac puncture from both animals that died and survivors'. Although this procedure is usually performed at terminal sacrifice, the animals may have been observed after the procedure. The author is no longer available for clarification on the length of the (possible) observation period.

1 **Results**

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Responded
Rat	2922	0.167	10	4
Rat	2970	0.167	10	2
Rat	3696	0.167	10	4
Rat	3714	0.167	10	3
Rat	3912	0.167	10	5
Rat	4290	0.167	10	7
Rat	6684	0.167	10	10
Rat	10230	0.167	10	10
Rat	773	1	10	3
Rat	1225	1	10	6
Rat	1989	1	20	13
Rat	2403	1	10	10
Rat	4169	1	10	10
Rat	217	5	10	0
Rat	317	5	10	1
Rat	382	5	10	2
Rat	439	5	10	3
Rat	485	5	10	7
Rat	628	5	10	4
Rat	658	5	10	10
Rat	888	5	10	10
Rat	87	30	10	0
Rat	110	30	10	0
Rat	130	30	10	1
Rat	138	30	10	5
Rat	160	30	10	5
Rat	167	30	10	2
Rat	180	30	10	6
Rat	189	30	10	5
Rat	193	30	10	8
Rat	215	30	10	7
Rat	68	60	10	0
Rat	86	60	10	0
Rat	95	60	10	0

Rat	134	60	10	5
Rat	146	60	10	2
Rat	156	60	10	4
Rat	182	60	10	6
Rat	205	60	10	10

LC_{50} values by the author (Ballantyne).

Duration (minutes)	LC_{50} (mg/m^3) 95%-C.I.
0.167	3788 (3371-4313)
1	1129 (664-1471)
5	493(372-661)
30	173 (159-193)
60	158 (144-174)

Probit function

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

$Pr = a + b \times \ln(C) + c \times \ln(t) + dxS$

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

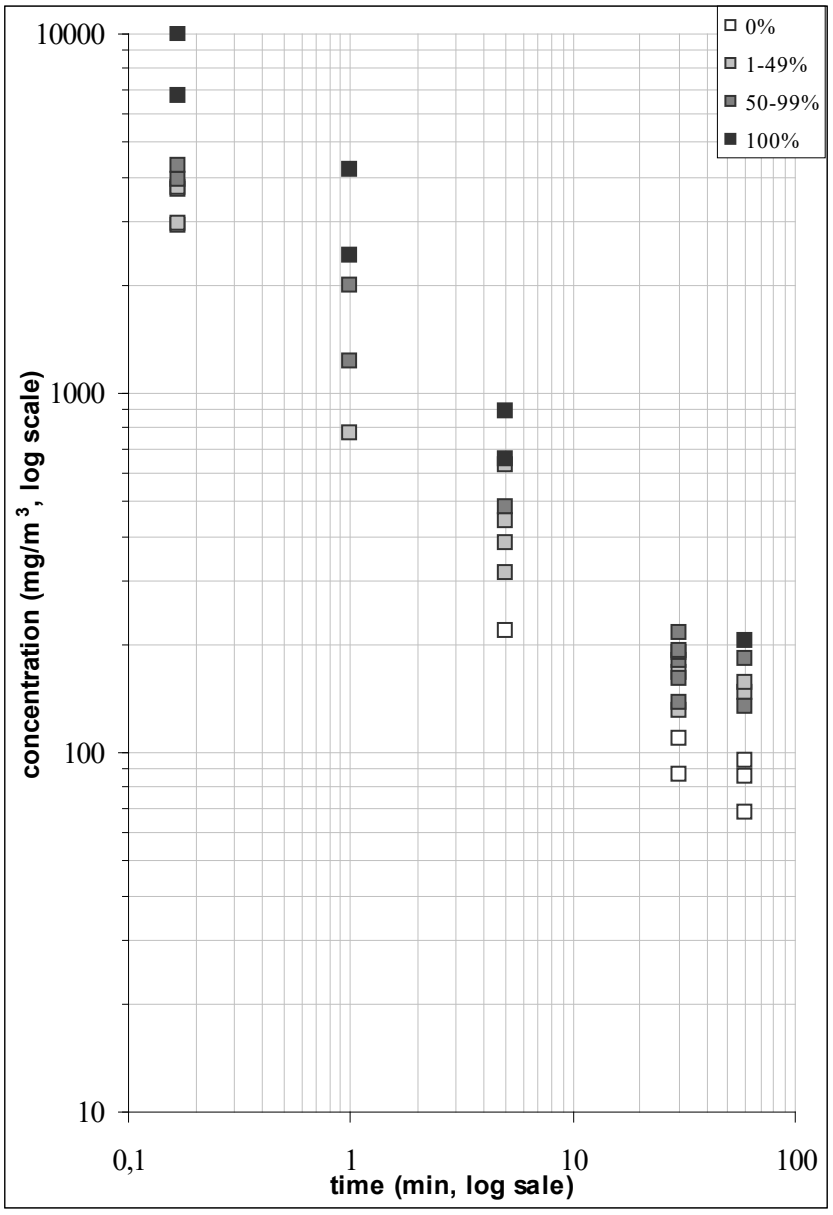
A probit function was calculated for all data, excluding 10 second data, and excluding both 10 second and 1 minute data. Excluding the 5 minute data in the analysis as well resulted in an unrealistic n-value of 7.96 with a confidence interval of -3.11-19.0.

Probit function	Species	a	b	c	n-value
All data	Rat	-12.2	2.40	1.34	1.79 (1.67-1.91)
Excl. 10 sec data	Rat	-11.3	2.30	1.20	1.92 (1.64-2.20)
Excl. 10 sec and 1 min data	Rat	-14.9	2.88	1.39	2.07 (1.66-2.48)

The table below presents the LC_{50} values for several exposure durations, based on all data.

Duration (minutes)	LC_{50} (mg/m^3) 95%-C.I. All data
10	365 (336-401)
30	198 (179-222)
60	135 (119-154)

A graphical overview of the data is presented below. Each concentration-time combination represents one point in the plot.


0%
1-49%
50-99%
100%
10000
1000
100
10
concentration (mg/m 3, log scale)
0,1
1
10
100
time (min, log sale)

1

Study ID: B2.2

Author, year: Ballantyne, 1994

Substance: Hydrogen cyanide
Species, strain, sex: Rabbit, New Zealand White, female
Number/sex/concentration group: 3 (one group of 6)
Age and weight: 2.20-2.80 kg
Observation period: not specified

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)	*No*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose only.*
Type of restrainer	*Please see the figure below. Make and model of the restrainer are unknown.*
Pressure distribution.	*Not specified*
Homogeneity of test atmosphere at breathing zone of animals	*HCN vapour was generated by mixing solutions of NaCN and HCl, and vaporizing the HCN so produced by an air stream. This led to a premixing chamber were various concentrations were produced by adjusting the rate of pumping of NaCN and HCl.*
Number of air changes per hour	*Unknown*
Equilibration time (t95)	*t95 cannot be derived as the flow rate is unknown.*
Start of exposure relative to equilibration	*Information is insufficient.*
Actual concentration measurement	*Chamber atmosphere concentrations were monitored by aspirating chamber air through bubblers containing 5 ml 1.0 N NaOH at a flow rate of 1 L min^{-1}. Concentrations of cyanide in the alkaline extract were determined using a cyanide-specific electrode (nominal concentrations).*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*
Assessment of Reliability	***B2*** *No information on the observation period was provided and therefore the study was not given the A status.*

The publication states that 'within a few minutes of the end of exposure, blood was collected by cardiac puncture from both animals that died and survivors'. Although this procedure is usually performed at terminal sacrifice, the animals may have been observed after the procedure. The author is no longer available for clarification on the length of the (possible) observation period.

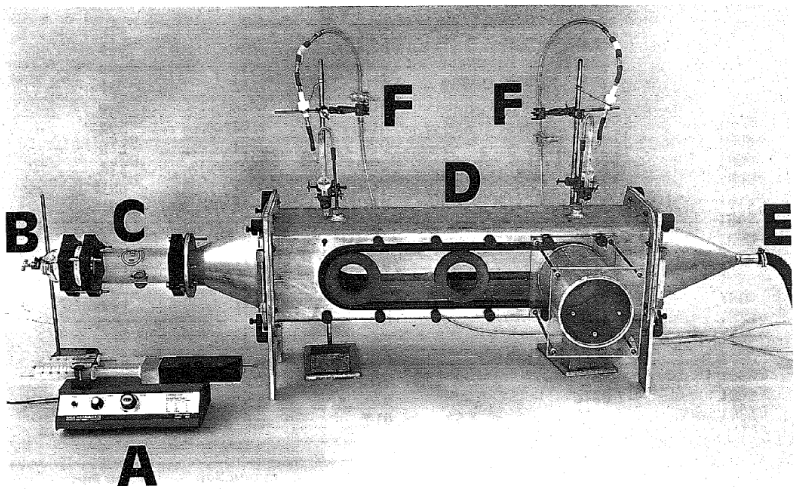


Figure 3. Nose-only exposure equipment for rabbits. A constant-rate pump (A) passes NaCN and HCl solutions to the microreactor (B), from which HCN passes to a premixing chamber (C) and then to the exposure chamber (D). Two seals are shown at the left and middle of the chamber, and a restrainer at the right. Air is drawn through the chamber by means of a flexible hose (E) attached to a constant-rate pump. HCN in the chamber is measured by samplers (F), which draw air through NaOH traps.

Figure from Ballantyne (1994) showing the nose-only exposure equipment for rabbits. Next to the two seals (below D) on the right side the restrainer is shown.

Results

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Responded
Rabbit	2116	0.75	3	0
Rabbit	2129	0.75	3	0
Rabbit	2167	0.75	3	0
Rabbit	2279	0.75	3	2
Rabbit	2372	0.75	3	1
Rabbit	2431	0.75	3	2
Rabbit	2471	0.75	3	2
Rabbit	2528	0.75	3	2
Rabbit	2519	0.75	3	1
Rabbit	2536	0.75	3	3
Rabbit	2539	0.75	3	2
Rabbit	2556	0.75	3	2
Rabbit	2611	0.75	3	2
Rabbit	2640	0.75	3	2
Rabbit	2679	0.75	3	2

Rabbit	2767	0.75	3	3
Rabbit	2977	0.75	3	3
Rabbit	358	5	3	1
Rabbit	365	5	3	1
Rabbit	379	5	3	1
Rabbit	398	5	3	1
Rabbit	401	5	3	2
Rabbit	417	5	3	0
Rabbit	418	5	3	3
Rabbit	424	5	3	3
Rabbit	427	5	3	1
Rabbit	429	5	3	1
Rabbit	430	5	3	2
Rabbit	554	5	6	5
Rabbit	560	5	3	2
Rabbit	567	5	3	3
Rabbit	581	5	3	3
Rabbit	71	35	3	0
Rabbit	141	35	3	0
Rabbit	153	35	3	2
Rabbit	159	35	3	2
Rabbit	162	35	3	1
Rabbit	166	35	3	3
Rabbit	169	35	3	0
Rabbit	177	35	3	1
Rabbit	178	35	3	2
Rabbit	188	35	3	0
Rabbit	224	35	3	1
Rabbit	233	35	3	2
Rabbit	258	35	3	0
Rabbit	266	35	3	2
Rabbit	277	35	3	2
Rabbit	289	35	3	3
Rabbit	384	35	3	2
Rabbit	468	35	3	3
Rabbit	469	35	3	3

Below an overview of the LC_{50} values by the author (Ballantyne) is given for several exposure durations.

Duration (minutes)	LC_{50} (mg/m^3) 95%-C.I.
0.75	2432 (2304-2532)
5	409 (321-458)
35	208 (154-276)

Probit function

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

$Pr = a + b \times \ln(C) + c \times \ln(t) + dxS$

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

Probit function	Species	a	b	c	n-value
All data	Rabbit	-1.67	0.92	0.55	1.69 (1.29-2.09)

Below the LC_{50} values based on all data is given for several exposure durations.

Duration (minutes)	LC_{50} (mg/m^3) 95%-C.I. All data
10	353 (235-452)
30	184 (116-268)
60	122 (71.6-199)

Study ID: C.1

Author, year: Matijak-Schaper, 1982

Substance: Hydrogen cyanide
Species, strain, sex: Mouse, Swiss-Webster, male
Number/sex/concentration group: 4
Age and weight: 26-28 g
Observation period: 10 minutes

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)	
Whole body / nose-only (incl. head/nose-only) exposure	*Head-only*
Type of restrainer	
Pressure distribution.	*No information*
Homogeneity of test atmosphere at breathing zone of animals	*No information*
Number of air changes per hour	*Unknown*
Equilibration time (t95)	*No information*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Concentrations were measured using titration. Samples were taken at the top of the exposure chamber.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*
Assessment of Reliability	***C*** *Few study details are available. Follow up time was very short. Study data were not suitable to derive a probit function. A 30 minute LC_{50} value could be calculated.*

Results

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Responded
Mouse	112	30	4	0
Mouse	169	30	4	2
Mouse	247	30	4	3
Mouse	371	30	4	4
Mouse	562	16	4	4
Mouse	854	2	4	4
Mouse	1124	2	4	4
Mouse	1292	2	4	4

The author calculated a 30 minute LC_{50} of 209 mg/m^3.

Probit function

No probit function could be calculated. A 30 minute LC_{50} was calculated using the DoseResp program using only the 30-min data (Wil ten Berge, version 2015).

Duration (minutes)	LC_{50} (mg/m^3) 95%-C.I. Male
30	187 (119-280)

Study ID: C.2

Author, year: Barcroft 1931 / McNamara, 1976

Original study by Barcroft (1931) was used to describe study parameters. McNamara presented data that were read off from a graph published by Barcroft (1931).

Substance: Hydrogen cyanide

Species, strain, sex: Rat, strain and sex not specified

Other species included: Monkey, goat, dog, cat, rabbit, guinea pig, mice, canaries, pigeons.

Number/sex/concentration group: 6 (two groups of 4)

Age and weight: not specified

Observation period: not specified

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 guideline(s)	*OECD guidelines did not exist*
Stability of test compound in test atmosphere	*Unstable, 22% loss in 38 minutes*
Use of vehicle (other than air)	*No*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution.	*No information*
Homogeneity of test atmosphere at breathing zone of animals	*Static exposure; circulation with ventilator*
Number of air changes per hour	*No information*
Equilibration time (t95)	*No information*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Nominal concentrations*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*
Assessment of Reliability	***C*** *Few study details are provided. Analysis of the study data did not result in a reliable probit function or LC_{50} values for exposure duration >10 minutes.*

Results

Barcroft presented two tables with acute lethal and non-lethal concentrations for a number of species.

Animal	*Lethal time of exposure to 1000 mg/m³ HCN (mins)*	*Animal*	*Highest appr. Concentration which can be breathed indefinitely (mg/m³)*
Dog	0.8	Dog	100
Mouse	1.0	Rat	100
Cat	1.0	Mouse	140
Rabbit	1.0	Rabbit	180
Rat	2.0	Monkey	180
Guinea Pig	2.0	Cat	180
Goat	3.0	Goat	240
Monkey	3.5	Guinea Pig	400

Experimental data were also presented in graphs by Barcroft (1931). No attempt was made to read off the experimental data by the author of the TSD. Barcroft concluded that the sensitivity of the species was in the following order of decreasing sensitivity: dog > mouse = cat = rabbit > rat = guinea pig > goat > monkey. The order of susceptibility is based on time of deaths at an exposure concentration of 1000 mg/m^3 HCN.

Probit function

No probit function was calculated.

Other studies (C quality)

Higgins et al. (1972; as cited in AEGL, 2002) exposed rats (Wistar) and mice (ICR) to HCN for five minutes at analytical concentrations ranging from 313 to 773 mg/m^3 (rats) and 224 to 478 mg/m^3 (mice). All deaths occurred during the exposure period or within 20 minutes thereafter. The 5-min LC_{50} for rats and mice were 563 (95% C.I. 451-701) mg/m^3 and 362 (95% C.I. 309-422) mg/m^3, respectively. Vernot et al. (1977; as cited in AEGL, 2002) observed a 5-min LC_{50} of 542 (95% C.I. 495-701) mg/m^3 in male Sprague-Dawley rats.

The AEGL document further gives reference to a number of studies that have only reported a LC_{50} value for a certain exposure duration. These studies have not been taken up in this document, because the derived LC_{50} values in those studies could not be evaluated.

Appendix 2 Derivation of lethal air concentration based on the Schulz et al. (1982) model.

Schulz et al. (1982) predicted the accumulation of cyanide after a single intravenous dose of SNP from the maximum detoxification rate and volume of distribution. Based on their model they estimated that a dose rate of 20 µg/kg bw/min SNP for 90 minutes would lead to the threshold concentration of 400 nmol CN^- /mL erythrocytes. The 20 µg/kg bw/min SNP for 90 minutes equals a dose rate of approximately 9 µg/kg bw/min CN^- for 90 minutes (based on molecular weight of the dihydrate form, i.e. 44% of SNP assuming complete release of the five CN ions (Friedrich and Butterworth, 1995)). If one assumes a person of 60 kg, inhaling 1.26 m^3/h (light exercise) and 100% absorption through the lungs, inhaling an air concentration of 25 mg/m^3 HCN for 90 minutes would result in an equal systemic dose as 9 µg/kg bw/min CN^- for 90 minutes. It should be noted that the model includes a large number of assumptions that do not hold for susceptible subjects, e.g. subjects with a reduced amount of sulphur donors in their body required for detoxification, will not reach the maximum detoxification rate.

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