Probit function technical support document

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Author: dr. ir. M. Ruijten, CrisisTox Consult, on behalf of the Netherlands' Ministry of Housing, Spatial Planning and the Environment
Contact: cev@rivm.nl

substance name	CAS number
Hydrazine	**302-01-2**

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 of 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 Housing, Spatial Planning and the Environment (VROM) 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.

Interested parties are invited to submit comments and suggestions concerning this document within 6 weeks after the issue date to the e-mail address mentioned above.

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 Hydrazine

1 Substance identification

CAS-number:	302-01-2
IUPAC name:	Hydrazine
Synonyms:	Diamine
Molecular formula:	N_2H_4 , structural formula: 
Molecular weight:	32.1 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	114°C (at 101.3 kPa)
Vapour pressure:	2.1 kPa (at 20°C)
Saturated vapor conc:	21000 ppm = 28 g/m^3 (at 20°C and 101.3 kPa)
Conversion factor:	1 mg/m^3 = 0.749 ppm (at 20°C and 101.3 kPa)
	1 ppm = 1.335 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	R: 45-10-23/24/25-34-43-50/53

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

Acute effects: The main target organs and tissues for inhalation exposure to hydrazine are the respiratory and nervous systems, the liver and the kidneys.
The irritant and corrosive properties of hydrazine produce liquefactive necrosis in the respiratory system, resulting in laboured breathing, secretions from nose, mouth and eyes and prostration. Hydrazine antagonizes the function of GABA and inhibits GABA formation, which produces seizures.
Damage in the respiratory system results in mucus secretion, upper airway and/or pulmonary oedema and laryngospasm. The resulting hypoxemia will cause CNS and cardiovascular effects. CNS effects are aggravated by the concomitant convulsions.
High exposure may produce hepatonecrosis and renal tubular necrosis.
Lethality results when the respiratory damage proceeds to inflammation, degeneration and necrosis of affected tissue, atelectasis, emphysema and finally death. Lethality may also result from liver or renal failure.
Long-term effects: Chronic exposure may produce cancers in the respiratory system. IARC considers hydrazine possibly carcinogenic to humans (2B).
Reactive Airways Dysfunction Syndrome, an acquired asthma-like condition has been described to develop after single exposure. Symptoms occur within hours after the initial exposure and may persist as non-specific bronchial hyper-responsiveness for months to years.

3 Human toxicity data

No informative reports on health effects in humans following acute inhalation exposure were identified. Such reports are considered informative if both health effects as well as the exposure have been documented in sufficient detail.

[1] AEGL interim TSD, AHLS provider manual.

4 Animal acute toxicity data

Animal lethal toxicity data considering acute exposure are described in Appendix 1. A total of 5 studies were identified -with 6 datasets for 3 species- with data on lethality following acute inhalation exposure. No datasets were assigned status A for deriving the human probit function, 2 datasets were assigned status B and 4 have been assessed to be unfit (status C) for human probit function derivation.

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

1. AEGL final TSD and ERPG documents and reference database for hydrazine, covering references before 1995.
2. An additional search covering publications from 1980 - 2008 was performed in HSDB, MEDline/PubMed, Toxcenter, IUCLID, RTECS, with the following search terms:
   - Hydrazine and synonyms
   - CAS number
   - lethal*
   - mortal*
   - fatal*
   - LC_{50}, LC
   - probit
3. Unpublished data were sought through networks of toxicological scientists.

Sensory irritation

No studies were identified in which sensory irritation was studied.

5 Probit functions

It was not possible to derive a probit function for hydrazine based on data from any of the available studies alone.

6 Evaluation

To derive the human probit function the results from study B.1 (HRC, 1993) have been used to derive a point of departure. This was the only available study that was well conducted and reported. The Comstock (1954) study (C.1) reported lethality for 3 exposure durations, but the generation of chamber atmosphere and the determination of analytical concentrations are highly questionable.

As point of departure for deriving the human probit function the 60 min LC_{50} value of 4160 mg/m^3 for the rat from the HRC (1993) 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} data available
Nominal concentration	1	Analytical concentrations reported
Adequacy of database:	2	Only 1 adequate B-study

The estimated human equivalent 60-minute LC_{50} value is 4160 / 6 = **693 mg/m^3**.

No reliable experimentally determined n-value was available, so the default n-value of **2.0** 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 = **1.0**.

The human probit function is then calculated on the human equivalent 60 min LC_{50} and using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1.0 \times \ln (693^{2.0} \times 60)$ resulting in the a-value of **-12.18**.

$Pr = -12.2 + 1 \times \ln (C^2 \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically weak basis. The probit function is based on 1 study in the rat with B quality, where 60 animals were exposed for 1 hour to 4 concentrations and one control group. The response rate ranged from 0% - 60%.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 219 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 150 mg/m^3.

Estimated level	**30 min (mg/m^3)**	**60 min (mg/m^3)**
1% lethality, this probit	309	219
0.1% lethality, this probit	212	150
AEGL-3 (2005, interim)	59	46
ERPG-3 (2008)		40
LBW (2007)		50

Comparing to equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with the probit function are higher than the other existing acute guidelines. The AEGL values are lower because a total uncertainty factor of 30 was used with the LC_{50} from the same study (HRC 1993) as point of departure.

Appendix 1 Animal experimental research

Study ID: B.1

Author, year: HRC (1993)

Substance: Hydrazine

Species, strain, sex: male and female Sprague-Dawley rats

Number/sex/concentration group: 5 / sex / concentration

Age and weight: 6-8 weeks old, 192 - 229 grams

Observation period: 14 days

Criteria	*Comment*
Study carried out according to GLP	*GLP statement provided*
Study carried out according to guideline(s)	*Statement of compliance with OECD guideline 403 provided*
Stability of test compound in test atmosphere	*Exposure was to an aerosol of a 64% aqueous solution of hydrazine*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose ('snout') only, with animals in a restraining tube*
Pressure distribution.	*No information provided.*
Homogeneity of test atmosphere at breathing zone of animals	*The 64% aqueous solution was aerosolized in an air stream of 25 ℓ/min and fed into the exposure unit.*
Number of air changes per hour	*2.5 ℓ/min/animal*
Actual concentration measurement	*3 samples per exposure were drawn through a fritted glass impinger*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*1 sample per exposure was taken to determine the particle size. Results listed in the table below.*
Assessment of Reliability	***B*** *Well conducted study, but only 1 exposure duration tested*

Results

Species	Concentration (mg/m^3)		MMAD (µm)	Exposure duration (min)	Lethality	
	aerosol	hydrazine			Male	Female
Rat	0	0		60	0/5	0/5
	1020	650	5.0[2]	60	0/5	0/5
	3170	2040	1.1	60	0/5	0/5
	5040	3240	2.4	60	1/5	3/5
	7760	4980	1.8	60	2/5	4/5

All lethality occurred within 3 days after exposure.

Probit function

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

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

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

Probit function	*Species*	*a*	*b*	*d*
Sex as covariate	*Rat*	*-16.6*	*2.65*	*-1.04*
Sexes combined	*Rat*	*-14.8*	*2.38*	

Since the LC_{50} for males was outside the experimental range, the comparison of response between the sexes was based on the LC_{25}. The LC_{25} 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.

Duration (minutes)	***LC_{25}*** *(mg/m^3) 95%-C.I.* ***Male***	***LC_{25}*** *(mg/m^3) 95%-C.I.* ***Female***	*LC_{50} (mg/m^3) 95%-C.I.* ***Combined***
60	3924 (2411 - 5942)	2647 (1267 - 3513)	4160 (3291 - 6849)

No concentration-time-response probit function could be calculated from these data alone. The LC_{50} value reported by the authors was 4.2 mg/ℓ - 4200 mg/m^3, which is essentially identical to the value calculated for this review.

[2] For this group a different aerosol generator was used.

Study ID: C.1

Author, year: Comstock (1954)

Substance: Hydrazine

Species, strain, sex: Male Wistar rats

Number/sex/concentration group: 6 / group

Age and weight: age unknown, weight 150-250 grams

Observation period: 14 days

Criteria	*Comment*
Study carried out according to GLP	*GLP did not exist at the time*
Study carried out according to guideline(s)	*OECD guideline 403 did not exist at the time. Some details missing, small exposure chamber.*
Stability of test compound in test atmosphere	*The chemical appeared to react with the tubing, the chamber and animals' furs.*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Pressure distribution.	*No information*
Homogeneity of test atmosphere at breathing zone of animals	*An attempt was made to generate saturated atmosphere by passing dried air through a bubbler containing anhydrous liquid hydrazine at 25 °C. The chamber atmosphere was not actively mixed. Chamber atmosphere leakage probably occurred when introducing the animals in the chamber.*
Number of air changes per hour	*2 ℓ/min through a 10 ℓ chamber, which equals 12 air changes/h.*
Actual concentration measurement	*Chamber air was passed through two bubblers in series containing 0.5N H_2SO_4 and titration of excess acid with NaOH. Sampling location in the exhaust, not necessarily in animals' breathing zone.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*No information*
Assessment of Reliability	***C*** *The generation of an adequate test atmosphere and the analytical determination were questionable. Lethality occurred on the last observation day.*

Results

One test series was only reported as nominal concentrations, the second series as nominal and analytical concentrations. Only the results of exposures with analytically determined concentration levels are reported here. The nominal / analytical ratio was very large.

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Nominal	analytical		exposed	died
Rat	18000	400	240	6	5
	20000	352	240	6	2
	20000	344	240	6	3
	17000	227	240	6	1
	18000	109	240	6	3
	21000	756	120	6	1
	20000	405	120	6	1
	16000	285	120	6	2
	19000	129	120	6	2
	18000	128	120	6	1
	21000	831	60	6	3
	17000	185	60	6	0
	19000	151	60	6	1
	19000	106	60	6	1

Probit function

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

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

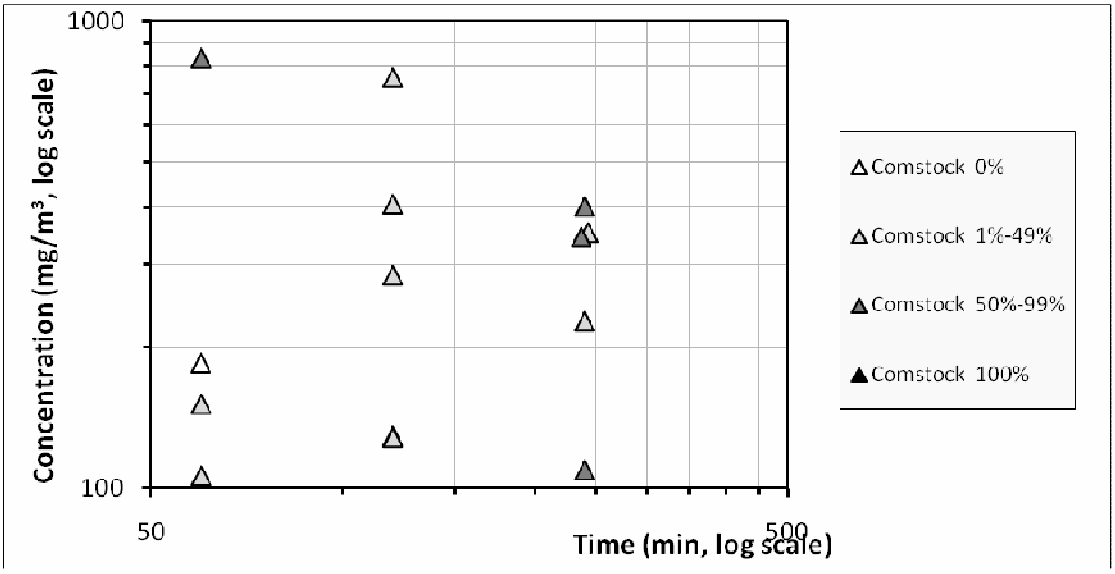
with C for concentration in mg/m^3 and t for time in minutes.

Probit function	*Species*	*a*	*b*	*c*	*n-value*
Sexes combined	*Rat*	*0.43*	*0.24*	*0.56*	*0.44 (-0.47 - 1.35)*

The model fit was very poor, which agrees well with the lack of systematic dose-response relationship which is apparent from the data. The data did not allow to calculate 95%-confidence intervals for any of the required exposure durations. While a concentration-time-response relationship could be calculated from these data alone, the outcome appears to be meaningless.

Duration (minutes)	*LC_{50} (mg/m^3) 95%-C.I.* ***Male***
10	7.3×10^5
30	5.9×10^4
60	1.2×10^4
120	2500
240	513

The lack of dose-response is also apparent from the graphical overview of the data, which is presented below. Each concentration-time combination (with 6 male animals) represents one point in the plot.



196 ***Study ID: C***
197
198 Jacobson (1955) Groups of 10 male rats and 10 female mice (strain, age and weight
199 unspecified) were exposed to hydrazine for 4 hours (probably whole-body) and
200 observed for 14 days. The authors reported LC_{50} values of 750 mg/m^3 for rats and 330
201 mg/m^3 for mice. Lethality percentages have to be assessed form a graph which also
202 includes datapoints for a number of methylated hydrazines.
203
204 Latendresse (1995) exposed rats (5/sex) and hamsters (10 males) to 1000 mg/m^3 for 1
205 hour as part of a study to determine the oncogenic potential of hydrazine. All animals
206 survived the exposure and the observation period that followed (duration unknown).
207
208 McEwen (1981) exposed male Syrian golden Hamsters (10/concentration) whole-
209 body to hydrazine for 1 hour, and observed the animals for 14 days. A 1-hour LC_{50} of
210 3450 mg/m^3 was reported.

Appendix 2 Reference list

Comstock, CC, LH Lawson, EA Greene,FW Oberst. Inhalation toxicity of hydrazine vapor. A. M. A. Arch. Ind. Hyg. Occupat. Med. 1954;10: 476-490.

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