National Institute for Public Health
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Probit function technical support document

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Author: L. Geraets (RIVM)
E-mail response to: safeti-nl@rivm.nl

substance name	CAS number
Acetonitrile	**75-05-8**

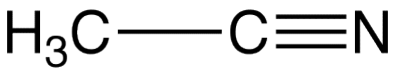
This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

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 Acetonitrile



1. Substance identification

CAS-number:	75-05-8
IUPAC name:	acetonitrile
Synonyms:	cyanomethane, methyl cyanide, ethanenitrile
Molecular formula:	C_2H_3N
Molecular weight:	41.1 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	81°C (at 101.3 kPa)
Vapour pressure:	9.7 kPa (at 20°C)
Saturated vapor conc:	97000 ppm = 165870 mg/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.585 ppm (at 20°C and 101.3 kPa)
	1 ppm = 1.710 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H302-H312-H319-H332

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

Acute effects: Toxic effects of acetonitrile are the result of metabolic cyanide formation and consistent with those produced after cyanide poisoning. The main target organs and tissues for inhalation exposure to acetonitrile are tissues with high oxygen demand such as the brain and heart. Cyanide interrupts cellular respiration by blocking the terminal step of electron transfer from cytochrome c oxidase to oxygen. This leads to increased oxygen tension and decreased unloading of oxyhaemoglobin. As oxidative metabolism slows down, the lack of energy results in central respiratory arrest and death.
The health endpoints are irritation to the eyes, skin and respiratory tract and depression of the CNS. Dyspnea, cyanosis, dizziness, hypoactivity, convulsions, coma, and pulmonary damage are symptoms after high exposure.
Long-term effects: Chronic exposure produces the same effects as acute exposure to cyanide.

3. Human toxicity data

No informative reports on human toxicity following acute inhalation exposure were identified in which details about both health effects and the exposure have been documented in sufficient detail.
Pozzani et al. (1959) studied three male volunteers (ages 31-47) who inhaled acetonitrile at 40 ppm (68.4 mg/m^3) for 4 h. The two older subjects reported no subjective symptoms during or after the inhalation period. The youngest subject reported no adverse subjective response during exposure, but experienced slight chest tightness that evening. The following morning, he reported a cooling sensation in the lungs, which persisted for 24 h and was described as being similar to that experienced when menthol was inhaled. The two older subjects were exposed 1 week later to acetonitrile at 80 ppm (136.8 mg/m^3) for 4 h; no symptoms were reported. Nine days after the 80 ppm (136.8 mg/m^3) exposure, the same two subjects were exposed at 160 ppm (273.6 mg/m^3) for 4 h. One subject reported a slight transitory flushing of the face 2 h after inhalation and slight bronchial tightness 5 h later, which resolved overnight.

[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 acetonitrile, 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. A total of 8 studies were identified -with 11 datasets for 4 species- with data on lethality following acute inhalation exposure. None of the datasets were assigned status A for deriving the human probit function, one dataset was assigned status B and 10 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

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

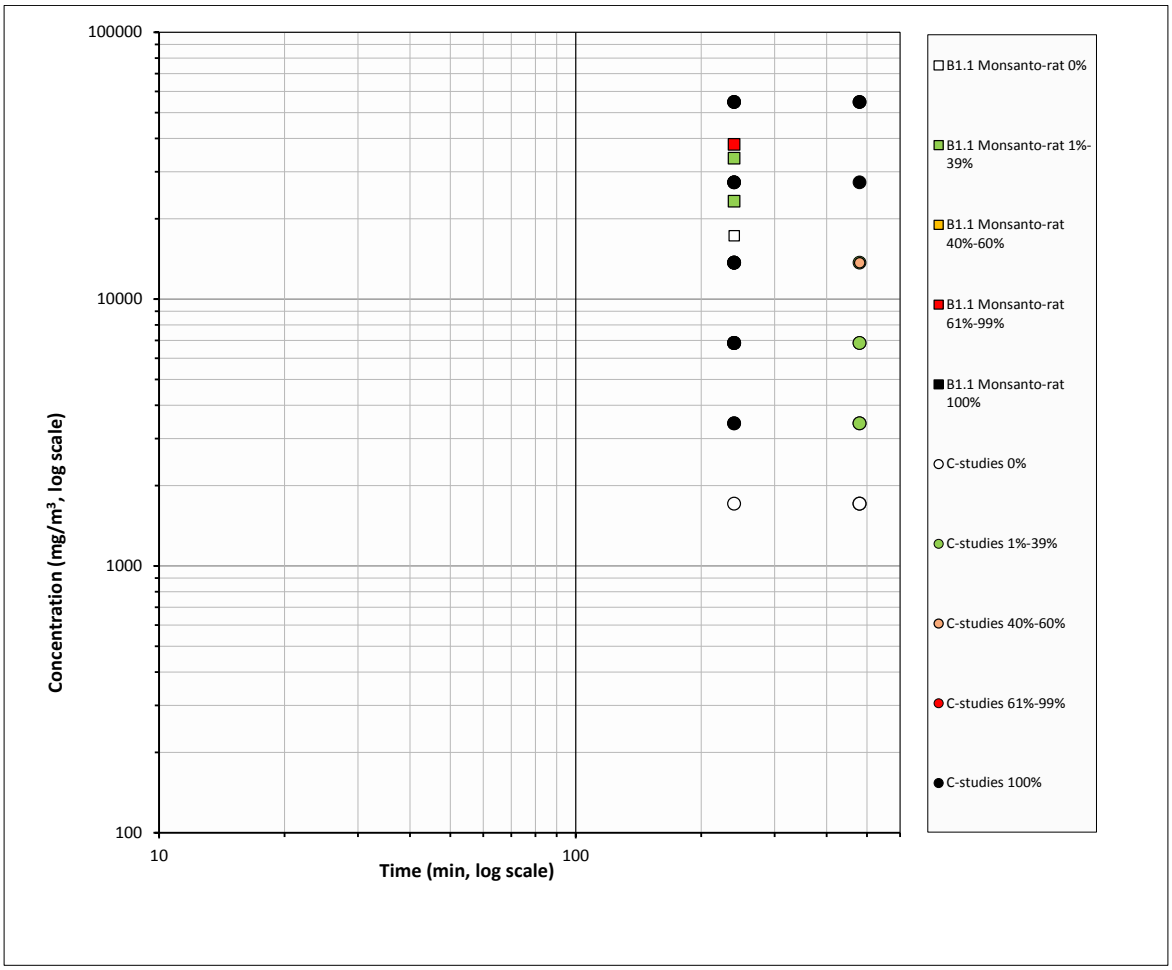


Figure 1 *All available acute lethality data for acetonitrile.*

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

It was possible to derive a probit function for acetonitrile based on the only available study with B1 quality. However, this B1 study did not enable to produce a concentration-time-lethality relationship.

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 1.

Table 1 *Data selected for initial analysis of the animal probit function of acetonitrile.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} at tested exposure duration (mg/m^3) 95% C.I.	n-value 95% C.I.
B1.1	rat	*240-min LC_{50}*	34080 (29590-41580)	N/A

The data of study B1.1 with rats are presented graphically below.

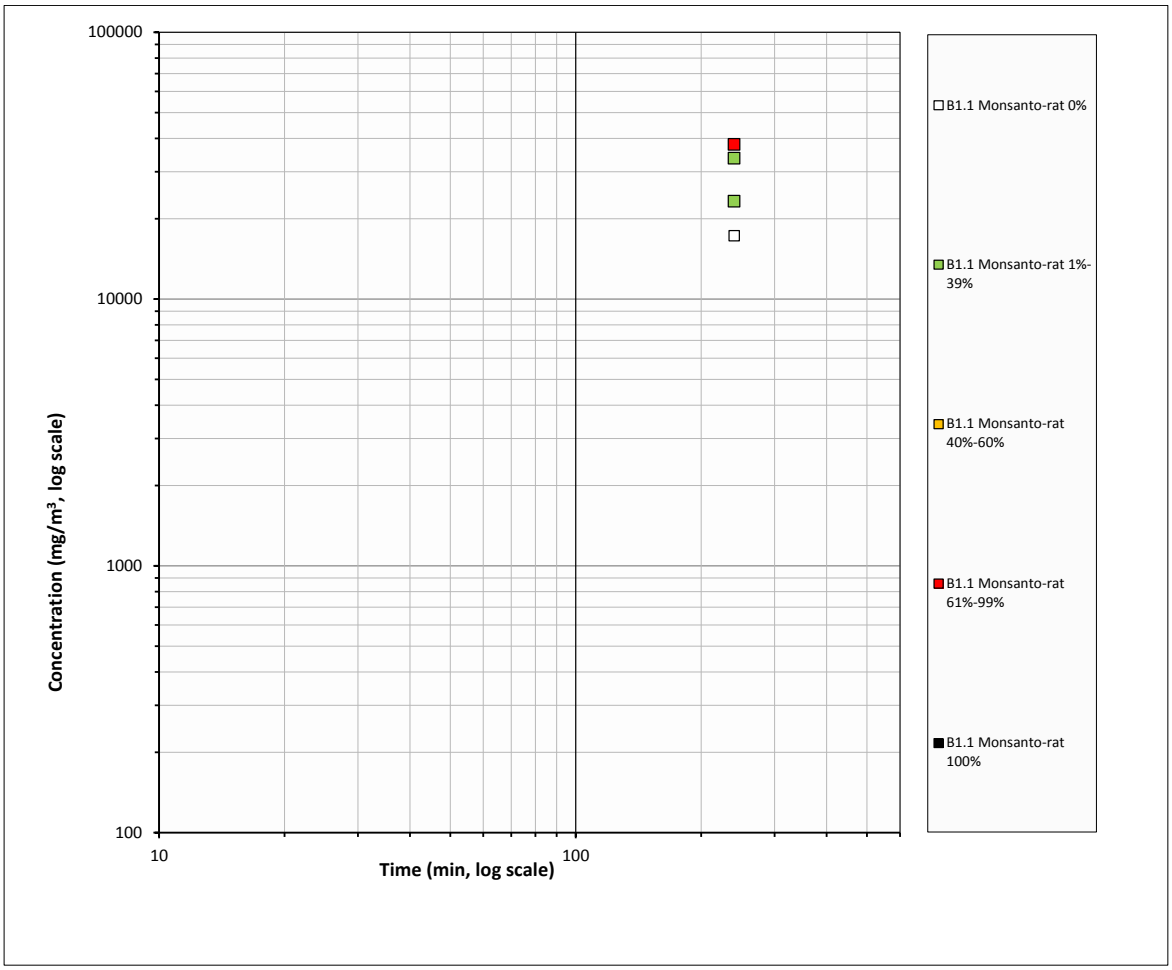


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of acetonitrile.*

Based on criteria outlined in the guideline the data from study B1.1 were selected for the final dataset for the derivation of the animal probit function. The data that were selected for final analysis of the animal probit function are presented in Table 2 and Figure 3.

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

Table 2 *Data selected for the derivation of the animal probit function of acetonitrile (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.	n-value 95% C.I.
B1.1	rat	*240-min LC_{50}*	34080 (29590-41580)	N/A

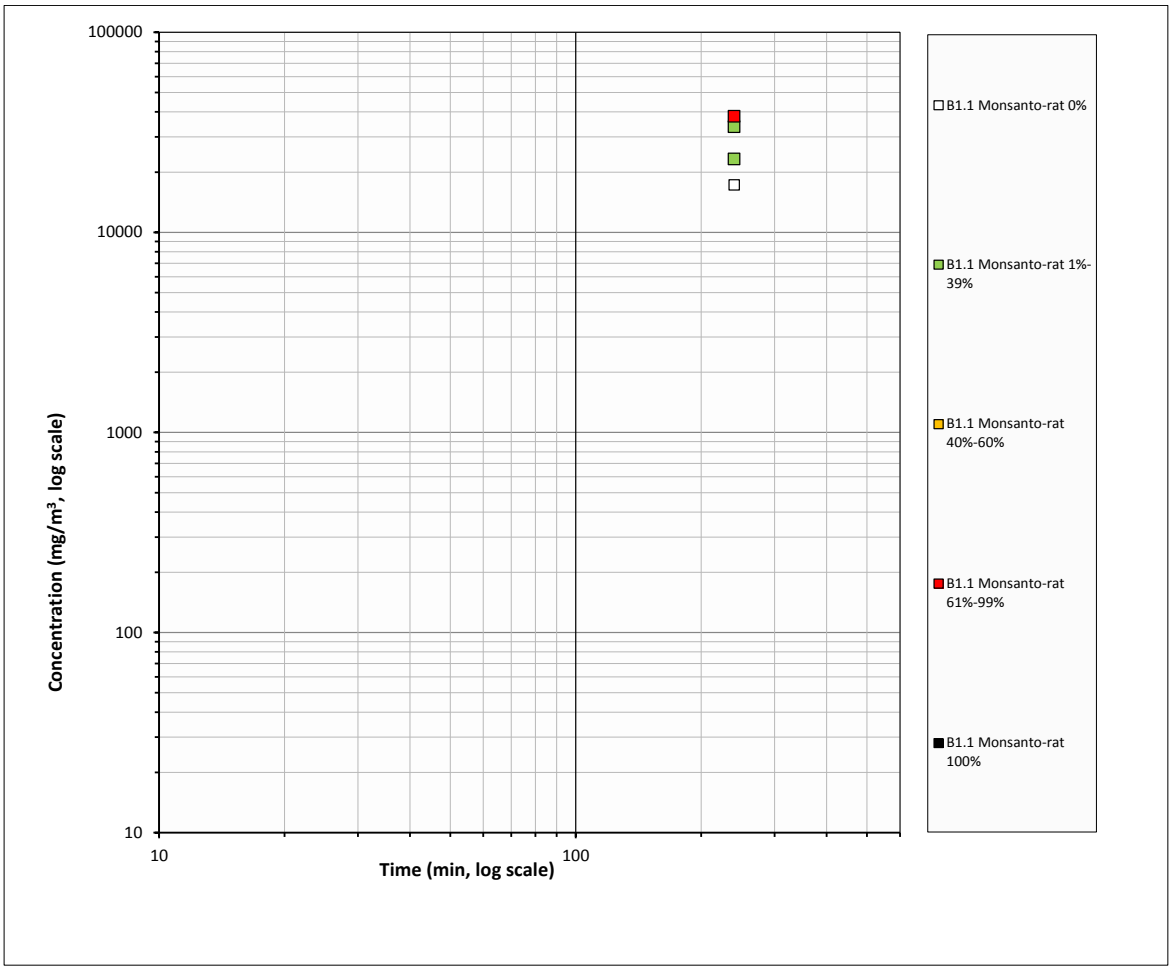


Figure 3 *Final data selected for derivation of the animal probit function of acetonitrile (identical to figure 2).*

6. Derivation of the human probit function

To derive the human probit function the results from study B1.1 (Monsanto 1986) have been used to derive a point of departure as outlined above.

The Point of Departure for the human probit function is a 240-minute animal LC_{50} value of 34080 mg/m^3 and a default n-value of 2.

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. It is noted that the data on rat, guinea pig, rabbit and dog indicate that rat seems not to be the most sensitive test species.
Nominal concentration	1	B1-study with analytically determined concentrations

Adequacy of database:	2	Only one B1-dataset was found. The study was performed using only one exposure duration well outside the exposure duration target range of 30-60 min. This creates a relative large uncertainty because of extrapolation over a large range of exposure duration.

The estimated human equivalent 240-minute LC_{50} value is 34080 / 6 = **5680 mg/m^3**.

No reliable experimentally determined n-value was available, so the default n-value of **2** was used. Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1**.

The human probit function is then calculated on the human equivalent 240 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1 \times \ln(5680^2 \times 240)$ resulting in the a-value of **-17.8**.

$Pr = -17.8 + 1 \times \ln(C^2 \times t)$ 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 B1 quality, including 40 animals, an exposure duration of 240 min and response rates between 0 and 100%.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 2460 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 3597 mg/m^3.

Table 4 *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	3479	2460
1% lethality, this probit	5087	3579
AEGL-3[2] (2014, final)	410	257
ERPG-3[3]	-	-
LBW (2017)	1800	1100

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

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

Appendix 1 Animal experimental research

Study ID: B1.1

Author, year: ***Monsanto 1986***
Substance: Acetonitrile
Species, strain, sex: rat, Sprague-Dawley, male
Number/sex/conc. group: 10
Age and weight: age not specified/218-280 g
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*No GLP statement provided*
Study carried out according to OECD 403 guideline(s)	*No statement of compliance with OECD guideline 403 provided*
Stability of test compound in test atmosphere	*No information*
Use of vehicle (other than air)	*N/A*
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	*Metered air was bubbled through a heated (80°C) bubbler of the acetonitrile. This stream was diluted with air at 15 L/min prior to entering the exposure chamber.*
Number of air changes per hour	*20 L inhalation chamber; at least 45 air changes per hour*
Equilibration time (t95)	*maximally 4 min*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*The acetonitrile exposure concentration was monitored continuously over the 4-h period using a gas analyser, and the concentration of acetonitrile vapour was determined with a calibration curve.*
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
				Dead/tested
Rat	17271	N/A	240	0/10
Rat	23256	N/A	240	1/10

Rat	33687	N/A	240	3/10
Rat	37962	N/A	240	8/10

Probit function

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

$Pr = a + b \times \ln C$

with C for concentration in mg/m^3.

Probit function	Species	a	b	n-value
	Rat	-37.5	4.07	N/A

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I.	LC_{50} (mg/m^3) 95%-C.I. (Monsanto, 1986)
240	34080 (29590-41580)	34115 (CI not specified by study author)

No C × t probit function could be calculated from these data alone.

Study ID: C.1

Author, year: ***Pozzani et al., 1959***
Substance: acetonitrile
Species, strain, sex: rat, Nelson albino (Carworth Farms), male and female
Number/sex/conc. group: 12/sex/group
Age and weight: not specified
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	*No information*
Use of vehicle (other than air)	*N/A*
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	*The vapour is generated by delivering the fluid via a 10 ml syringe into an evaporator through which metered air is forced. The rats are exposed to the vapour-air mixture which flows through a 9-liter desiccator fitted with inlet and outlet ports built in a standard taper stopper. Vapours enter beneath the desiccators grid upon which the rats are supported.*
Number of air changes per hour	*Air flow was not higher than 20 L/min. Size of exposure chamber was not specified.*
Equilibration time (t95)	*Insufficient information to calculate t95*
Start of exposure relative to equilibration	*Not specified*
Actual concentration measurement	*No analytical concentrations determined. The reported concentrations appear to be target concentrations.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***C*** *No actual concentrations measured (only target concentrations provided) and some study details are lacking.*

* the authors refer to Carpenter et al. (1949) for details on the methodology

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Target	Adjusted		Male	Female
				Dead/tested	
Rat	6840	N/A	240	0/12	0/12
Rat	13680	N/A	240	3/12	0/12
Rat	27360	N/A	240	3/12	6/12
Rat	54720	N/A	240	12/12	12/12
Rat	1710	N/A	480	0/12	0/12
Rat	3420	N/A	480	0/12	1/12
Rat	6840	N/A	480	1/12	1/12
Rat	13680	N/A	480	6/12	1/12
Rat	27360	N/A	480	12/12	9/12
Rat	54720	N/A	480	12/12	12/12

An additional group of 6 animals/group (sex not specified; a different rat strain, i.e. Carworth Farms Wistar, was used) was tested preliminarily. A “concentrated vapour”, generated by bubbling dried air at 2.5 L/min through a fritted glass disc immersed in 50 mL of acetonitrile resulted in mortality ratios of three of six and zero of six Carworth Farms Wistar rats in 30 and 15 minutes, respectively. The authors stated that saturated vapour of the liquid would approximate 96,000 ppm (*164,160 mg/m^3*) at room temperature, but the volatility of acetonitrile caused the temperature of the liquid phase to fall considerably during aeration and resulted in mean measured concentrations approximating 53,000 ppm (*90,630 mg/m^3*). The data of this preliminary test was not analysed in combination with the data as presented in the table above, as this considered a different rat strain. Given the mentioned shortcomings and the fact that the preliminary experiment included only one exposure concentration at two exposure durations, this additional dataset should also be given the C-status.

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 (0 = male, 1 = female).

Analysis of 240 min data

Probit function	Species	a	b	d	n-value
Sex as variable	Rat	-18.0	2.26	-0.107	N/A
Sexes combined	Rat	-17.9	2.25		N/A

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
240	26130 (13520-51440)	27390 (14200-54120)	26720 (22420-31940)

Pozzani et al reported the following LC_{50} values:
Male: 27360 mg/m^3 (21290-35161)
Female: 27360 mg/m^3 (22293-33578)

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.

Analysis of 480 min data

Probit function	Species	a	b	d	n-value
Sex as variable	Rat	-10.9	1.68	-0.580	N/A
Sexes combined	Rat	-10.8	1.64		N/A

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
480	12830 (5453-30360)	18130 (7774-43590)	15380 (10900-21830)

Pozzani et al reported the following LC_{50} values
Male: 12912 mg/m^3 (10217-16317)
Female: 21264 mg/m^3 (18872-23959)

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.

No C × t probit function could be calculated from these data alone.

Study ID: C.2

Author, year: ***Pozzani et al., 1959***
Substance: acetonitrile
Species, strain, sex: guinea pig, strain not specified, male and female
Number/sex/conc. group: 6/group (male and female combined)
Age and weight: not specified
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	*No information*
Use of vehicle (other than air)	*N/A*
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	*The vapour is generated by delivering the fluid via a 10 ml syringe into an evaporator through which metered air is forced. The rats are exposed to the vapour-air mixture which flows through a 9-liter desiccator fitted with inlet and outlet ports built in a standard taper stopper. Vapours enter beneath the desiccators grid upon which the rats are supported.*
Number of air changes per hour	*Air flow was not higher than 20 L/min. Size of exposure chamber was not specified.*
Equilibration time (t95)	*Insufficient information to calculate t95*
Start of exposure relative to equilibration	*Not specified*
Actual concentration measurement	*No analytical concentrations determined. The reported concentrations appear to be target concentrations.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***C*** *No actual concentrations measured (only target concentrations provided) and some study details are lacking. Limited to one exposure duration. Only zero or 100% mortality.*

* the authors refer to Carpenter et al. (1949) for details on the methodology

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Target	Adjusted		
				Dead/tested
Guinea pig	6840	N/A	240	0/6
Guinea pig	13680	N/A	240	6/6
Guinea pig	27360	N/A	240	6/6

Probit function

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

$Pr = a + b \times \ln C$

with C for concentration in mg/m^3.

Probit function	Species	a	b	n-value
	Guinea pig	-93.7	10.8	N/A

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I.	LC_{50} (mg/m^3) 95%-C.I. (reported by Pozzani et al.)
240	9738 (no CI estimated due to large variances)	9670

No C × t probit function could be calculated from these data alone.

Study ID: C.3

Author, year: ***Pozzani et al., 1959***
Substance: acetonitrile
Species, strain, sex: rabbit, strain not specified, male
Number/sex/conc. group: 4/sex/group
Age and weight: not specified
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	*No information*
Use of vehicle (other than air)	*N/A*
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	*The vapour is generated by delivering the fluid via a 10 ml syringe into an evaporator through which metered air is forced. The rats are exposed to the vapour-air mixture which flows through a 9-liter desiccator fitted with inlet and outlet ports built in a standard taper stopper. Vapours enter beneath the desiccators grid upon which the rats are supported.*
Number of air changes per hour	*Air flow was not higher than 20 L/min. Size of exposure chamber was not specified.*
Equilibration time (t95)	*Insufficient information to calculate t95*
Start of exposure relative to equilibration	*Not specified*
Actual concentration measurement	*No analytical concentrations determined. The reported concentrations appear to be target concentrations.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***C*** *No actual concentrations measured (only target concentrations provided) and some study details are lacking. Limited to one exposure duration. Only zero or 100% mortality.*

* the authors refer to Carpenter et al. (1949) for details on the methodology

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Target	Adjusted		Male
				Dead/tested
Rabbit	1710	N/A	240	0/4
Rabbit	3420	N/A	240	0/4
Rabbit	6840	N/A	240	4/4

Probit function

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

$Pr = a + b \times \ln C$

with C for concentration in mg/m^3.

Probit function	Species	a	b	n-value
	rabbit	-78.8	10.8	N/A

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I.	LC_{50} (mg/m^3) 95%-C.I. (reported by Pozzani et al.)
240	2434 (no CI estimated due to large variances)	4836

No C × t probit function could be calculated from these data alone.

Study ID: C.4

Author, year: ***Pozzani et al., 1959***
Substance: acetonitrile
Species, strain, sex: dog, strain not specified, male
Number/sex/conc. group: 1-3/sex/group
Age and weight: not specified
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	*No information*
Use of vehicle (other than air)	*N/A*
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	*The vapour is generated by delivering the fluid via a 10 ml syringe into an evaporator through which metered air is forced. The rats are exposed to the vapour-air mixture which flows through a 9-liter desiccator fitted with inlet and outlet ports built in a standard taper stopper. Vapours enter beneath the desiccators grid upon which the rats are supported.*
Number of air changes per hour	*Air flow was not higher than 20 L/min. Size of exposure chamber was not specified.*
Equilibration time (t95)	*Insufficient information to calculate t95*
Start of exposure relative to equilibration	*Not specified*
Actual concentration measurement	*No analytical concentrations determined. The reported concentrations appear to be target concentrations.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***C*** *No actual concentrations measured (only target concentrations provided) and some study details are lacking. Limited to one exposure duration. Only zero or 100% mortality*

* the authors refer to Carpenter et al. (1949) for details on the methodology

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Target	Adjusted		Male
				Dead/tested
Dog	3420	N/A	240	0/2
Dog	13680	N/A	240	0/1
Dog	27360	N/A	240	3/3
Dog	54720	N/A	240	1/1

Probit function

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

$Pr = a + b \times \ln C$

with C for concentration in mg/m^3.

Probit function	Species	a	b	n-value
	Dog	-93.7	10.0	N/A

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Male
240	19060 (no CI estimated due to large variances)

No C × t probit function could be calculated from these data alone.

Study ID: other C studies

In a lethality study conducted at Haskell Laboratory (E.I. du Pont de Nemours and Company (1981)), groups of young adult male ChR-CD rats (248-268 g) were exposed to acetonitrile (tested concentrations not specified) for 4 hours. The test chamber atmosphere was analyzed at least every half hour by gas chromatography. Test animals were observed for 14 days. Irregular respiration, hyperemia, incoordination, and face pawing were observed at sublethal concentrations during exposure, and irregular respiration, hyperemia followed by pale ears, face pawing, incoordination, and unresponsiveness were observed at lethal concentrations during exposure. Moderate to severe weight loss for 1-3 days, followed by normal weight gain, was observed after the exposure period at sublethal concentrations. Severe weight loss for 1-3 days, followed by normal weight gain, was observed after the exposure period at lethal concentrations. Deaths occurred from 3 h during exposure through 24-h post-exposure. An LC_{50} of 17,100 ppm (CI: 14,600-20,000 ppm) (29241 mg/m^3 (CI: 24966-34200 mg/m^3)) was calculated. No other experimental details were reported and the individual animal lethality data were not presented.

Haguenoer et al. (1975) exposed three rats to acetonitrile at 25,000 ppm (42750 mg/m^3); all rats died within 30 min after the start of exposure after exhibiting difficult breathing and cyanosis.

Groups of five male and five female Crl:CD-1 (ICR) BR mice were exposed to acetonitrile (>99.9%) vapor for 4 h via whole-body exposure methods (MPI 1998; as cited in AEGL 2014). Mean analytic concentrations determined by infrared spectrometer analysis were 3039, 5000, 4218, and 3568 ppm (Groups 1-4, respectively).
Combined sex mortalities were 20, 80, 90, and 50%, respectively. All mortalities occurred on the day of exposure, except for a single male that died in the low exposure group on post-exposure day 1. Clinical signs observed during the exposure and up to 4-h post-exposure included death, decreased activity, abnormal gait, loss of righting reflex, slow respiration, labored breathing, rapid respiration, gasping, cold-to-the-touch splayed limbs, leaning to the right, and yellow body surface staining. Surviving animals from Groups 2-4 were judged normal by study day 2. Clinical signs observed during the 14-day observation period for animals exposed at 3,039 ppm included death, decreased activity, and decreased defecation; survivors in this group were judged normal by study day 5. At necropsy, no test article-related macroscopic findings were observed in male or female mice. All tissues were considered to be within normal limits. A 4 h LC_{50} of was calculated to be 3587 ppm (6134 mg/m^3), with 95% confidence limits of 2938-4039 ppm (5023-6907 mg/m^3). Original data could not be retrieved.

Smyth and Carpenter (1948) reported mortality of 1 out of 6 rats (strain not specified) upon a 4 hour exposure to 8000 ppm (13680 mg/m^3). A 14 day postexposure observation period was included. Details on the exposure chamber were not presented.

Groups of 30 rats were exposed to 6840, 13680 or 54720 mg/m3 acetonitrile for four hours. Mortality was 10%, 33% and 57%, respectively (Union Carbide, 1970; as cited in AEGL 2014). Original data could not be retrieved.

Willhite (1981) exposed groups of 10 male CD-1 mice to five or six concentrations of acetonitrile ranging from 500-5,000 ppm (855-8550 mg/m^3) for 60 min and observed for 14 days. Actual individual group exposure concentrations were not reported. Acetonitrile was mixed with a stream of dehumidified air (10 L/min) and delivered to a single pass 45-L glass inhalation chamber. Samples were collected every 5 min using a gas-tight syringe and were analyzed by gas chromatography. The

mice exhibited dyspnea, tachypnea, gasping, tremors, convulsions, and corneal opacity 30-300 min following initial contact with acetonitrile. All mice exposed at 5,000 ppm (8550 mg/m^3) died within 180 min of initial exposure and delayed deaths were observed for up to 3 days after exposure at lower (unspecified) concentrations. The livers of exposed mice were bright red compared with controls. An LC_{50} of 2,693 ppm (1,955-4,247 ppm), 4605 mg/m^3 (3343-7262 mg/m^3), was calculated. No details on the individual animal lethality were presented.

Appendix 2 Reference list

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