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

Probit function technical support document

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substance name	CAS number
Acetone cyanohydrin	**75-86-5**

This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA).

This document has been checked for completeness by the Netherlands' National Institute for Public Health and the Environment (RIVM). The contents of this document, including the probit function, have been approved by the scientific expert panel on probit functions on scientific grounds. The status of this document was therefore raised to "interim", pending a decision on its formal implementation.

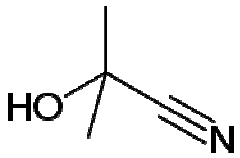
Subsequently the Ministry of Infrastructure and the Environment will perform a second tier evaluation to decide whether the probit function will be formally implemented. The decision on actual implementation will primarily be based on the results of a consequence analysis.

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

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:	81°C (at 101.3 kPa)
Vapour pressure:	0.13 kPa (at 20°C)
Saturated vapor conc:	1300 ppm = 4615 mg/m^3 (at 20°C and 101.3 kPa)
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:	R26/27/28-50/53
	H330-310-300-410



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

Acute effects of acetone cyanohydrin: 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 exposure results in neurological symptoms, loss of consciousness and inhibition of the respiratory system caused by inhibition of cellular respiration, which is especially detrimental in the brain. 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 of acetone cyanohydrin: No information. 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

[1] AEGL (2005) final. The final AEGL report on acetone cyanohydrin applied the HCN values for acetone cyanohydrin, because of rapid and complete decomposition of acetone cyyanohydrin to HCN and acetone.

informative if both health effects as well as the exposure have been documented in sufficient detail.

4. Animal acute toxicity data

Animal lethal toxicity data considering acute exposure to acetone cyanohydrin are described in Appendix 1. A total of 5 studies were identified -with 5 datasets for 2 species- with data on lethality following acute inhalation exposure. One dataset was assigned status A for deriving the human probit function, no datasets were assigned status B and 4 were assessed to be unfit (status C) for human probit function derivation. In addition, lethality after a single exposure in a repeated toxicity study was reported.

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

1. AEGL final TSD (2005), 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, RTECS, IRIS and ToxNet with the following search terms:
   - Acetone cyanohydrin and synonyms
   - CAS number
   - lethal*
   - mortal*
   - fatal*
   - LC_{50}, LC
   - probit
3. Unpublished data were sought through networks of toxicological scientists.
4. In this specific case the TSD on probit function for hydrogen cyanide was also taken into consideration.

Sensory irritation

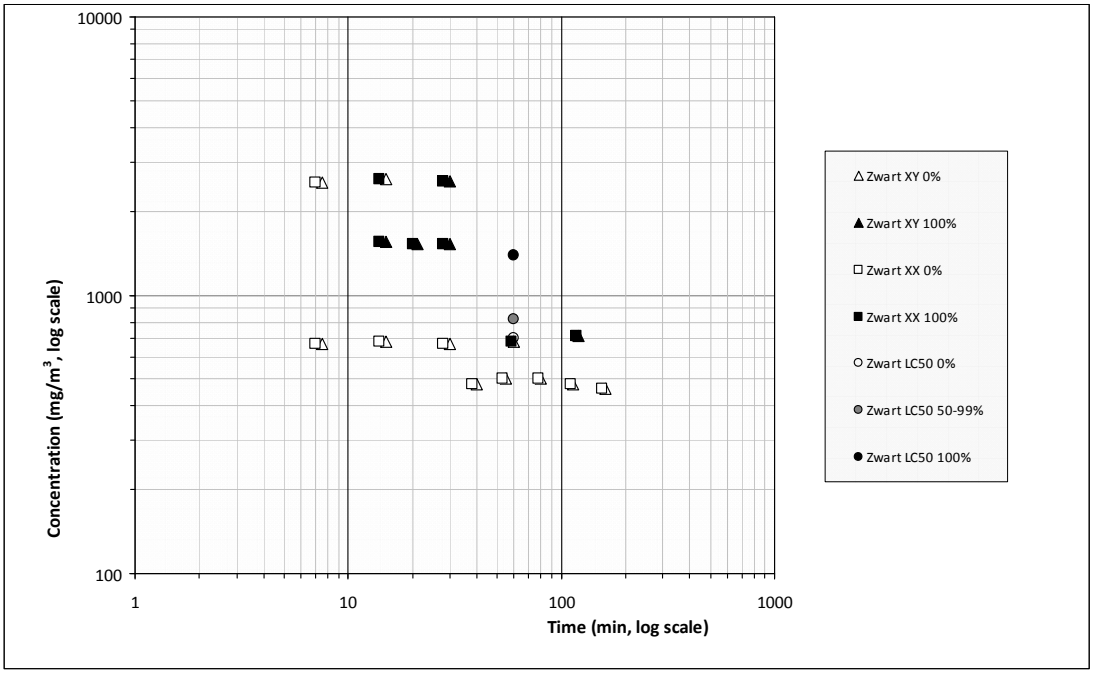
No studies on sensory irritation were found.

5. Probit functions

Probit functions have been calculated and reported in Appendix 1 for the reported study with A status. The results of the calculations are presented in the table below.

Study ID	*Species*	*Probit (C in mg/m^3, t in min)*	*LC_{50}, 30 minutes (mg/m^3) 95% C.I.*
A.1	*Rat*	*$-27.5 + 3.68 \times lnC + 1.91 \times lnt$*	1168 (930 – 1397)

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



6. Evaluation

To derive the human probit function the results from Zwart (1989, study ID A.1) have been used to derive a point of departure. The reason was that the study was well performed and reported. The study included 16 concentration-time combinations, where the concentrations were determined analytically.

Although it is believed that health effects are predominantly caused by free cyanide formation, the use of the Zwart (1989) study was preferred over using HCN data as point of departure for probit function derivation for acetone cyanohydrin, in contrast to the derivation of the AEGL values. The reason was that there is an A study available in which 16 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), which should cover the rapid hydrolysis process of acetone cyanohydrin to form HCN. Although as a general rule the <10-min. data are excluded[2], it is decided to include the 7.5-min. data for the derivation of the animal probit function for the following reasons:

- Animals were exposed to acetone cyanohydrin once the concentrations in the atmosphere were stabilized. The concentrations were regularly measured by gas chromatography and automatic integration.
- There are no signs that acetone cyanohydrin is a sensory irritant. Gross pathology findings in the Zwart study seem to indicate that the substance predominantly leads to pulmonary irritation. Hence, there is no indication that exposed animals did reduce their respiration which would have influenced the results.

[2] As a general rule, data from exposure durations less than 10 minutes are excluded because of possible uncertainties in chamber conditions and the ability of animals to temporarily reduce their minute volume.

In addition, in Zwart et al. (1992) was noted that the lethality data of acetone cyanohydrin shows a certain curvature over time and thus could be described with a non-linear probit function. The analysis with the non-linear probit function showed a fit equally well as with a linear probit function. It seems that a lethality threshold for acetone cyanohydrin exists independent of time and suggest a 'steep' curve at short durations and a 'shallow' curve at longer durations.
Since in this framework the linear probit function is desired, the probit function incl. the 7.5-min. data was preferred over the probit function excl. the 7.5-min. data, because of the fact that the former describes short exposure durations the best ('steeper' curve with n-value of 1.93) and is conservative for longer exposure durations and moreover provides a better fit of the data (see appendix 1).

For these reasons, the results by Zwart including those after 7.5-min. exposure are considered sufficiently reliable.

It should be noted that if the HCN 30 min. LC_{50} value of 96 ppm (108 mg/m^3) was used as point of departure a 30 min. LC_{50} value of 341 mg/m^3 acetone cyanohydrin would have been derived, based on the fact that one mole of acetone cyanohydrin can produce one mole of cyanide. This value is approximately 3.5-fold lower than the 30 min LC_{50} of 1168 mg/m^3 calculated from the Zwart study (1989).

As point of departure for deriving the human probit function the 30-min LC_{50} value of 1168 mg/m^3 for the rat from the A.1 study was taken. The human equivalent LC_{50} was calculated by applying the following assessment factors:

Assessment factor for:	**Factor**	**Rationale**
Animal to human extrapolation:	3	
RD_{50}	1	No RD_{50} study was found.
Nominal concentration	1	Analytically determined concentrations were reported
Adequacy of database:	1	Well performed A study

The estimated human equivalent 30-minute LC_{50} value is 1168 / 3 = **389 mg/m^3**.

The experimentally determined n-value was **1.93** (Zwart, 1989). 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 30 min LC_{50} and using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1.04 \times \ln (389^{1.93} \times 30)$ resulting in the a-value of **-10.45**.

$$Pr = -10.5 + 1.04 \times \ln (C^{1.93} \times t)$$ with C in mg/m³ and t in min.

The derived human probit function has an acceptable scientific basis. The probit function is based on one study in the rat with A quality, including 16 concentration-time combinations.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 85 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 58 mg/m^3.

Estimated level	**30 min (mg/m^3)**	**60 min (mg/m^3)**
1% lethality, this probit	121	85
0.1% lethality, this probit	83	58
AEGL-3 (2005, final)	74	53
ERPG-3	-	-
LBW (2007)		100

Comparing to equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are approximately similar even though the AEGL-3 values are based on HCN.

Appendix 1 Animal experimental research

Study ID: A.1

Author, year: Zwart, 1989

Substance: acetone cyanohydrin

Species, strain, sex: male and female SPF-reared, Wistar rats (strain code: Bor:WISW)

Number/sex/concentration group: 1/sex/concentration group (16 groups in C × t protocol) and 5/sex/concentration group (3 groups, 1h-LC_{50} protocol)

Age and weight: age not specified. Mean body weights at beginning were 274 g (M) and 185 g (F) in the C × T protocol and 193 g (M) and 149 g (F) in the 1h-LC_{50} study.

Observation period: 14 days

Evaluation of study quality

Criteria	*Comment*
Study carried out according to GLP	*Yes*
Study carried out according to guideline(s)	*Both protocols are in accordance with OECD 403*
Stability of test compound in test atmosphere	*No information on whether HCN formation occured.*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Nose-only in C × t protocol* *Whole body in 1h-LC_{50} protocol*
Pressure distribution.	*No information*
Homogeneity of test atmosphere at 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 (nose only) or chamber (whole body, 1h-LC_{50} protocol). Animals were exposed once concentrations were stabilized.*
Number of air changes per hour (ACH)	*Flow in nose-only exposure: 6 l/animal/min.* *Number of ACH (whole body exposure): 56.3 ACH (15 l/min in 16 l chamber.*
Actual concentration measurement	*Actual concentrations were determined at regular intervals by gas chromatography and automatic integration. Numbers of determinations are provided in the table with results.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*
Assessment of Reliability	***A*** *Well conducted study using the C × t protocol.*

Results

C x t protocol

Species	Concentration (mg/m^3) (No. of determinations)	Exposure duration (min)	Lethality	
			Male	Female
Rat	2540 (2)	7.5	0/1	0/1
	2610 (4)	15	0/1	1/1
	2560 (6)	30	3/3	3/3
	670 (3)	7.5	0/1	0/1
	680 (4)	15	0/1	0/1
	670 (6)	30	0/1	0/1
	680 (9)	60	0/1	1/1
	710 (12)	120	1/1	1/1
	480 (3)	40	0/1	0/1
	500 (5)	55	0/1	0/1
	500 (7)	80	0/1	0/1
	480 (10)	113	0/1	0/1
	460 (13)	160	0/1	0/1
	1550 (2)	15	1/1	1/1
	1520 (4)	21	1/1	1/1
	1530 (5)	30	3/3	3/3

The author (Zwart, 1989) derived the following probit functions based on the C × t protocol data:

Probit function	*Species*	*a*	*b*	*c*	*d*	*n-value*
Sex as covariate	*Rat*	*-3.22*	*4.25*	*2.13*	*0.70*	*2.0 (1.4 - 2.6)*
Sexes combined	*Rat*	*-2.35*	*3.94*	*1.99*		*Not given by author. (but should be 1.98).*

The derived 1h-LC_{50} values were 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.

1h-LC_{50} protocol

Species	Concentration (mg/m^3) (No. of determinations)	Exposure duration (min)	Lethality	
			Male	Female
Rat	700 (11)	60	0/5	0/5
	820 (12)	60	2/5	4/5
	1390 (12)	60	5/5	5/5

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, December 2006) as

$Pr = a + b \times \ln C + c \times \ln t + d \times S$

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

The data from the C × t protocol were analysed with DoseResp software. Since different exposure methods (nose-only versus whole body) were applied the data were not pooled. The data were analysed, including and excluding data from exposures durations <10min. The 7.5-min data were included because the exposures were analysed regularly.

C x t Protocol

Probit function	*Species*	*a*	*b*	*c*	*d*	*n-value*
Sex as covariate	*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)*
Data excl. 7.5-min. Sexes combined	*Rat*	*-22.4*	*3.33*	*1.25*		*2.67 (-0.52 – 5.86)*

The LC_{50} values for both sexes did not differ by more than a factor 2. This does not support that sex differences exist in the lethal response. For this reason the data from both sexes were pooled and analyzed to derive the animal probit function. Below results are given for the analyses with and without the 7.5-min. data.

Results incl. 7.5-min. data.

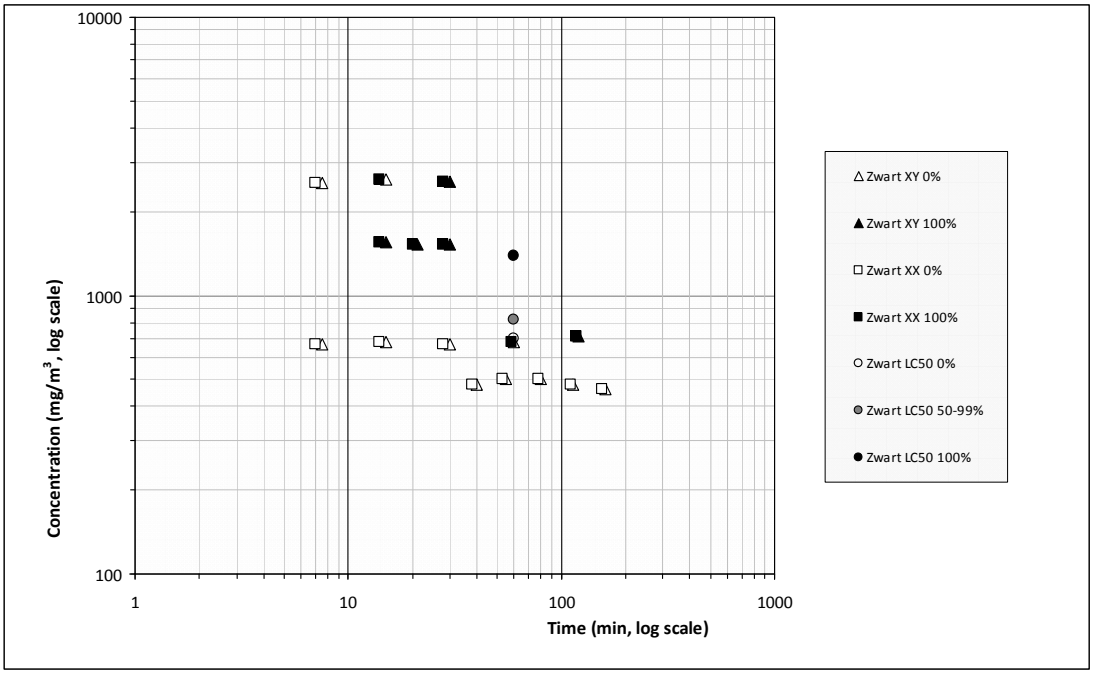
Duration (minutes)	*LC_{50} (mg/m^3) 95%-C.I.* ***Male***	*LC_{50} (mg/m^3) 95%-C.I.* ***Female***	*LC_{50} (mg/m^3) 95%-C.I.* ***Combined***
10	2238 (1451 – 3081)	1892 (1129 – 2603)	2066 (1333 – 2736)

30	1269 (970 – 1648)	1073 (771 – 1362)	1168 (930 – 1397)
60	887 (687 – 1216)	750 (561 – 979)	815 (664 – 1020)

Results excl. 7.5-min. data

Duration (minutes)	*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	1784 (39.7 – 3910)	1380 (16.4 – 3307)	1587 (C.I. could not be calculated)
30	1189 (282 – 2569)	920 (135 – 1873)	1051 (C.I. could not be calculated)
60	920 (439 – 4349)	712 (297 – 2248)	811 (C.I. could not be calculated)

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



Study ID: C studies

References discussed in AEGL document:

Smyth et al. (1962; as cited in AEGL document) exposed groups of 6 albino rats to acetone cyanohydrin that was 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 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 an 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; as cited in AEGL document) reported an LC_{40} of 185 mg/m³ (51.8 ppm) for 2 hours in rats (no details were reported).

Sunderman and Kincaid (1953; as cited in AEGL document) exposed rats to saturated vapors of commercially available acetone cyanohydrin. After 1.5 minutes all rats (6/6) died.

Monsanto (1986a; as cited in AEGL document) exposed groups of 10 female and 10 male Sprague-Dawley rats to acetone cyanohydrin at 0, 10, 30 or 60 ppm (0, 36, 107, 213 mg/m^3) for 6 hours/day, 5 days/week for 20 exposure days (28 days in total). Concentrations in the exposure chamber were calculated by dividing the net amount of acetone cyanohydrin delivered to the chamber per unit time by the airflow per unit time and, in addition, measured by an infrared analyzer (using the C-N triple bond frequency, which detects both acetone cyanohydrin and hydrogen cyanide) four times daily. In the highest exposure group respiratory distress and tremors or convulsions or both, foaming at the mouth, and prostration were observed in 4 males, of which 3 animals died, following the first exposure. No deaths occurred in the lower exposure groups. In three other studies conducted under similar protocols no deaths were observed at 60 ppm for 6 hours/day (Monsanto, 1982b; 1982c; 1986b, all as cited in AEGL document)

Appendix 2 Reference list

NAC/AEGL. Acute Exposure Guideline Levels for Selected Airborne Chemicals. TSD for Acetone cyanohydrin. Washington, US EPA, 2005.

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

Also reported in:
Zwart A. Arts JHE, ten Berge WF and Appelman LM. (1992) Alternative acute inhalation toxicity testing by determination of the concentration-time-mortality relationship: experimental comparison with standard LC_{50} testing. Reg. Tox. Pharm. 15, 278-290.

LBW (2007) Interventie waarden gevaarlijke stoffen, 2007.