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
Acetone cyanohydrin	**75-86-5**

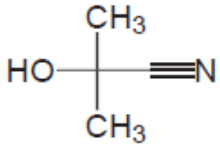
This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

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 Acetone cyanohydrin



1. Substance identification

CAS-number:	75-86-5
IUPAC name:	Acetone cyanohydrine
Synonyms:	2-propanone cyanohydrine, 2-cyano-2-propanol, 2-cyano-2-hydroxypropane.
Molecular formula:	$(CH_3)_2C(OH)CN$
Molecular weight:	85.1 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	82°C (at 3.1 kPa)
Vapour pressure:	0.11 kPa (at 20°C)
Saturated vapor conc:	1100 ppm = 3894 mg/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.282 ppm (at 20°C and 101.3 kPa) 1 ppm = 3.55 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H330-310-300

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

Acute effects: The main target organs and tissues for inhalation exposure to acetone cyanohydrin are irritation effects to the nose, eye and throat. The systemic toxicity of acetone cyanohydrin is related to the formation of free cyanide after decomposition to hydrogen cyanide (HCN) and acetone. The main target organs and tissues for inhalation exposure to HCN 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. Hydrogen cyanide exposure results in neurological symptoms, loss of consciousness. Besides, 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: No information on acetone cyanohydrin. The long-term effects of inhalation exposure to HCN are given below: 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 can be uneventful without any permanent adverse health effects.

3. Human toxicity data

No informative reports on health effects in humans following acute inhalation exposure to acetone cyanohydrin 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 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 acetone cyanohydrin, 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*

[1] AEGL (2009)

- 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. Five studies were identified -with seven datasets for two species- with data on lethality following acute inhalation exposure. One dataset was assigned status A for deriving the human probit function, one dataset was assigned status B and five datasets were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation
No studies on sensory information were found.

5. Probit functions from individual studies

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

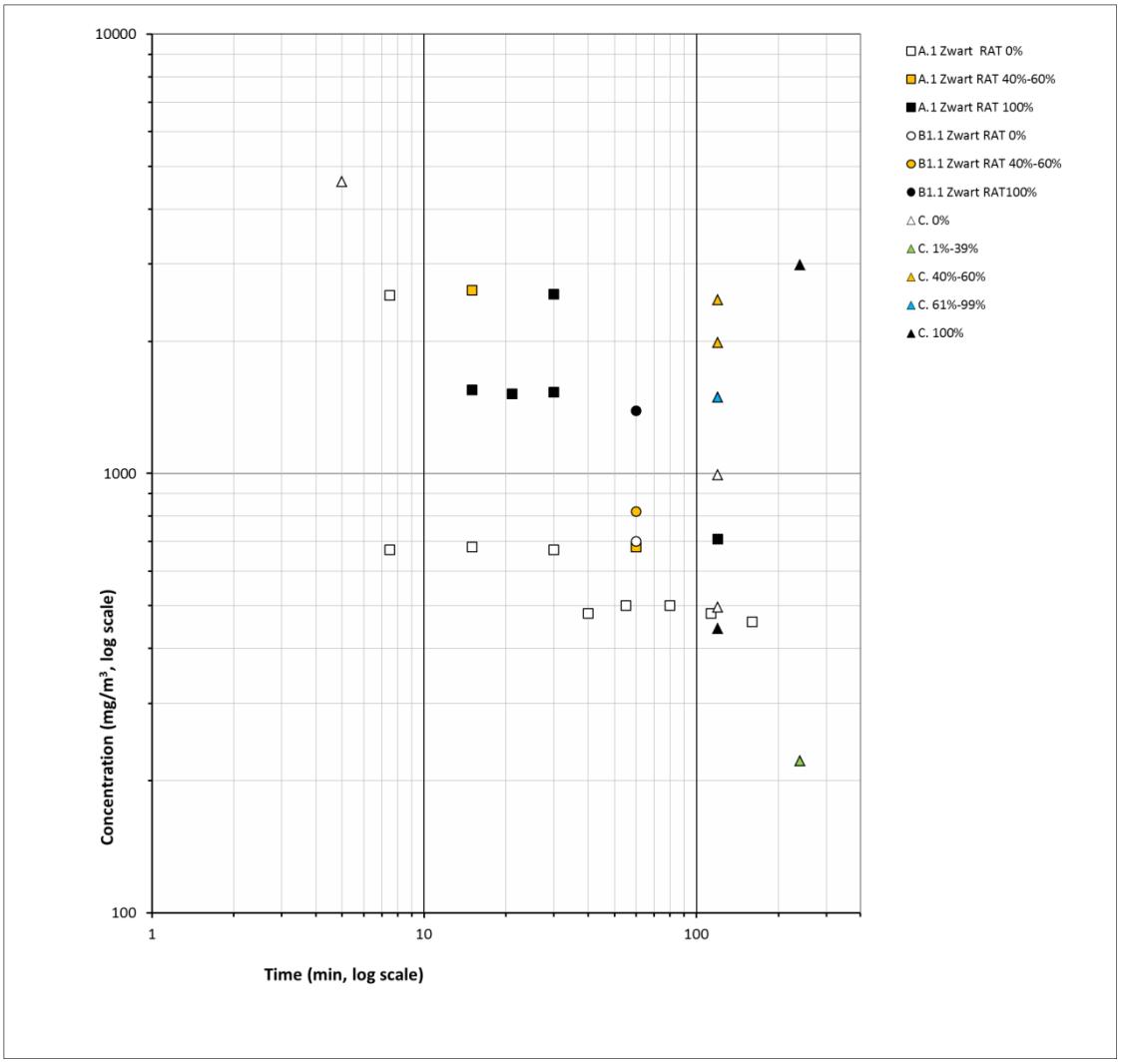


Figure 1 *All available acute lethality data for acetone cyanohydrin*

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

All A and B1 studies were selected for derivation of the animal probit function for acetone cyanohydrin.

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 1 *Data selected for initial analysis of the animal probit function of acetone cyanohydrin.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} at tested exposure duration (mg/m^3) 95% C.I. (60 min.)	LC_{50}, 30 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
A.1	Rat	-27.5 + 3.68×lnC + 1.91×lnt	815 (664-1020)	1168 (930-1397)	1.93 (1.31-2.55)
B1.1	Rat	*60-min LC_{50}*	811 (766-902)		N/A

The data of the A and B1.1 studies with rats are presented graphically below.

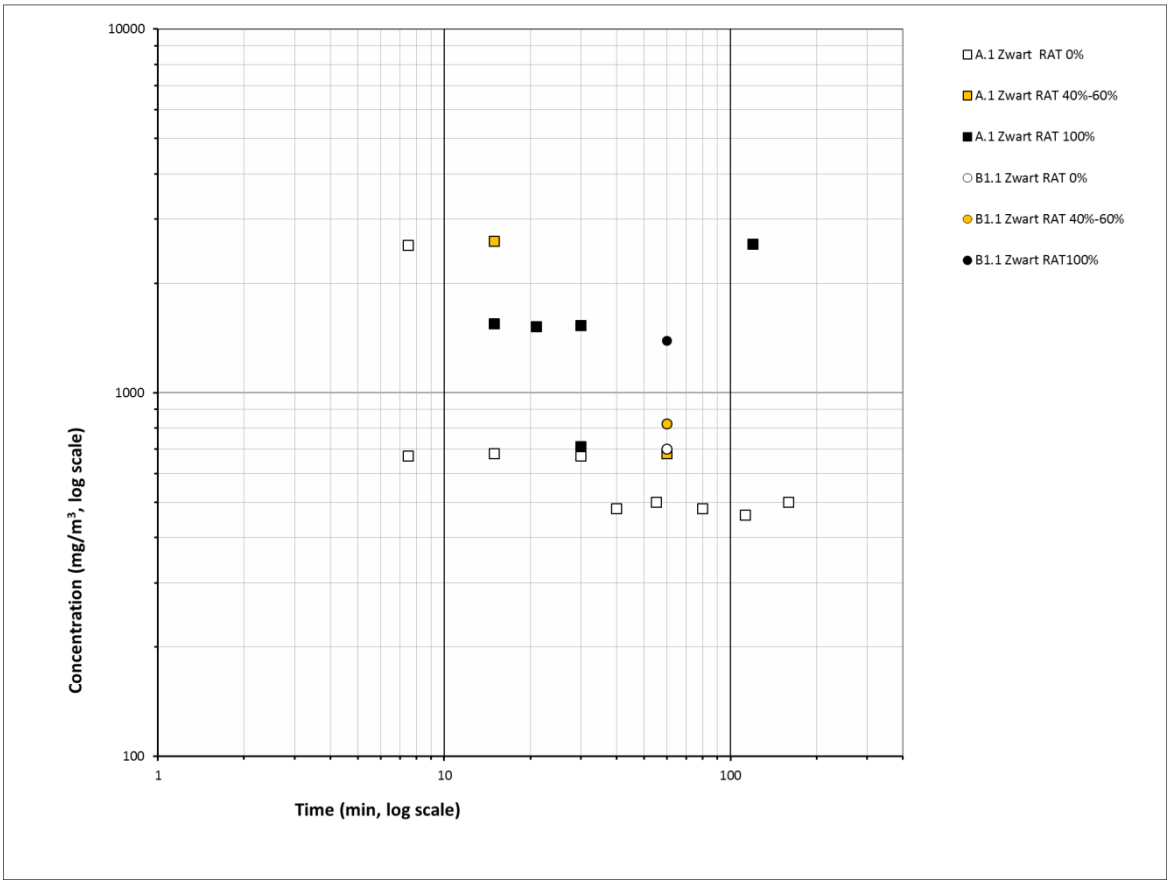


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of acetone cyanohydrin.*

Based on criteria outlined in the guideline the data from rat studies A.1 and B1.1 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 2 and Figure 4.

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

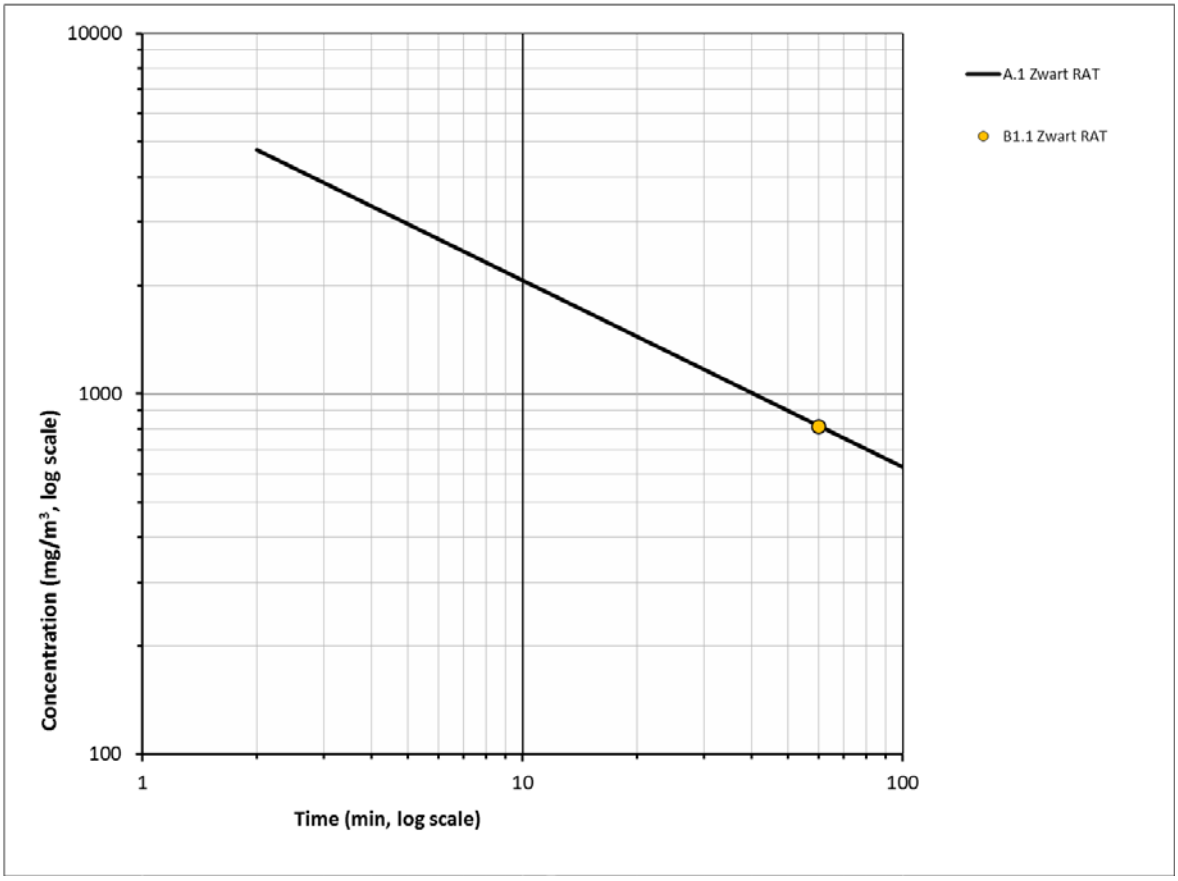


Figure 3 *LC_{50} values of A1 and B1.1 datasets for acetone cyanohydrin, over time where available.*

Table 2 *Data selected for the derivation of the animal probit function of acetone cyanohydrin (identical to table 1).*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} at tested exposure duration (mg/m^3) 95% C.I. (60 min.)	LC_{50}, 30 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
A.1	Rat	-27.5 + 3.68×lnC + 1.91×lnt	815 (664-1020)	1168 (930-1397)	1.93 (1.31-2.55)
B1.1	Rat	*60-min LC_{50}*	811 (766-902)		N/A

The data of the selected datasets are presented graphically below.

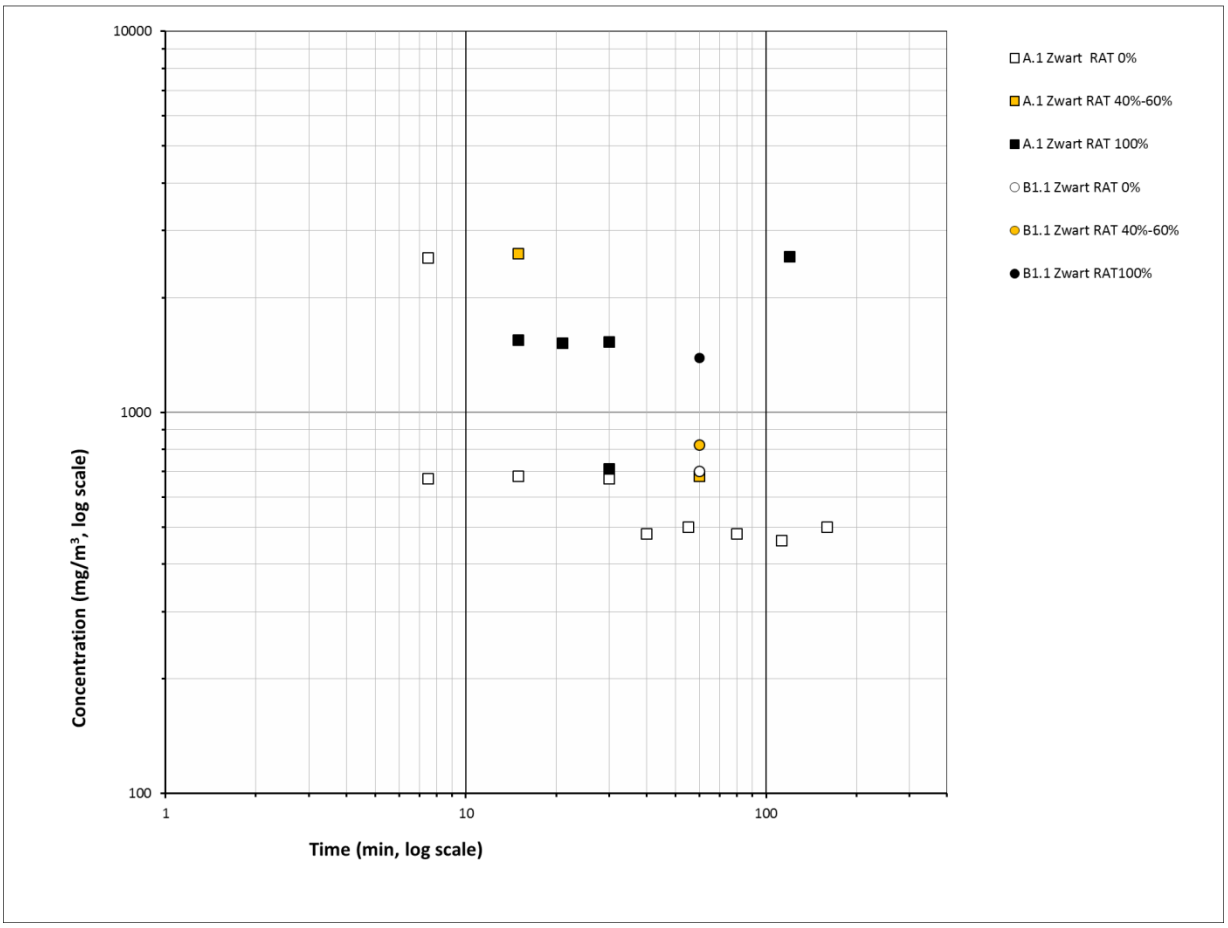


Figure 4 *Final data selected for derivation of the animal probit function of acetone cyanohydrin (identical to figure 2).*

6. Derivation of the human probit function

To derive the human probit function the results from rat studies A.1 and B1.1 have been used to derive a point of departure as outlined above.

Although it is believed that health effects are predominantly caused by free cyanide formation, the use of the A.1 and B1.1 datasets of Zwart (1989) were preferred over using HCN data as point of departure for probit function derivation for acetone cyanohydrin. A substance-specific A and B1 dataset is available, in which 19 concentration-time combinations were tested with acetone cyanohydrin. The concentrations of acetone cyanohydrin in the Zwart study were regularly analysed by gas chromatography and automatic integration (see Appendix 1, study A.1 and study B1.1), which should cover the rapid hydrolysis process of acetone cyanohydrin to form HCN.
It was noted that if the HCN 30-minute LC_{50} value of 181 mg/m^3 (161 ppm) (see Probit TSD HCN) was used as point of departure, a 30 minute LC_{50} value of 570 mg/m^3 (161 ppm) acetone cyanohydrin would have been derived. This is based on the fact that one mole of acetone cyanohydrin can produce one mole of cyanide. This value is approximately 2-fold lower than the 30 min LC_{50} of 1168 mg/m^3 calculated from the Zwart study (1989) for acetone cyanohydrin.

The rat-specific n-value was 1.93 (study A.1).
Second, the LC_{50}-values of all applicable A- and B1-studies were calculated for a common exposure duration of 60 minutes.

Finally, the rat geometric mean LC_{50}-value was calculated from the 60-min LC_{50} values of studies A.1 and B1.1. The rat geometric mean 60-min was 813 mg/m^3. The formula for the geometric mean of time-scaled LC_{50}-values from 1 species is as follows:

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

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 (i=1…m).

The Point of Departure for the human probit function is a 60-minute geometric mean animal LC_{50} value of 813 mg/m^3 and an n-value of 1.93.

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

Table 3 *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	Default.
Nominal concentration	1	Studies with analytically determined concentrations available.
Adequacy of database:	1	Database consists of well conducted A and B1 studies

The estimated human equivalent 60-minute LC_{50} value is 813 / 3 = **271 mg/m^3**.

The experimentally determined n-value was 1.93 (A.1). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1.04**.

The human probit function is then calculated on the human equivalent 60-min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept):
5 = a + 1.04 × ln ($271^{1.93}$ × 60) resulting in the a-value of **-10.4**.

Pr = -10.4 + 1.04 × ln ($C^{1.93}$ × t) with C in mg/m^3 and t in min.

The derived human probit function has a scientifically acceptable basis. The probit function is based on one study in the rat with A quality and one study in the rat with B1 quality. Furthermore, these data included in total 19 C x t combinations, including durations from 7.5 minutes to 160 minutes and lethality range of 0-100%.

The calculated human 60-min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 57 mg/m^3 and the calculated human 60-min LC_1 (Pr = 2.67) is 83 mg/m^3.

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

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
0.1% lethality, this probit	81	57
1% lethality, this probit	118	83
AEGL-3[2] (2009, final)	74	53
ERPG-3	-	-
LBW (2017)	69	59

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

[2] AEGL values were converted from ppm to mg/m^3 with the conversion factor calculated in section 1. Therefore, the AEGL values in mg/m^3 can deviate slightly from those reported in the AEGL TSD.

Appendix 1 Animal experimental research

Study ID: A.1

Author, year:	***Zwart, 1989. (C x t protocol)***
Substance:	acetone cyanohydrin
Species, strain, sex:	male and female SPF-reared, Wistar rats (strain code: Bor:WISW)
Number/sex/conc. group:	1/sex/C x t group
Age and weight:	Age not specified. Mean body weights at beginning were 274g (M) and 185g (F).
Observation period:	14 days.

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes*
Study carried out according to OECD 403 guideline(s)	*Yes, but instead of 4-h exposures, a C x t protocol was used*
Stability of test compound in test atmosphere	*No information whether HCN formation occurred*
Use of vehicle (other than air)	*No*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose-only*
Type of restrainer	*Modified Battelle restraining tubes*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere was generated by bubbling a small flow of air through test material and diluting the saturated flow with fresh air. The mixture was passed to the exposure unit*
Number of air changes per hour	*6 l/animal/min*
Equilibration time (t95)	*Insufficient data available to calculate t95; however, animals were places in the exposure system after target concentration was reached*
Start of exposure relative to equilibration	*Animals were exposed once concentrations were stabilized*
Actual concentration measurement	*Actual concentrations were determined at regular intervals by gas chromatography and automatic integration*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***A*** *Well performed study, including multiple C x t combinations*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	2.54×10^3	N/A	7.5	0/1	0/1
Rat	2.61×10^3	N/A	15	0/1	1/1
Rat	2.56×10^3	N/A	30	3/3	3/3
Rat	0.67×10^3	N/A	7.5	0/1	0/1
Rat	0.68×10^3	N/A	15	0/1	0/1
Rat	0.67×10^3	N/A	30	0/1	0/1
Rat	0.68×10^3	N/A	60	0/1	1/1

Rat	0.71×10^3	N/A	120	1/1	1/1
Rat	0.48×10^3	N/A	40	0/1	0/1
Rat	0.50×10^3	N/A	55	0/1	0/1
Rat	0.50×10^3	N/A	80	0/1	0/1
Rat	0.48×10^3	N/A	113	0/1	0/1
Rat	0.46×10^3	N/A	160	0/1	0/1
Rat	1.55×10^3	N/A	15	1/1	1/1
Rat	1.52×10^3	N/A	21	1/1	1/1
Rat	1.53×10^3	N/A	30	3/3	3/3

Probit function
The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, 2016) as
$Pr = a + b\times lnC + c\times lnt + d\times S$
with C for concentration in mg/m^3, t for time in minutes and S for sex (1 = female, 0 = male).

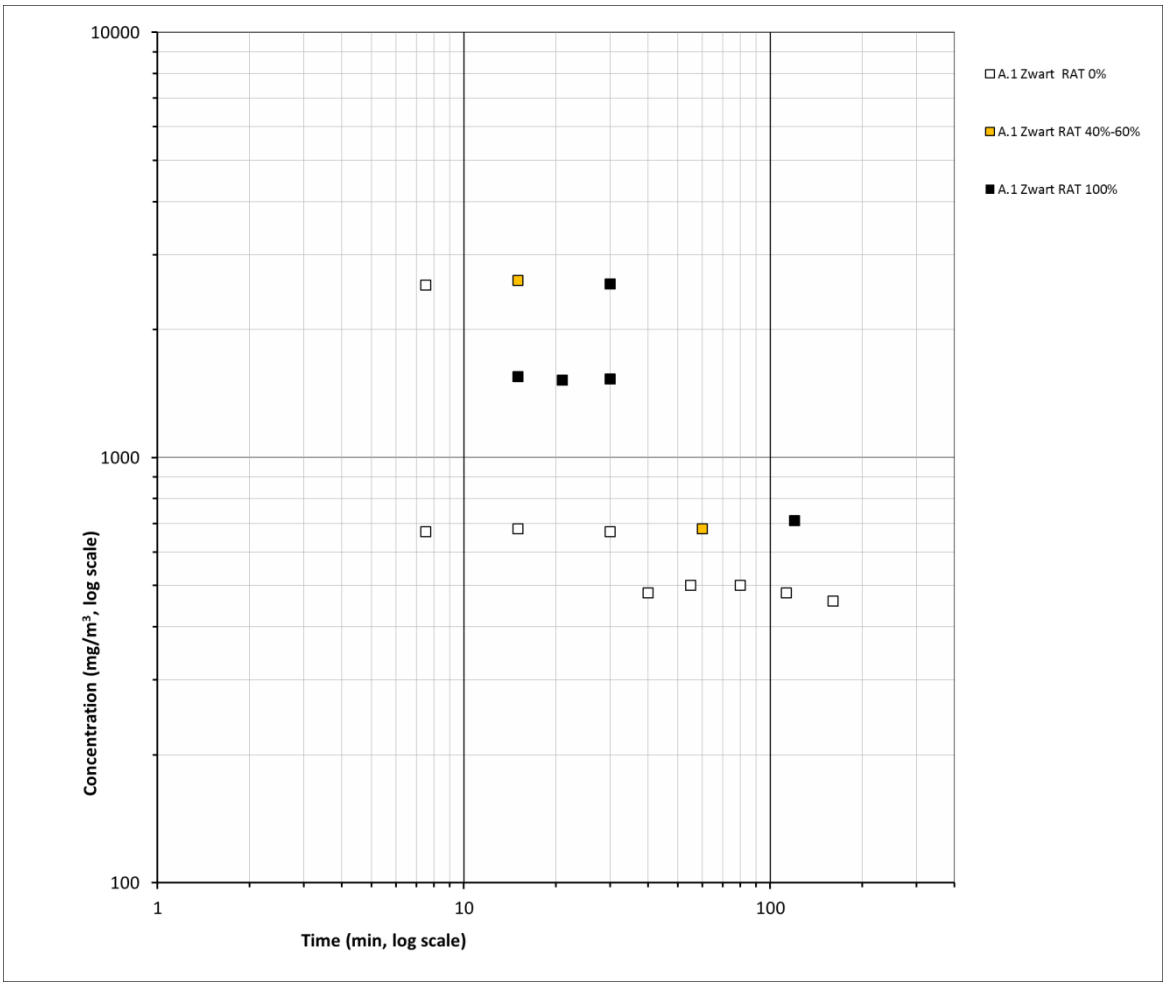
Probit function	Species	a	b	c	d	n-value
Sex as variable	Rat	-30.0	3.94	2.03	0.66	1.94 (1.34-2.53)
Sexes combined	Rat	-27.5	3.68	1.91		1.93 (1.31-2.55)

The LC_{50} values for both sexes did not differ by more than a factor of 2. This does not support the proposition that sex differences exist in the lethal response. For this reason the data from both sexes were pooled and analysed to derive the animal probit function.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Male	LC_{50} (mg/m^3) 95%-C.I. Female	LC_{50} (mg/m^3) 95%-C.I. Combined
10	2238 (1451-3081)	1892 (1129-2603)	2066 (1333-2736)
30	1269 (970-1648)	1073 (771-1361)	1168 (930-1397)
60	887 (687-1216)	750 (561-979)	815 (664-1020)

Zwart (1989) derived 1h LC_{50} values of 890 (700-1190), 750 (570-970) and 820 (670-1010) mg/m^3 based on the probit function with sex as covariate for males and females, and for the sexes combined, respectively.

A graphical overview of the data is presented below. Each concentration-time combination (with 1 male and 1 female animal) represents one point in the plot.


10000
1000
100
1
10
100
Concentration (mg/m³, log scale)
Time (min, log scale)
A.1 Zwart RAT 0%
A.1 Zwart RAT 40%-60%
A.1 Zwart RAT 100%

Study ID: B1.1

Author, year:	***Zwart, 1989. (1h-LC50 protocol)***
Substance:	acetone cyanohydrin
Species, strain, sex:	male and female SPF-reared, Wistar rats (strain code: Bor:WISW)
Number/sex/conc. group:	5/sex/ group
Age and weight:	Age not specified. Mean body weight at beginning was 193g (M) and 149g (F).
Observation period:	14 days.

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes*
Study carried out according to OECD 403 guideline(s)	*Yes, except for a 1-h instead of a 4-h duration*
Stability of test compound in test atmosphere	*No information on whether HCN formation occurred*
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 in breathing zone of animals	*Test atmosphere was generated by bubbling a small flow of air through test material and diluting the saturated flow with fresh air. The mixture was passed to the chamber.*
Number of air changes per hour	*Number of ACH: 56.3 ACH (15 l/min in 16 l chamber)*
Equilibration time (t95)	*t95 is 3.2 min*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Actual concentrations were determined at regular intervals by gas chromatography and automatic integration*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Well performed study, limited to one exposure duration*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	0.70×10^3	N/A	60	0/5	0/5
Rat	0.82×10^3	N/A	60	2/5	4/5
Rat	1.39×10^3	N/A	60	5/5	5/5

Probit function

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

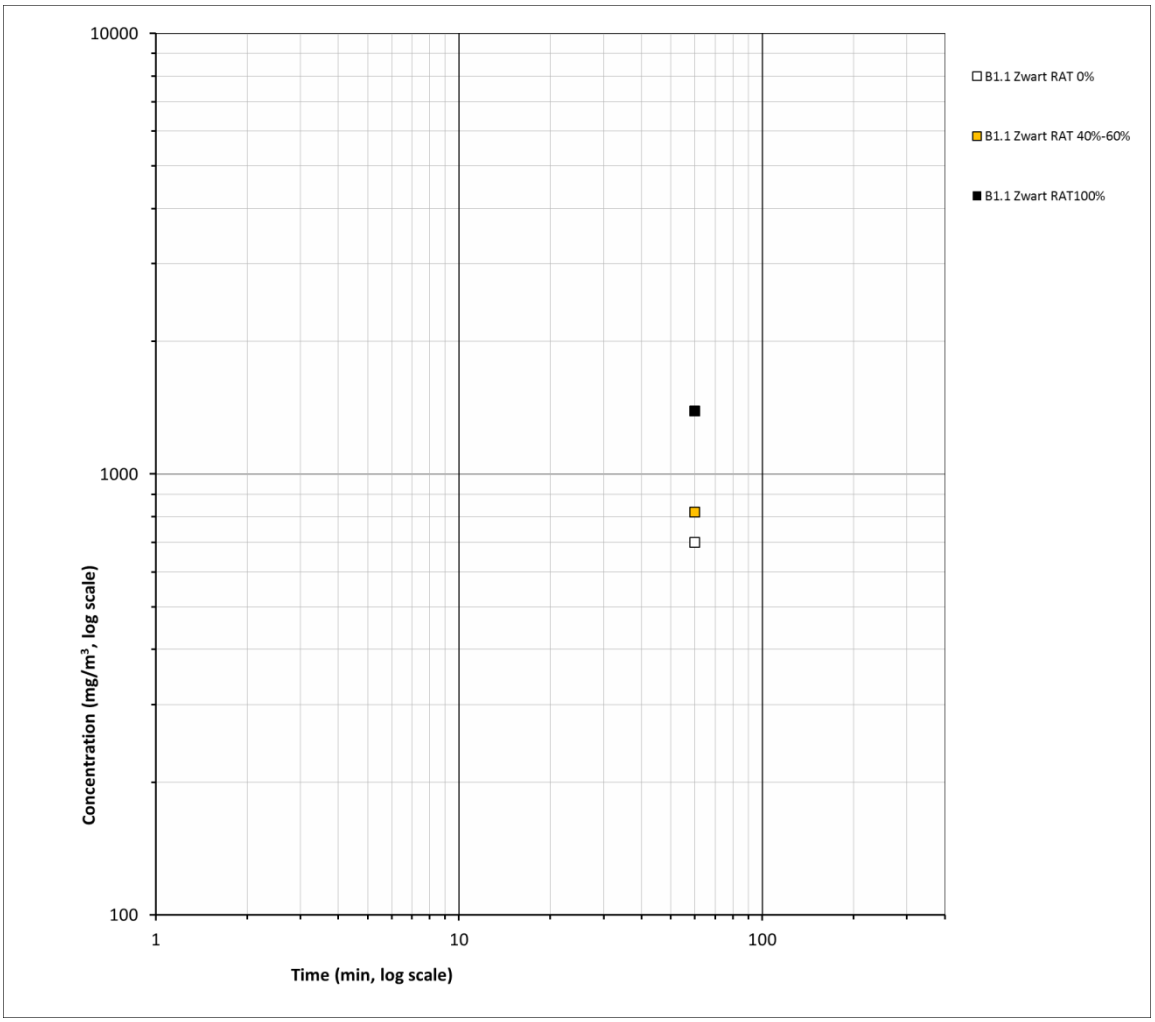
$Pr = a + b\times lnC + d\times S$

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

Probit function	Species	a	b	d	n-value
Sex as variable	Rat	-70.7	11.2	0.82	-
Sexes combined	Rat	-69.0	11.0		-

The LC_{50} values for both sexes did not differ by more than a factor of 2. This does not support the proposition that sex differences exist in the lethal response. For this reason the data from both sexes were pooled and analysed to derive the animal 60-min value.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Male	LC_{50} (mg/m^3) 95%-C.I. Female	LC_{50} (mg/m^3) 95%-C.I. Combined
60	843 (772-984)	783 (721-871)	811 (766-902)



Study ID: C studies

Smyth et al. (1962) exposed groups of 6 albino rats to acetone cyanohydrin administered by passing a 2.5-l/min-air stream through a fritted glass disc immersed in 50 ml acetone cyanohydrin. Concentrations were logarithmically distributed, differing by a factor of two (concentrations were not stated explicitly). After exposure for 4 hours, 2/6 rats were killed at 62.5 ppm (222 mg/m^3) and 6/6 rats were killed at 125 ppm (444 mg/m^3). The maximum time rats could be exposed to a saturated vapor (about 1300 ppm; 4615 mg/m^3) without producing any deaths was 5 minutes. The observation period was 14 days.

Gabor et al. (1962: Rumanian; as cited in AEGL document) exposed albino mice to different acetone cyanohydrin concentrations for 2 hours. Deaths were reported as 0/10 at 140 ppm and 280 ppm (497 and 994 mg/m^3), 8/10 at 420 ppm (1491 mg/m^3), 18/44 at 560 ppm (1988 mg/m^3), 4/10 at 700 ppm (2485 mg/m^3), and 10/10 at 840 ppm (2982 mg/m^3). The authors calculated a 2-h LC_{50} of 574 ppm (2038 mg/m^3). The mouse strain, analytical methods and post-exposure observation period were not reported.

Izmerov et al. (1982) reported an LC_{40} of 185 mg/m^3 (51.8 ppm) for 2h in rats and an LC_{30} of 70 mg/m^3 (19.6 ppm) for 2 h in mice (no details were reported).

Sunderman and Kincaid (1953) exposed rats to saturated vapors of commercially available acetone cyanohydrin. After 1.5 minutes all rats (6/6) died. When the free HCN contained in the acetone cyanohydrin was removed by precipitation with silver nitrate before exposure, the authors found that collapse occurred after an average time of 4 minutes and 50 % mortality after 10 minutes with a time to death of 11 minutes (the exact number of animals not stated).

Appendix 2 Reference list

Chemiekaarten. Ed 33. Den Haag. TNO/SDU uitgevers, 2018.

Gabor, S., C. Raucher, M. Leoca and R. Geleru. 1962. Experimental studies on the toxicity of some chemical substances used in the manufacturing or organic glass (plexiglass) [in Rumanian]. Igiena 11:27-30. (as cited in AEGL report)

Izmerov, N.F., I.V. Sanotsky and K.K. Sidorov. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. Centre of International Projects, GKNT, Moscow. (as cited in AEGL report)

NAC/AEGL. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Final TSD for acetone cyanohydrin. Washington, US EPA, 2009.

RIVM 2017. Interventiewaarden gevaarlijke stoffen. http://www.rivm.nl/rvs/Normen/Rampen_en_incidenten/Interventiewaarden.

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

Smyth, H.F., C.P. Carpenter, C.S. Weil, U.C. Pozzani, and J.A. Striegel. Range finding toxicity data: List VI. American Industrial Hygiene Association Journal. 1962.

Sunderman, F.W., and J.F. Kincaid. 1953. Toxicity studies of acetone cyanohydrin and ethylene cyanohydrin. Arch. Ind. Hyg. Occup. Med. 8(4):371-376.

Zwart (1989) Acute (1-hour) inhalation toxicity of acetone cyanohydrine in rats. TNO report number V89.155.