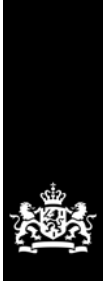


National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Probit function technical support document

Date: 6 June 2017
Document id: 20170606-ammonia-INTERIM
Status: interim
Author: Dr Ir Marc Ruijten
Commissioned by RIVM

substance name	CAS number
Ammonia	**7664-41-7**

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

This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have had the opportunity to comment on the derivation of the proposed probit function. The status of this document has now been raised to "interim", pending a decision on its formal implementation.

The decision on actual implementation depends on the results of a further consequence analysis.

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

Technical support document Ammonia

1. Substance identification

CAS-number:	7664-41-7	NH_3
IUPAC name:	ammonia	
Synonyms:	gaseous anhydrous ammonia, azane	
Molecular formula:	NH_3	
Molecular weight:	17 g/mol	
Physical state:	gas (at 20°C and 101.3 kPa)	
Boiling point:	-33°C (at 101.3 kPa)	
Vapour pressure:	860 kPa (at 20°C)	
Saturated vapor conc:	NA (gas)	
Conversion factor:	1 mg/m^3 = 1.41 ppm (at 20°C and 101.3 kPa)	
	1 ppm = 0.707 mg/m^3 (at 20°C and 101.3 kPa)	
Labelling:	H314-331	

2. Mechanism of action and toxicological effects following acute exposure

Acute effects: The main target organs and tissues for inhalation exposure to ammonia are the respiratory tract (AHLS 2014). The corrosive and exothermic properties of ammonia can result in immediate damage (severe irritation and burns) to the eyes, skin, and mucous membranes of the oral cavity and respiratory tract. In addition, ammonia is effectively scrubbed in the nasopharyngeal region of the respiratory tract because of its high solubility in water. The health endpoints are related to corrosive effects of ammonia such as damage to the respiratory tract and resulting hypoxemia. Symptoms of high exposure are broncho- and laryngospasm, chest pain, stridor, breathing difficulties, and altered mental status because of hypoxemia. Lethality results from pulmonary edema.

Long-term effects: Long-term effects after acute exposure to ammonia are related to the corrosive effects in the trachea, bronchus, and alveoli. Health effects are chronic cough, chemical pneumonitis, asthma, RADS, and lung fibrosis. Chronic exposure produces similar effects.

3. Human toxicity data

Many case reports on fatal and non-fatal accidental ammonia exposures exist. In addition, human volunteer studies with ammonia have been performed.

Exposures reported to be immediately fatal in humans, ranged from 5,000 ppm (3540 mg/m^3) to higher concentrations. Based on this, most handbooks consider the 5,000 ppm concentration as threshold for lethality.

It should be noted, however, that the underlying exposure estimates of the reported lethal concentrations are mostly based on reconstructions of accidents, such as the Potchefstroom accident (Michaels, 1999), which show many uncertainties regarding the exact location of the exposed subjects, the air concentrations and the duration of the exposure. In their evaluation of the Potchefstroom incident Mudan and Mitchell (1996) describe that all surviving individuals were either downwind and sheltered indoors or upwind. They report that within 50 m of the release point all 4 workers who were outside died while 4 out of 6 workers who were inside survived. For the zone between 50 and 100 m from the release point 8 out of 15 workers who were outside survived while all 16 workers who were inside buildings survived. This makes it difficult to relate survival rates to model estimations of outside concentrations. For

these reasons, the data from the described accidents are insufficient for quantitative estimates of survival rates related to specific concentrations.
In another accident in Houston, where a road tanker containing anhydrous ammonia crashed releasing its ammonia content, resulted in the death of 6 persons and the hospitalisation of 78 persons. Skin burns were reported amongst other symptoms, indicating that subjects were dermally exposed to anhydrous ammonia via the air. All fatalities occurred within 60m of the accident site (NTSB report, 1977). During the accident exposures were not monitored, but it was reported that a cloud had formed at the site of the accident suggesting high concentrations in the order of tenthousands ppm.

In the report by NIOSH (1974; original reference: Mulder and Van der Zalm, 1967)) a worker died from an acute exposure to an estimated level of 10,000 ppm (7080 mg/m^3) ammonia despite hospital treatment, when a tank of ammonium hydroxide overflowed.

Several volunteer studies have been described in literature. Two studies are particularly noteworthy.

Silverman et al. (1949) exposed 7 volunteers nose/mouth only to 500 ppm (354 mg/m^3) for 30 minutes. Respiratory effects included an *increase* in the minute volume of the volunteers over control values by 50-250%. The author reported that 'respiratory rates were consistently increased during ammonia inhalation, but to a much smaller degree than ventilation increases'. At the 500 ppm exposure level no coughing was induced. The authors report that 'Previous experiments with higher concentrations of ammonia (1000 ppm) had caused immediate coughing'. A slightly increased pulse rate and blood pressure in 1 of 2 tested volunteers was also reported. No changes in nitrogen metabolism (as tested by standard clinical parameters) were found.

Verberk (1977) exposed 8 members of a toxicological expert group and 8 students for 2 hours in a whole-body chamber to concentrations of 35, 57, 78 and 99 mg/m^3. Lung function parameters (before and after exposure) as well as subjective symptoms (15 min intervals during exposure) were recorded. Subjective symptoms included smell, taste, eye-, nose-, throat- and chest irritation, urge to cough, headache and general discomfort. The volunteers did not perform physical exercise. Post exposure changes in VC, FEV_1 or FIV_1 of >10% of pre-exposure values were not observed. All subjective symptoms were scored higher at higher exposure levels. The students did not complete the 2-hour exposure to the 99 mg/m^3 exposure level because of unbearable discomfort, whereas the (older) expert group all completed the exposures.

4. Animal acute toxicity data

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

1. AEGL final TSD 2008, ERPG document and EU RAR and reference database for ammonia, covering references before and including 1995.
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, ECHA, IUCLID, 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 9 datasets for 2 species- with data on lethality following acute inhalation exposure. One dataset was assigned status A for deriving the human probit function, 3 datasets were assigned status B and 5 were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

A total of 2 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 ammonia*

Species/strain	RD_{50} (mg/m^3)	Exposure duration (min)	Author/year
Mouse, Swiss webster	215 (NS)	30	Barrow *et al.*, 1978
Rat Wistar	972 (P) dry air	45	Li *et al*, 2010
Rat Wistar	905 (P) wet air	45	Li *et al*, 2010
Mouse OF1	582 (P) dry air	45	Li *et al*, 2010
Mouse OF1	782 (P) wet air	45	Li *et al*, 2010

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

5. Probit functions from individual studies

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

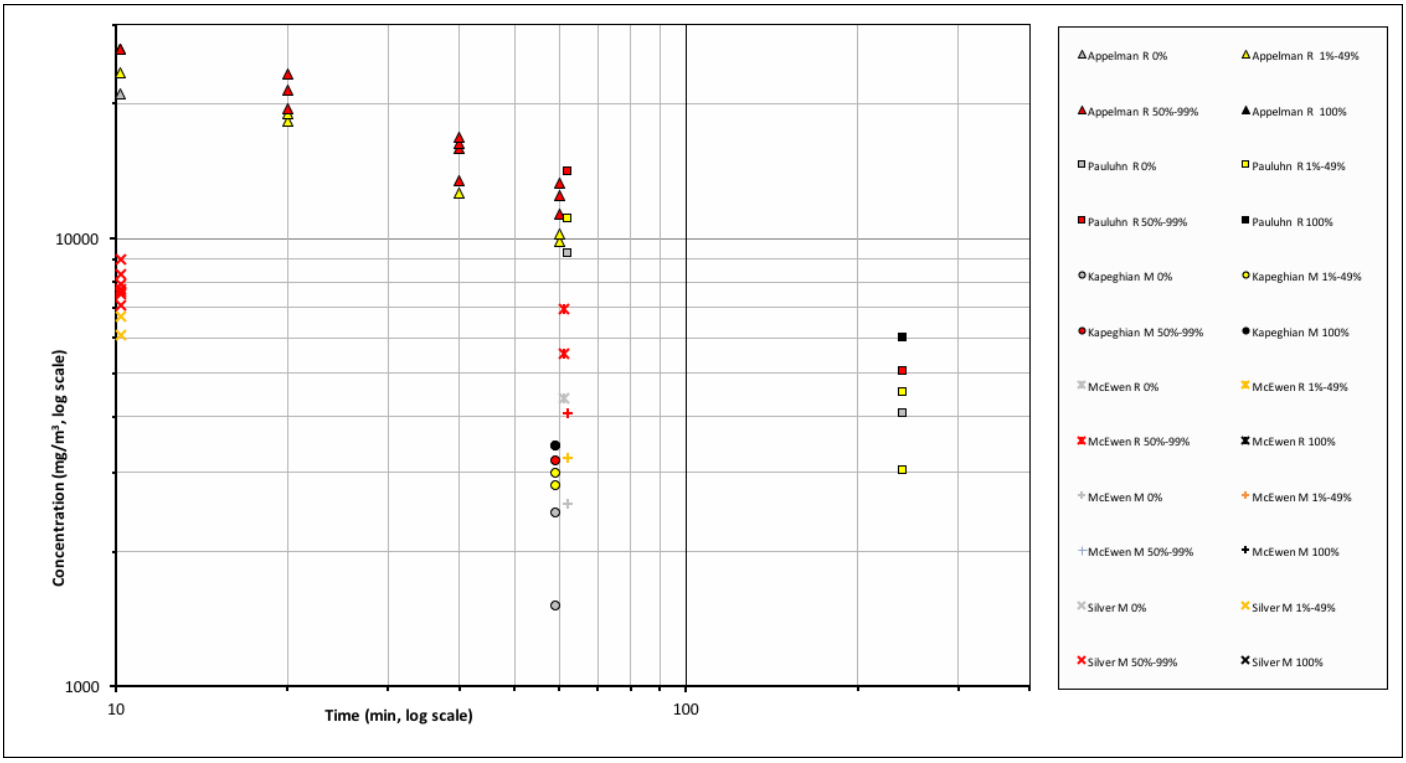


Figure 1 *All available acute lethality data for ammonia.*

The data selected for the primary analysis of the animal probit function are presented in Table 2 and Figure 3. All A and B1 studies were selected for derivation of the animal probit function for ammonia.

To enable intra-species pooling, LC_{50}-values of B1-studies were scaled using the rat n-value of 2.02 for ammonia with the following formula (section 6):

$$LC_{50,c} = LC_{50,test}\left(\frac{t_{test}}{t_c}\right)^{(1/n)}$$

With $LC_{50,c}$ = scaled LC_{50} value for common exposure duration t_c
$LC_{50,test}$ = observed LC_{50} value for tested exposure duration
t_c = common exposure duration for intra-species pooling
t_{test} = tested exposure duration
n = species specific (for [species]) / overall / default n-value

Probit functions have been calculated and reported in Appendix 1 for each of the reported studies. The results of the calculations are presented in the table below. The LC_{50}-values of all A- and B1-studies were calculated for a common exposure duration of 60 minutes, and are presented in table 2.

Table 2 *Data selected for the initial analysis for derivation of the animal probit function of ammonia.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} at tested exposure duration (mg/m^3) 95% C.I.	LC_{50}, 60 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
A.1	Rat	-47.9 + 4.65×lnC + 3.30×lnt		11590 (1234 – 7654)	2.022 (1.62-2.43)
B1.1	Rat	*60-min LC_{50}*		13490 (12230-16620)	N/A
B1.2	Mouse	*60-min LC_{50}*		3008 (2876 – 3121)	N/A
B1.3	Mouse	*10-min LC_{50}*	7066 @ 10 min (6631 – 7371)	*2913*	N/A

The data of the A study and study B1.1-B1.3 with rats and mice are presented graphically below.

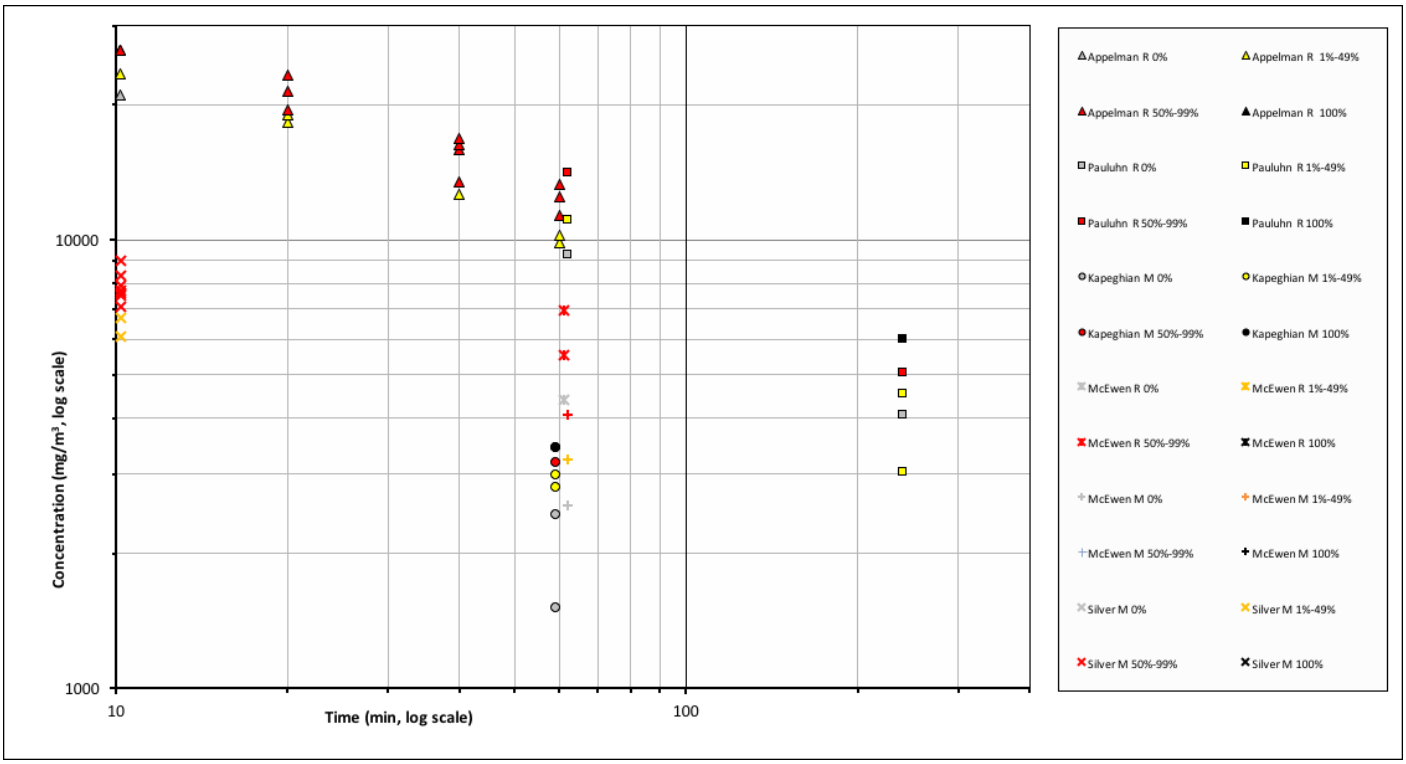


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of ammonia (identical to figure 1).*

Based on the availability of data for the appropriate exposure duration (for pooling of LC_{50} values), the data from studies A.1, B1.1 (rat data) and B1.2 (mouse data) were selected for the final dataset for the derivation of the animal probit function. The mouse data from study B1.3 were excluded because the 10-minute exposure duration was more than a factor 2 shorter than the target range of 30-60 minutes. The data from study B1.3, however, support the mouse study B1.2.

Thus, the final dataset used to calculate the animal probit function contains 3 datasets from 3 studies and includes data from 2 animal species.

Table 3 Data selected for the derivation of the animal probit function of ammonia.

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} at tested exposure duration (mg/m^3) 95% C.I.	LC_{50}, 60 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
A.1	Rat	-47.9 + 4.65×lnC + 3.30×lnt		11590 (10210 – 12970)	2.022 (1.62-2.43)
B1.1	Rat	*60-min LC_{50}*		13490 (12230-16620)	N/A
B1.2	Mouse	*60-min LC_{50}*		3008 (2876 – 3121)	N/A

The data of the selected datasets are presented graphically below.

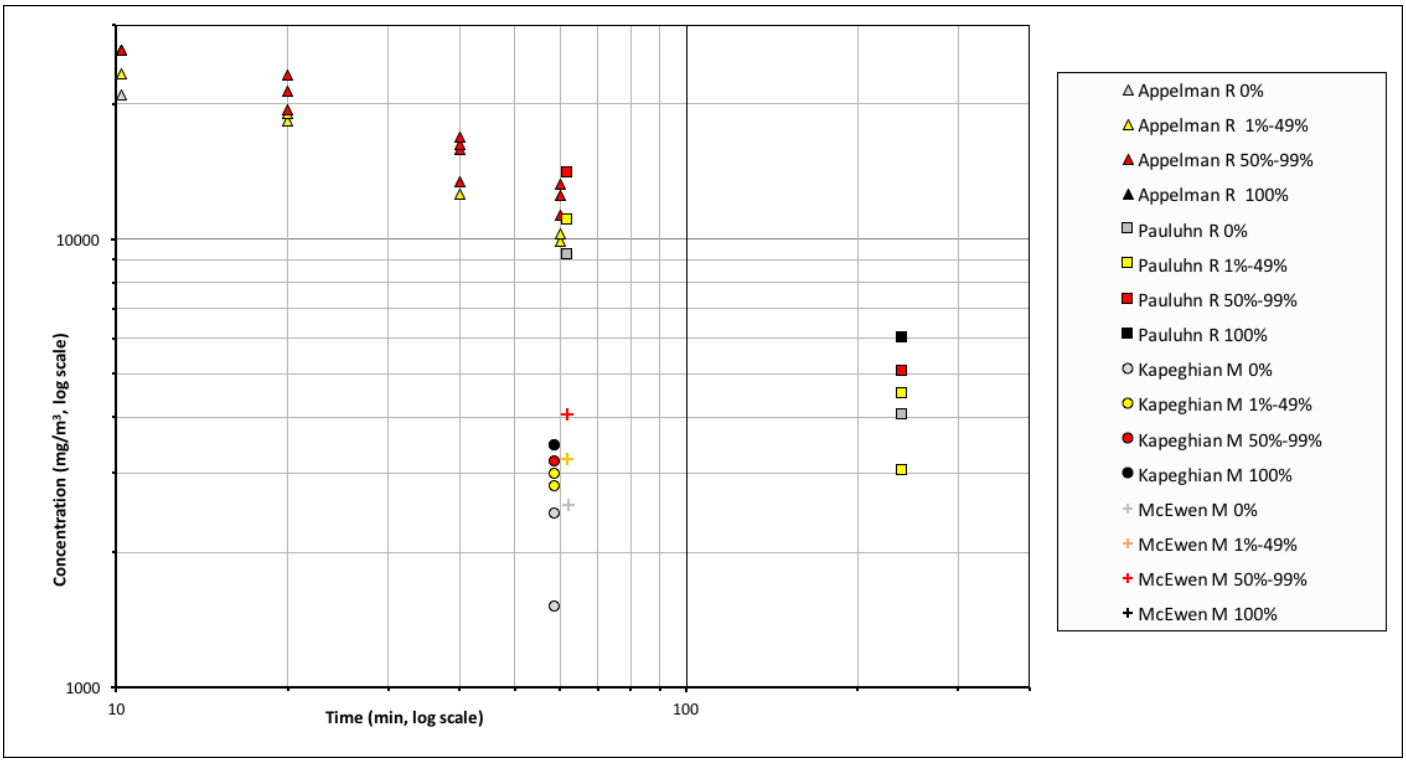


Figure 3 *Final data selected for derivation of the animal probit function of ammonia.*

6. Derivation of the human probit function

As a point of departure for deriving the human probit function, the studies A.1, B1.1 and B1.2 were used as outlined above.

The (rat) n-value from study A.1 was calculated to be 2.022.

Second, the LC_{50}-values of all applicable A- and B1-studies were calculated for a common exposure duration of 60 minutes.

Finally, the species-specific geometric mean LC_{50}-values were calculated from the LC_{50} values of studies A.1, B1.1 and B1.2. The species-specific LC_{50}-value was 12504 for the rat and 3008 for the mouse. Finally, a geometric mean overall LC_{50}-value was calculated. The overall formula for the geometric mean of LC_{50}-values is as follows:

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

With $\overline{LC_{50}}$ = geometric mean LC_{50}-value across species
$LC_{50,i}$ = 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…s).

The Point of Departure for the human probit function is a 60-minute geometric mean animal LC_{50} value of 6133 mg/m^3 and an arithmetic mean n-value of 2.022.

Relevant quantitative human data exist in addition to the animal data. Silver at al reported 30-min exposure of human volunteers up to 350-700 mg/m^3 (60-min

equivalent assuming n=2.02: 248-497 mg/m^3) without fatalities or serious objective effects. Verberk exposed volunteers to 99 mg/m^3 for 2 hours (60-min equivalent assuming n=2.02: 140 mg/m^3) which produces subjective complaints in students but not in an older toxicology expert group; all objective lung functions changes were less than 10%.

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	1	Application of the default assessment factor would produce a probit function that predicts lethality at exposures that are in the range where human volunteer studies and occupational experience did not produce serious health effects.
Nominal concentration	1	Well conducted studies with analytically determined concentrations.
Adequacy of database:	1	4 datasets in 2 animal species available for probit derivation (3 actually used), including one C×t dataset.

The estimated human equivalent 60-minute LC_{50} value is 6133 / 1 = **6133 mg/m^3**.

The experimentally determined n-value was **2.022** (study 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 = **0.989**.

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): $5 = a + 0.989 \times \ln (6133^{2.022} \times 60)$ resulting in the a-value of **-16.49**.

$Pr = -16.5 + 0.99 \times \ln (C^{2.02} \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically sound basis. The human probit function is based on 3 studies in the rat and mouse with A and B1 quality, where a total of 378 rats and mice were exposed to concentrations ranging from 800 - 26,000 mg/m^3 for exposure durations ranging from 10 – 240 minutes.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 1918 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 1312 mg/m^3.

Table 5 *LC-vales 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	1849	1312
1% lethality, this probit	2703	1918
AEGL-3 (2007, final)	1119	769
ERPG-3 (2014)		1061
LBW (2015)	1100	780

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are higher. This is mainly the result of the use of an interspecies assessment factor of 1 instead of 3 based on human data. AEGL and ERPG values were based solely on rat data, with a roughly 4 times higher LC_{50} value than mice, but with application of higher assessment factors.

Appendix 1 Animal experimental research

Study ID: A.1

Author, year: ***Appelman 1982***
Substance: Ammonia
Species, strain, sex: Rat, Cpb:WU, Wistar, males and females.
Number/sex/conc. group: 5/sex/exposure group
Age and weight: age at arrival 7-8 weeks (age at start study unknown), weights were 150-170g for males and 130-140 for females.
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	*Stable*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A (whole body)*
Pressure distribution	*no information on pressure distribution*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere was generated by diluting a measured flow of ammonia with filtered compressed air-derived auxiliary flow to obtain the concentration. The mixture was passed into horizontally placed glass exposure cylinder (0.90m by 0.15m, approx. 16 L) with sampling ports (inlet and outlet).*
Number of air changes per hour	*Flow of 25 L/min, resulting in 9.4 air changes per hour.*
Equilibration time (t95)	*2 minutes*
Start of exposure relative to equilibration	*At start of concentration build-up*
Actual concentration measurement	*The ammonia concentrations were monitored by passing a known volume of air through an impinger, filled with a known excess of 0.1N HCl. Back-titration with 0.1N NaOH. Ammonia concentrations were then calculated.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***A*** *Four exposure durations tested, concentrations were analytically determined.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Rat	20950		10	0/5	0/5
	23380		10	1/5	0/5
	26410		10	5/5	1/5
	27220		10	5/5	0/5
	37820		10	5/5	4/5
	18290		20	3/5	0/5
	19030		20	1/5	0/5
	19450		20	5/5	2/5
	21420		20	3/5	3/5
	23200		20	5/5	4/5
	12620		40	2/5	0/5
	13410		40	4/5	1/5
	15870		40	4/5	1/5
	16290		40	5/5	3/5
	16840		40	5/5	2/5
	9870		60	2/5	1/5
	10230		60	4/5	0/5
	11300		60	5/5	0/5
	12500		60	5/5	1/5
	13240		60	5/5	2/5

Probit function

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

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

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

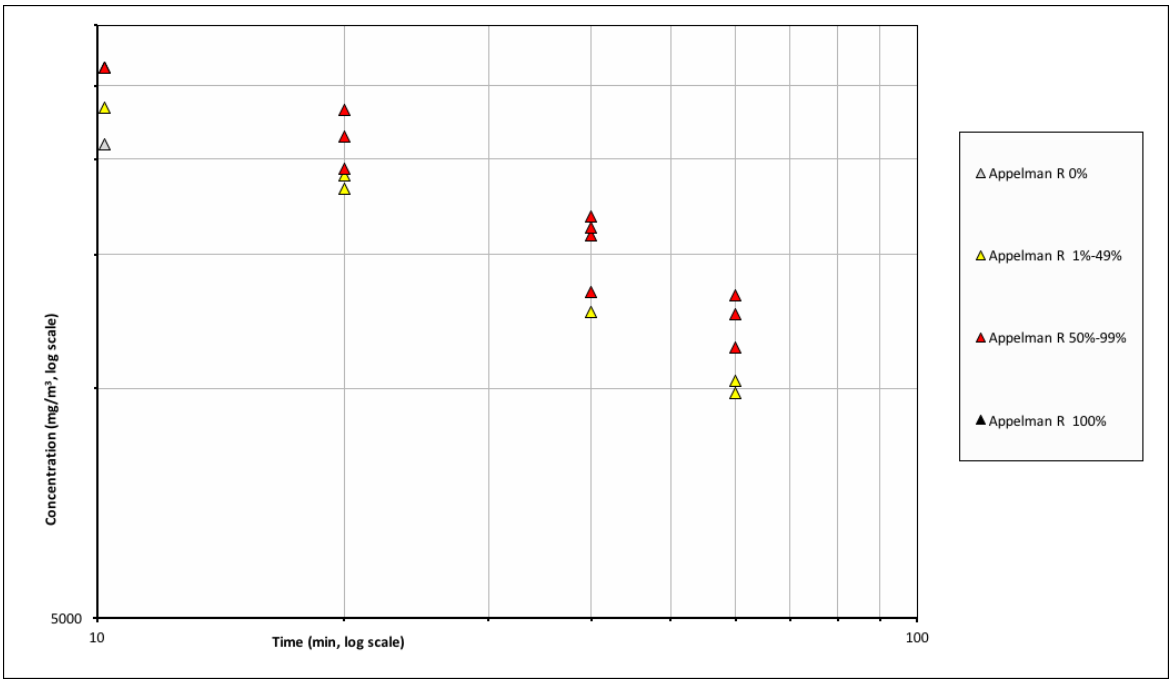
Probit function	Species	a	b	c	d	n-value
Sex as variable	Rat	-69.9	6.50	3.23	1.80	2.012 (1.81 – 2.22)
Sexes combined	Rat	-47.9	4.65	2.30		2.022 (1.62 – 2.43)

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	24540 (22710 - 26250)	32360 (30090 - 35580)	28120 (24740 - 32860)
30	14220 (13330 - 14920)	18740 (17850 - 20000)	16330 (15130 - 17660)
60	10070 (9264 - 10740)	13280 (12510 - 14280)	11590 (10210 - 12970)

The results for males and females were derived from the analysis with sex as covariate.

A graphical overview of the data is presented below. Each concentration-time combination (with 5 male and 5 female animals) represents one point in the plot.


Concentration (mg/m³, log scale)
5000
10
Time (min, log scale)
100
Appelman R 0%
Appelman R 1%-49%
Appelman R 50%-99%
Appelman R 100%

1
2
3

Study ID: B1.1

Author, year: ***Pauluhn 2013***
Substance: ammonia
Species, strain, sex: Hsd Cpb:WU Wistar rats, males and females
Number/sex/conc. group: 5/sex/concentration
Age and weight: 180 ± 15 gram
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*GLP statement provided*
Study carried out according to OECD 403 guideline(s)	*In accordance with OECD guideline 403*
Stability of test compound in test atmosphere	*No stability issues reported*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Directed-flow nose-only system*
Type of restrainer	*Not specified*
Pressure distribution	*Test atmospheres were generated by metering pure ammonia gas from a tank into a push-pull dilution system to a directed-flow nose-only testing system. Each exposure port received an airflow of 0.75 l/min.*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere generation, particularly for liquids (spraying, evaporation, other) and solids. Mixing of test atmosphere in the exposure system.*
Number of air changes per hour	*Flow was 15 l/min per exposure port*
Equilibration time (t95)	*t95 was reached < 1 min (according to author)*
Start of exposure relative to equilibration	*At start of concentration build-up.*
Actual concentration measurement	*Real-time analytical measurement (FTIR) and verified by samples in gas-sampling bags.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Well performed study with 2 exposure durations tested.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Rat	9222		60	0/5	0/5
	11015		60	1/5	0/5
	14044		60	4/5	2/5
	3028		240	0/5	1/5
	4046		240	0/5	0/5
	4516		240	2/5	1/5
	5053		240	4/5	4/5

Probit function

The probit function and associated LC-values can 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 (0 = female, 1 = male).

Probit function	Species	a	b	c	d	n-value
Sex as variable	Rat	-61.5	5.23	4.03	0.28	1.299 (0.93 - 1.67)
Sexes combined	Rat	-61.7	5.26	4.04		1.302 (0.99 - 1.613)

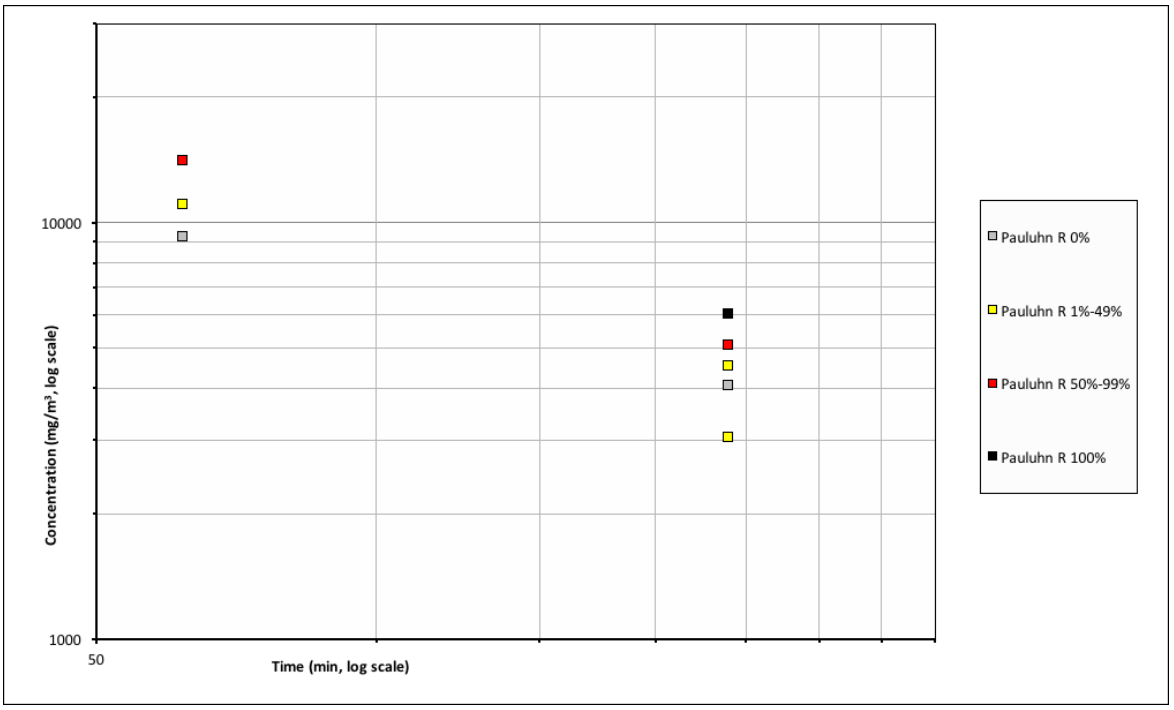
The model with all data did not allow to calculate confidence intervals for the LC_{50} in male and female rats, but did produce a confidence interval for the LC_{50} in the analysis with sexes combined. The LC_{50} values for both sexes did not differ by more than a factor of 2 (actually <10%). This 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.

Since this study only tested 2 exposure durations, only the 60-min data will be used in the derivation of the human 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, all data
60	12470 (10810-15040)	14590 (12670-19560)	13490 (12230-16620)
240	4847 (no cfd-i)	4745 (no cfd-i)	4797 (no cfd-i)

The 60- and 240-minute LC_{50} values were calculated with only the 60- and 240-minute data respectively. The 60-min LC_{50} value estimated with the model including all data was 13740 (95% cfd-i 10910–79260). The point estimate is almost identical to that of the analysis with only 60-min data, but the cfd-i is much wider. Probably the 1 fatality in the females at 3028 mg/m^3 for 240 min is difficult to model.

A graphical overview of the data is presented below. Each concentration-time combination (with 5 male and 5 female animals) represents one point in the plot.


10000
1000
50
Concentration (mg/m³, log scale)
Time (min, log scale)
Pauluhn R 0%
Pauluhn R 1%-49%
Pauluhn R 50%-99%
Pauluhn R 100%

Study ID: B1.2

Author, year: ***Kapeghian 1982***
Substance: ammonia
Species, strain, sex: Mouse, albino ICR, male
Number/sex/conc. group: 12/concentration
Age and weight: Age unknown, weight ranged from 25-30g
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	*Stable.*
Use of vehicle (other than air)	*Air.*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body.*
Type of restrainer	*N/A (whole body)*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere was generated by mixing ammonia with air in a mixing board. From the mixing board a flow is delivered to 4 glass distributing tubes generating different concentrations and consequently the flow is directed to the 4 chamber tubes. The 4 exposure groups are exposed simultaneously.*
Number of air changes per hour	*No information*
Equilibration time (t95)	*10-15 min (as stated by authors)*
Start of exposure relative to equilibration	*Uncertain, but probably at the start of concentration build-up*
Actual concentration measurement	*Air concentrations are analytically measured at 3-5 minute intervals during the air flow from a mixing board to distributing tubes, distributing tubes to chamber tubes, and from chamber tubes to exhaust.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Well performed study, only one exposure duration tested.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Mouse	843		60	0/12	
	949		60	0/12	
	1508		60	0/12	
	2436		60	0/12	
	2797		60	4/12	
	2988		60	5/12	
	3179		60	8/12	
	3441		60	10/12	
	3441		60	12/12	

The authors report that 'at high concentrations deaths usually occurred within 30 min; 90% occurred in the initial 15-20 min of exposure. Lower lethal concentrations produced death as late as 45 min after initiation of the 1-h exposure'. They also stated that 'Delayed deaths were detected up to 14 days after exposure, however 97% of the mortalities generally occurred within 72 hours'.

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.

Species	a	b	n-value
Mouse	-73.8	9.84	N/A

There were insufficient data to evaluate sex differences in the response to inhalation of ammonia.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Male
60	3008 (2876 – 3121)

The authors report an LC_{50} of 2995 mg/m^3 (95% cfd-i 2882 – 3115 mg/m^3).

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

Study ID: B1.3

Author, year: Silver 1948
Substance: ammonia
Species, strain, sex: mice, sex unknown
Number/sex/conc. group: 20 animals
Age and weight: age and weight unspecified
Observation period: 10 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. Many details missing.*
Stability of test compound in test atmosphere	*No mention of stability issues.*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*NA, whole-body exposure*
Pressure distribution.	*Not specified*
Homogeneity of test atmosphere in breathing zone of animals	*The compound was volatilized from a temperature controlled bubbler by aeration with nitrogen and mixed with inlet air prior to introduction into the chamber. No description of the method to mix the chamber atmosphere.*
Number of air changes per hour	*250 ℓ/min in a 629 ℓ chamber which equals 25 Air Changes/h.*
Equilibration time (t95)	*Calculated t95 = 7.55 min*
Start of exposure relative to equilibration	The initiation of exposure and gas sampling was governed by the time of equilibration to 99% constant concentration. Animals were introduced into the chamber in their cages by means of a gas-tight cradle.
Actual concentration measurement	*Air samples were captured in an impinger and analysed by titration. Sampling location and number of samples per exposure unspecified.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	N/A
Assessment of Reliability	***B1*** Study does not allow calculation of a concentration/time/lethality response. The observation period was short: one animal died at the last observation day.

Results

Species	Reported Concentration (mg/m^3)	Exposure duration (min)	Lethality	
			exposed	Fatal
Mouse	6.1×10^3	10	20	5
	6.7×10^3	10	20	5
	7.1×10^3	10	20	11
	7.5×10^3	10	20	13
	7.6×10^3	10	20	17
	7.7×10^3	10	20	14
	7.9×10^3	10	20	11
	8.3×10^3	10	20	16
	9.0×10^3	10	20	16

Probit function

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

$Pr = a + b\times lnC$

with C for concentration in mg/m^3.

Probit function	*Species*	*a*	*b*	*n-value*
Sex unknown	*mouse*	*-35.9*	*4.61*	*N/A*

Duration (min)	*LC_{50} (mg/m^3) 95%-C.I.* ***Sex unknown***
10	7066 (6631 – 7371)

The authors report an LC_{50} of 7060 mg/m^3 (95% cfd-i 6740 – 7380 mg/m^3).

No graphical overview of the data is presented.

Study ID: C

Author, year: ***McEwen and Vernot 1972***
Substance: ammonia
Species, strain, sex: male CFE rats and male CFI mice.
Number/sex/conc. group: 10/concentration group
Age and weight: rats: 200-300 grams; mice: 20-30 grams.
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	*Stable.*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A (whole body)*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*Exposure generation method according to Weil, no further information given or referred to.*
Number of air changes per hour	*No information*
Equilibration time (t95)	*No information*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Chamber concentrations were analysed with an IR spectrophotometer. Actual concentrations were found to range from 25-40% lower than nominal concentrations.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***C*** *One exposure concentration tested, many uncertainties about the exposure technique.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Rat	4397		60	0/10	
	5537		60	8/10	
	6967		60	9/10	
Mouse	2549		60	0/10	
	3221		60	3/10	
	4050		60	9/10	

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

Species	a	b	n-value
Rat	-56.2	7.13	N/A
Mouse	-63.0	8.35	N/A

There were insufficient data to evaluate sex differences in the response to inhalation of ammonia.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Rat	LC_{50} (mg/m^3) 95%-C.I. Mouse
60	5350 (no cfd-i)	3453 (3139 – 3805)

The authors reported 60-minute LC_{50} values (95% CI) of 5100 (95% cfd-i 4840-5370) mg/m^3 for rats and 3360 (95% cfd-i 3064-3687) mg/m^3 for mice.

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

Study ID: Other C studies

In a study by Hilado et al. (1977, as cited in AEGL), four Swiss mice per group were exposed in a static 4.2 liter exposure chamber to 7143-28571 ppm (5057-20228 mg/m^3) of ammonia for 30 min. Exposure concentrations were calculated rather than measured analytically. One mouse died at 19048 ppm (13486 mg/m^3), two at 21429 ppm (15172 mg/m^3), three at 23810 ppm (16857 mg/m^3), and four each at 26190 and 28,571 ppm (18543 and 20228 mg/m^3). All deaths occurred during exposure except the death at the lowest concentration, which occurred 1 day after exposure. No deaths occurred after exposure to concentrations of 14286 ppm (10114 mg/m^3) or lower. The LC_{50} value was reported as 21000 ppm (rounded value; 14868 mg/m^3) for the 30-min exposure.

In another inhalation study, an LC_{50} value for the rat, with a 2-h exposure, was 7600 mg/m^3 (Alpatov, 1964, as cited in WHO 1986). No further information on the study was available.

Prokop'eva et al. (1973) reported that white rats exposed to high concentrations of ammonia (6000, 3000, 1000 mg/m^3) for periods of 5, 15, 30, and 60 min. The number and sex of animals exposed per dose, the observation period and many important experimental details are reported to be missing.
Exposed rats were reported to exhibit dyspnoea, irritation of the respiratory tract and eyes, cyanosis of the extremities, and increased excitability. Animals exposed to high concentrations of ammonia (exact concentration not specified) developed pneumonia. The reported LC_{50} values were 18693 mg/m^3 (5 min), 12160 mg/m^3 (15 min), 7035 mg/m3 (30 min) and 7939 mg/m^3 (60 min). The table and the conclusion section list slightly different LC_{50} values.
All reported LC_{50} values seem to be *above* the highest tested concentration. Because of this unusual finding, the many missing study details and the inability to verify the information, this study was assigned a C status.

Appendix 2 Reference list

AHLS. University of Arizona Emergency Medicine Research Center. Advanced Hazmat Life Support (AHLS). Provider Manual, 4th ed. Tucson, AZ, 2014.

Appelman, L.M., W.F. ten Berge, and P.G. Reuzel. Acute inhalation toxicity study of ammonia in rats with variable exposure periods. Am. Ind. Hyg. Assoc. J. 1982;43(9):662-665.

Barrow, C.S., Y. Alarie, and M.F. Stock. Sensory irritation and incapacitation evoked by thermal decomposition products of polymers and comparisons with known sensory irritants. Arch. Environ. Health 1978;33(2):79-88.

Hilado, C.J., C.J. Casey, and A. Furst. Effect of ammonia on Swiss albino mice. J. Combust. Toxicol. 1977;4:385-388.

Kapeghian, J.C., H.H. Mincer, and A.B. Jones, A.J. Verlangieri, and I.W. Waters. Acute inhalation toxicity of ammonia in mice. Bull. Environ. Contam. Toxicol. 1982:29(3):371-378.

Li W-L, J. Pauluhn. Comparative assessment of the sensory irritation potency in mice and rats nose-only erxposed to ammonia in dry and humidified atmospheres. Toxicology 2010;276:135-142.

MacEwen, J.D., and E.H. Vernot. 1972. Toxic Hazards Research Unit Annual Technical Report: 1972. AMRL-TR-72-62. NTIS AD-755 358. Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH.

Michaels, R.A. Emergency planning: Critical evaluation of proposed AEGLs for ammonia. Process Saf. Prog. 1998;17(2):134-137.

Mudan, K., and K. Mitchell (1996) Report on the Potchefstroom, South Africa Ammonia Incident. Four Elements, Inc, Columbus, OH. 14 pp

National Research Council. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Volume 6. Washington, DC. The National Academies Press, 2008.

NTSB (1977). National Transportation Safety Board, Washington, DC 20594, Highway accident report, Transport company of Texas, tractor-semitrailer (tank) collision with bridge column and sudden dispersal of anhydrous ammonia cargo, I-610 at Southwest Freeway, Houston, Texas. May 11, 1976 Report number: NTSB-HAR-77-1. US Government.

Pauluhn J. Acute inhalation toxicity of ammonia: Revisiting the importance of RD_{50} and $LCT_{01/50}$ relationships for setting emergency response guideline values. Reg Toxicol Pharmacol 2013;66:315-325.

Prokop'eva, A.S., G.G. Yushkov, and I.O. Ubasheev. Data on the toxicologic characteristics of the single, brief effect of ammonia on animals. GIG TR PROF ZABOL 1973;17(6):56-57. Original publication in Russian; as cited on ECHA website (visited 23 June 2016).

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.

Silver, S.D., and F.P. McGrath. 1948. A comparison of acute toxicities of ethylene imine and ammonia to mice. J. Ind. Hyg. Toxicol. 30(1):7-9. (as cited in NRC 2007).

Silverman, L., J.L. Whittenberger, and J. Muller. Physiological response of man to ammonia in low concentrations. J. Ind. Hyg. Toxicol. 1949;31(2):74-78.

Verberk MM. Effects of ammonia in volunteers. Int. Arch. Occup. Environ. Health 1977;39:73-81.

WHO 1986. Ammonia. Environmental Health Criteria 54. Geneva, WHO, 1986.