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

Probit function technical support document

Date: 23 April 2019
Comments before: 14 June 2019
Document id: 20190423-hydrazine-PROPOSED
Status: proposed
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substance name	CAS number
Hydrazine	**302-01-2**

This draft 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 for Public Health and the Environment (RIVM) and has been assigned the status "proposed". The scientific expert panel on probit functions has approved this document for public discussion and comments. Interested parties are invited to submit comments and suggestions concerning this document within 6 weeks after the issue date to the email address mentioned above.

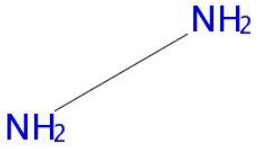
If the proposed probit function is approved by the expert panel on scientific grounds, after review and revisions following of public comments, the status of the document and probit function will be raised to "interim".

Subsequently, the Ministry of Infrastructure and Water Management will 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 the derivation, evaluation and formalization of probit functions is available at http://www.rivm.nl/en/Topics/P/Probit_functions.

Technical support document hydrazine

1. Substance identification



CAS-number:	302-01-2
IUPAC name:	Hydrazine
Synonyms:	Diamine
Molecular formula:	N2H4 , 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)
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:	Human H301-311-314-317-331-350

2. Mechanism of action and toxicological effects following acute exposure

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 (Gamma Amino Butyric Acid, a cerebral neurotransmitter) and inhibits GABA formation, producing seizures that are refractory to diazepam.
Damage in the respiratory system results in mucus secretion, laryngospasm and upper airway and/or pulmonary oedema. 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 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 probably 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 hyperresponsiveness for months to years.

3. Human toxicity data

No informative reports on human toxicity following acute inhalation exposure were identified in which details about both lethal or non-lethal health effects and the exposure have been documented in sufficient detail.

4. Animal acute toxicity data

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

1. AEGL final TSD, ERPG document and EU RAR and reference database for hydrazine, 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 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 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 hydrazine are displayed in Figure 1.

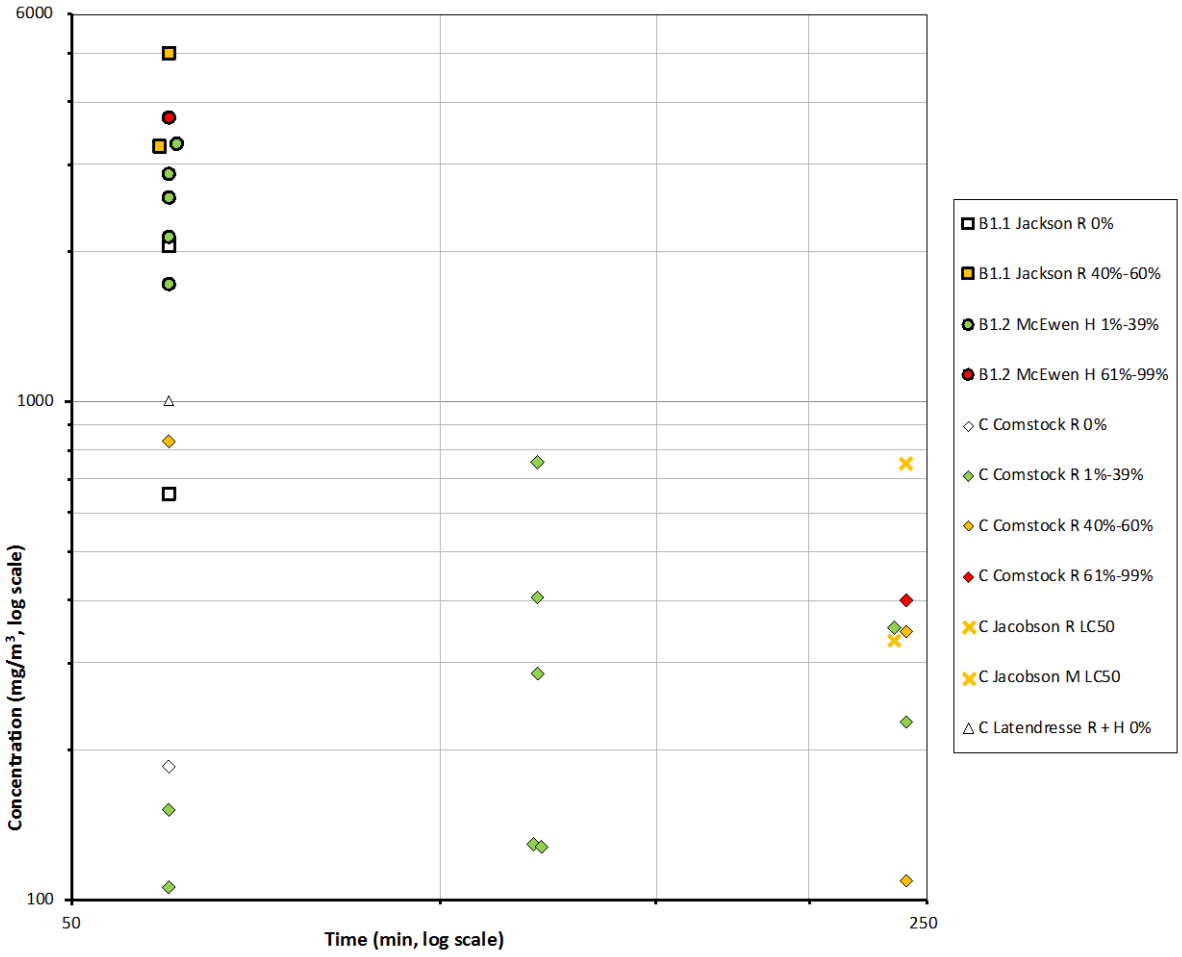


Figure 1 *All available acute lethality data for hydrazine.*

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

Both B1 studies were selected for derivation of the animal probit function for hydrazine. No time-scaling was required, since both LC_{50} values were derived for a 60-minute exposure duration.

Probit functions have been calculated and reported in Appendix 1 for each of the reported studies. The results of the calculations are presented in Table 2.

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

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}, 30 minutes (mg/m^3) 95% C.I.	n-value 95% C.I.
B1.1	Rat	*60-min LC_{50}*	4160 (3291 – 6856)		N/A
B1.2	Hamster	*60-min LC_{50}*	3185 (2676 – 5336)		N/A

The data of the 2 B.1-studies with rats and hamsters are presented graphically below.

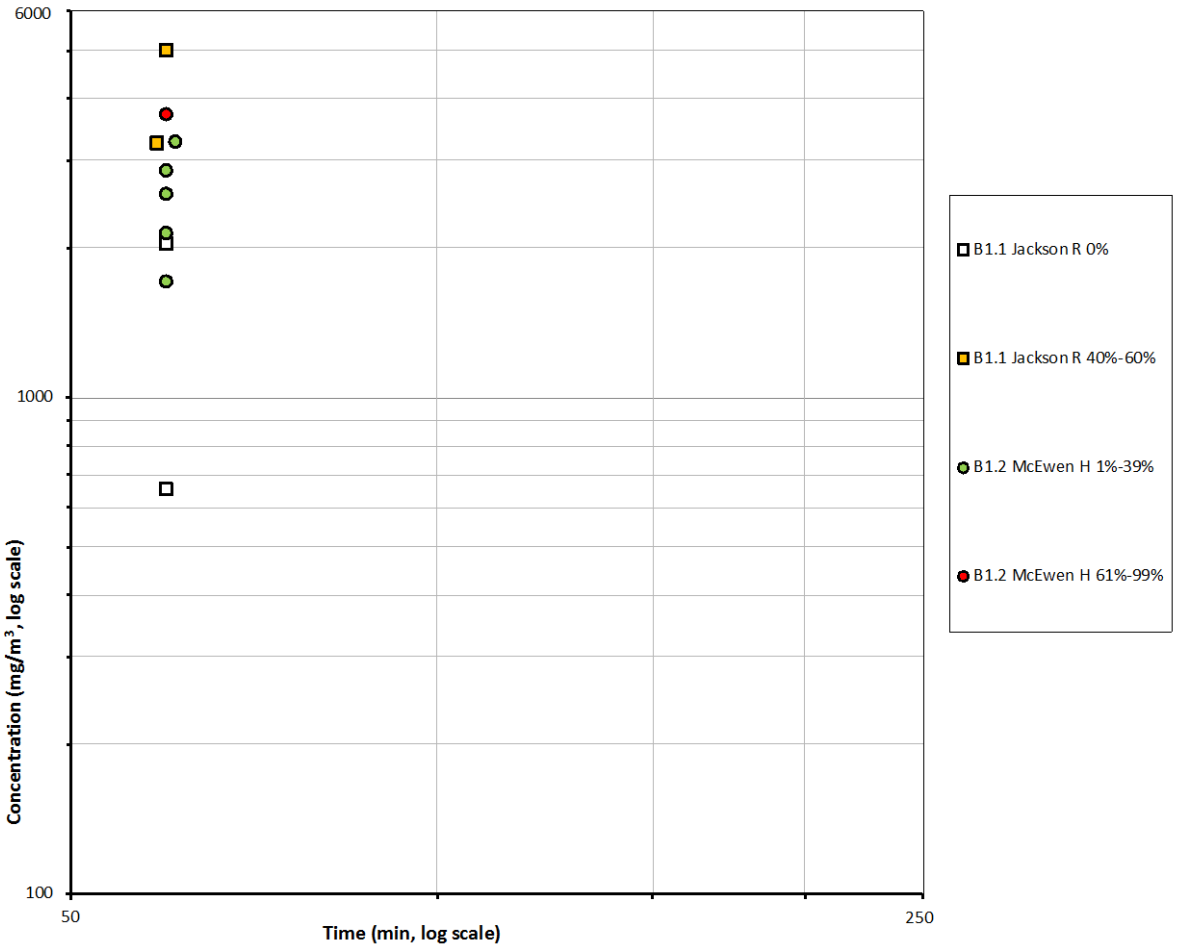


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of hydrazine.*

Based on criteria outlined in the guideline the data from studies B1.1 and B1.2 were selected for the final dataset for the derivation of the animal probit function. These were the only eligible data. 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 2 datasets from 2 studies and includes data from 2 animal species.

Table 2 *Data selected for the derivation of the animal probit function of hydrazine.*

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}, 30 minutes (mg/m^3) 95% C.I.	n-value 95% C.I.
B1.1	Rat	*60-min LC_{50}*	4160 (3291 – 6856)		N/A
B1.2	Hamster	*60-min LC_{50}*	3185 (2676 – 5336)		N/A

The data of the selected datasets are presented graphically below.

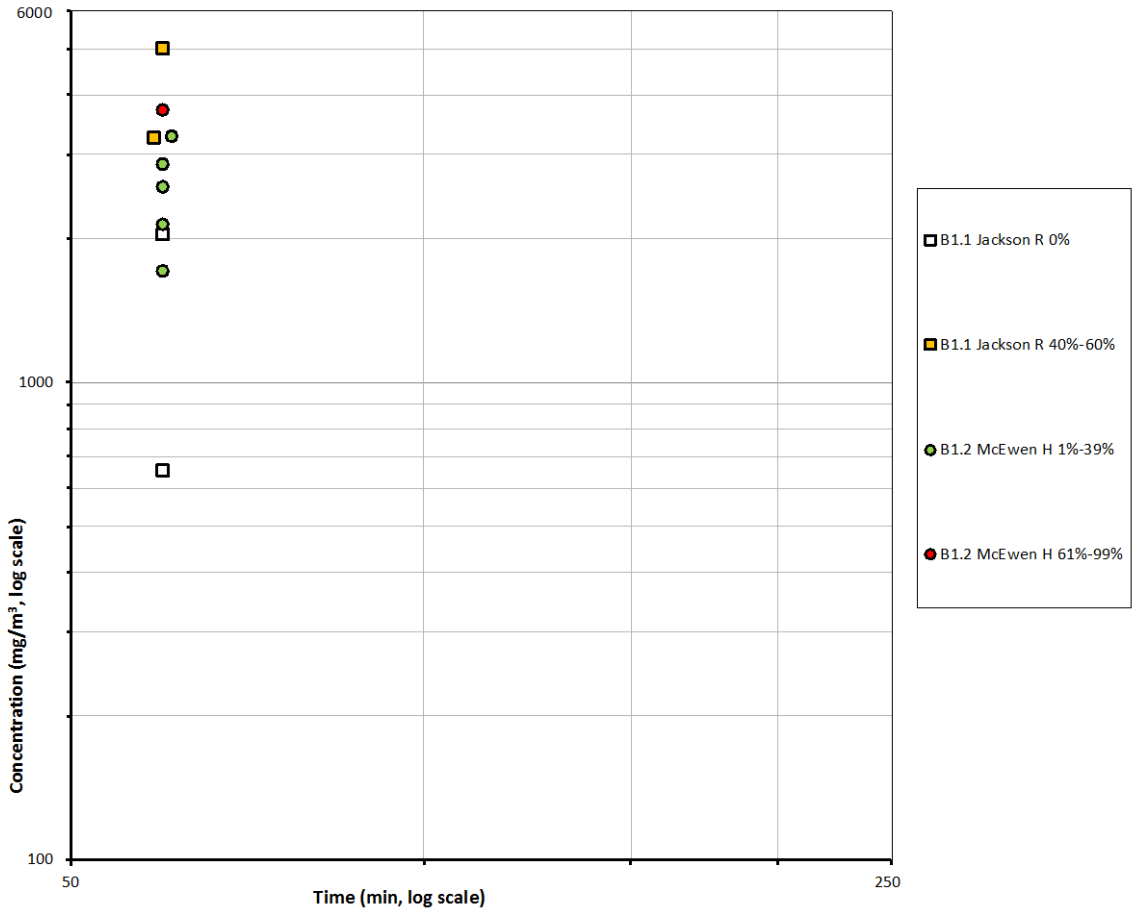


Figure 3 *Final data selected for derivation of the animal probit function of hydrazine (identical to figure 2).*

6. Derivation of the human probit function

To derive the human probit function the results from studies B1.1 and B1.2 have been used to derive a point of departure as outlined above. These were the only eligible studies for derivation of a probit function.

The LC_{50}-values of both B1-studies were available for a common exposure duration of 60 minutes with a rat LC_{50} of 4610 mg/m^3 and a hamster LC_{50} of 3185 mg/m^3.

A geometric mean overall LC_{50}-value was calculated from the 2 available LC_{50} values. The formula for the geometric mean of LC_{50}-values between species is as follows:

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

With $\overline{LC_{50}}$ = geometric mean LC_{50}-value across species
$LC_{50,j}$ = LC_{50}-value of species j.
s = number of species for which LC_{50}-values are pooled (j= 1…s).

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

The Point of Departure for the human probit function is a 60-minute geometric mean animal LC_{50} value of 3640 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	No rationale to deviate from the default interspecies AF.
Nominal concentration	1	Two studies with analytically determined concentrations.
Adequacy of database:	1	Reasonable database with 2 B1 studies in 2 species.

The estimated human equivalent 60-minute LC_{50} value is 3640 / 3 = **1213 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 60 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): 5 = a + 1 × ln (1213^2× 60) resulting in the a-value of **-13.297**.

Pr = -13.3 + 1 × ln (C^2 × 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 2 studies in the rat and hamster with B1 quality, 100 animals, an exposure duration of 60 minutes and a response rate from 0%-90%.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 259 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 379 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	367	259
1% lethality, this probit	536	379
AEGL-3[1] (2010, final)	60	47
ERPG-3[1] (2013)		40
LBW (2016)	90	71

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

[1] 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: ***Jackson (Huntingdon RC) 1993***
Substance: Hydrazine
Species, strain, sex: Rat, male and female Sprague-Dawley
Number/sex/conc. group: 5 / sex / group
Age and weight: 6-8 weeks old, 192 - 229 grams
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)	*Statement of compliance with OECD guideline 403 provided*
Stability of test compound in test atmosphere	*Exposure was to an liquid droplet 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*
Type of restrainer	*Perspex restraining tube, n.o.s.*
Pressure distribution	*No information provided.*
Homogeneity of test atmosphere in breathing zone of animals	*The 64% aqueous solution was aerosolized in an air stream of 25 ℓ/min and fed into the exposure unit. For the 650 mg/m^3 concentration a Devilbiss nebuliser was used, for the other groups a stainless-steel atomiser.*
Number of air changes per hour	*2.5 l/min/animal*
Equilibration time (t95)	*25 l/min through an approx. 50 l chamber: t95 = 6 min.*
Start of exposure relative to equilibration	*At start of concentration build-up; after completion of the exposure the chamber was allowed to clear before removal of the test animals.*
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. Apparently large variability in MMAD.*
Assessment of Reliability	***B1*** *Reasonably well conducted study. Only 1 exposure duration.*

Results

Species	Concentration (mg/m^3)	MMAD ± GSD (µm)	% respirable (<6 µm)	Exposure duration (min)	Lethality	
Rat	Hydrazine				Male	Female
Group	0			60	0/5	0/5
2	650	5.0 ± 2.56 [2]	53.8	60	0/5	0/5
3	2040	1.1 ± 3.56	89.5	60	0/5	0/5
4	3240	2.4 ± 3.04	84.4	60	1/5	3/5
5	4980	1.8 ± 2.40	83.1	60	2/5	4/5

All lethality occurred within 3 days after exposure.

The nominal-to-analytical ratio was about 3, except for group 2 (where it equalled 8). Due to the deviating aerosol generation system, nominal-to-analytical ratio and MMAD, the data from group 2 were not included in the calculations.

Probit function

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

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

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

Probit function	Species	a	b	d
Sex as variable	Rat	-17.6	2.65	1.04
Sexes combined	Rat	-14.8	2.38	

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
60	5060 (3643 – 10730)	3413 (2345 – 5175)	4160 (3291 – 6856)

The authors calculated the LC50 values as follows: 5.8 mg/l (males), 3.4 mg/l (females), 4.2 mg/l (sexes combined). No C × t probit function could be calculated from these data alone.

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

Study ID: B1.2

Author, year: ***McEwen 1975***
Substance: Hydrazine
Species, strain, sex: Male golden Syrian hamsters
Number/sex/conc. group: 10 / 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	*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	*The entire chamber air flow was sent through an evaporator which consisted of a heated Teflon line. The temperature was chosen to ensure evaporation but prevent decomposition.*
Number of air changes per hour	*1-2 cubic feet / min in a 3.5 cubic feet chamber, therefore 17-34 air changes/hour.*
Equilibration time (t95)	*Calculated t95 = 5-10.5 minutes*
Start of exposure relative to equilibration	*After complete equilibration of the chamber atmosphere, the animals were quickly introduced into the chamber with a sliding cage drawer.*
Actual concentration measurement	*Iodine absorber solution was mixed with the sampled air inside the chamber and determined with colorimeter outside the chamber. Colorimetric method was calibrated daily with standard gas mixtures.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*No mention of aerosol*
Assessment of Reliability	***B1*** *Well conducted study.* *Only 1 exposure duration.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality males
Hamster	Measured	Adjusted		Dead/tested
	1709		60	2/10
	2136		60	2/10
	2564		60	3/10
	2857		60	3/10
	3271		60	3/10
	3699		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.

Probit function	Species	a	b
	Hamster	-10.7	1.95

Sex differences could not be examined because only male animals have been tested.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Male
60	3185 (2676 – 5336)

Study ID: C studies

Comstock et al (1954) exposed male and female rats (5/sex/concentration) whole-body to essentially saturated hydrazine vapor for 0.5, 1, 2 and 4 hours. In test series 1, animals were exposed after equilibration of the test chamber, but only nominal concentrations were reported. In test series 2, build-up of the test atmosphere was initiated with the animals in the chamber but concentrations were determined analytically. The nominal-to-analytical ratio was 40-80, possibly partially due to the lack of active mixing of the chamber atmosphere in combination with a sampling rate that equalled the rate of introduction of test substance into the chamber (both 2 l/min in a 440 l exposure chamber). C×t calculations with the data produced a very poorly fitting model, which corresponds well with the lack of an apparent systematic dose response relationship in the data.

Jacobson (1955) Groups of 10 male rats and 10 female mice (strain, age and weight unspecified) were exposed to hydrazine for 4 hours (probably whole-body) and observed for 14 days. The authors reported LC_{50} values of 750 mg/m^3 for rats and 330 mg/m^3 for mice. Lethality percentages have to be assessed form a graph which also includes datapoints for a number of methylated hydrazines.

Latendresse (1995) exposed rats (5/sex) and hamsters (10 males) to 1000 mg/m^3 for 1 hour as part of a study to determine the oncogenic potential of hydrazine. All animals survived the exposure and the observation period that followed (duration unknown).

Appendix 2 Reference list

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Jacobson, KH, JH Clem, HJ Wheelwright Jr., WE Rinehart, N Mayes. The acute toxicity of the vapors of some methylated hydrazine derivatives. A.M.A. Arch. Ind. Health 1955;12: 609-616.

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