



Probit function technical support document

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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). 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.

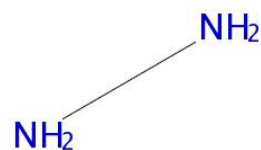
The decision on actual implementation depends on the results of a further consequence analysis.

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

1 Technical support document hydrazine

1. Substance identification

CAS-number:	302-01-2
IUPAC name:	Hydrazine
Synonyms:	Diamine
Molecular formula:	N ₂ H 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)
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:

- 1 • Substance name and synonyms
- 2 • CAS number
- 3 • lethal*
- 4 • mortal*
- 5 • fatal*
- 6 • LC₅₀, LC
- 7 • probit

8 3. Unpublished data were sought through networks of toxicological scientists.

9

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

15

16 **Sensory irritation**

17 No studies were identified in which sensory irritation was studied.

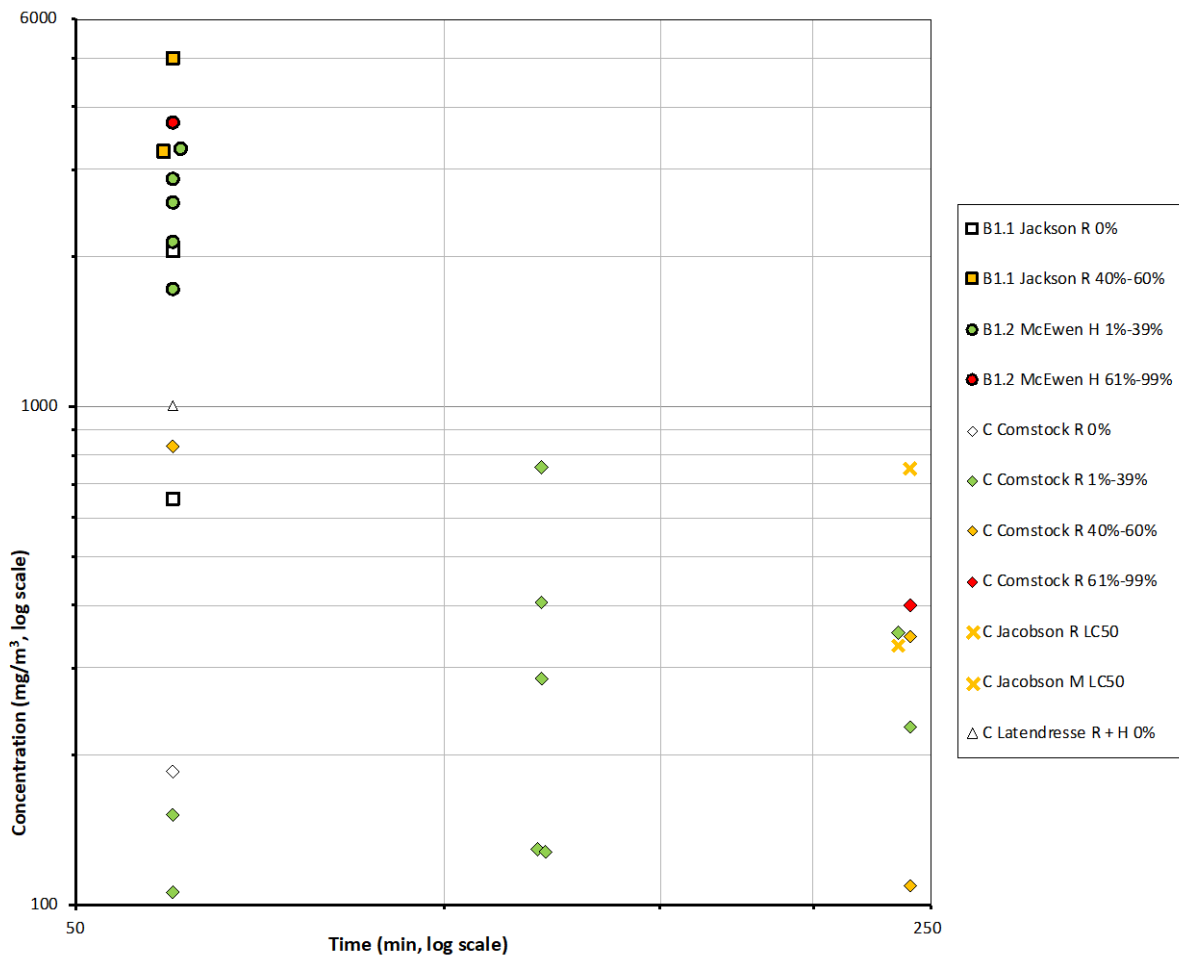
18

19

20 **5. Probit functions from individual studies**

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

22



23

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

25

26

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

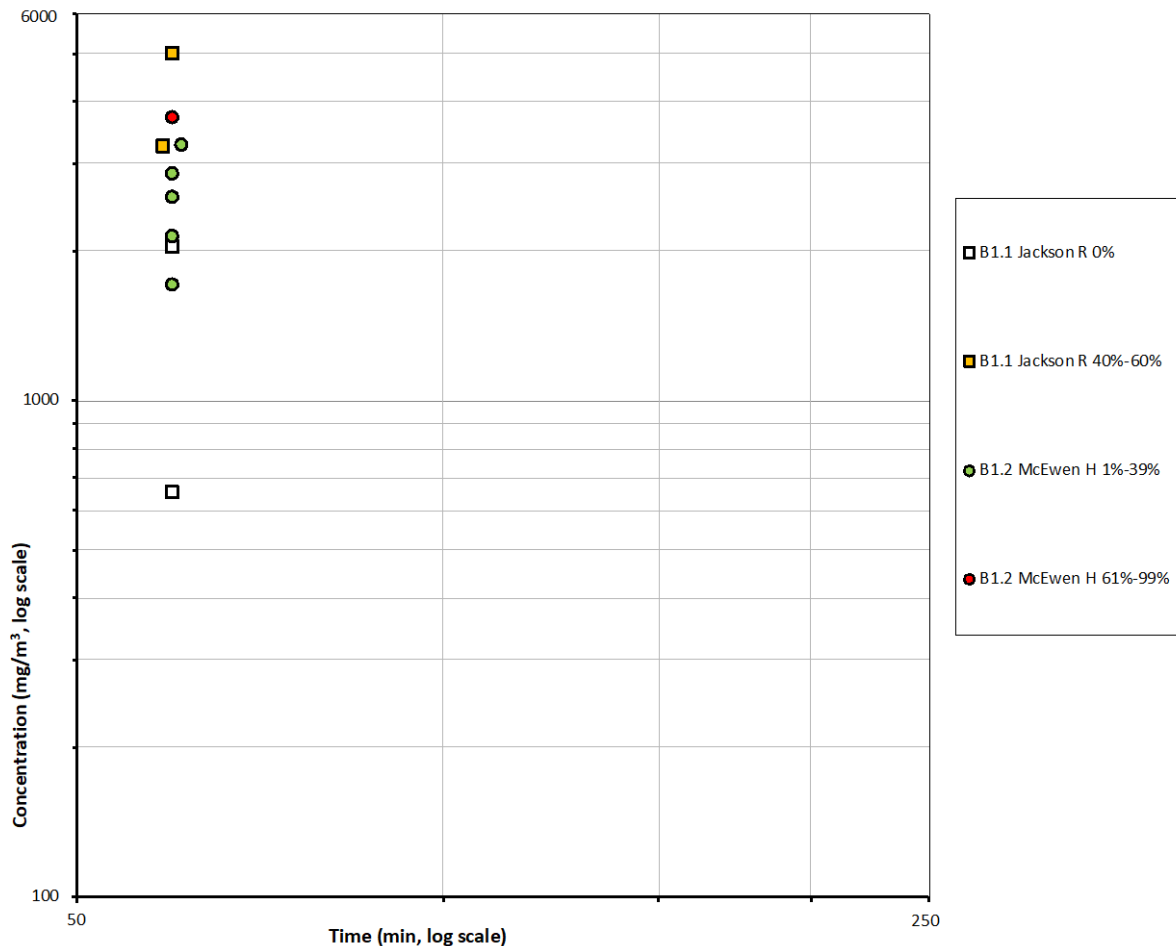
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 2 Both B1 studies were selected for derivation of the animal probit function for
 3 hydrazine. No time-scaling was required, since both LC₅₀ values were derived for a
 4 60-minute exposure duration.

5
 6 Probit functions have been calculated and reported in Appendix 1 for each of the
 7 reported studies. The results of the calculations are presented in Table 2.

8
 9 **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

10
 11 The data of the 2 B.1-studies with rats and hamsters are presented graphically below.
 12



13
 14 **Figure 2** Data selected for the initial analysis for the derivation of the animal probit
 15 function of hydrazine.
 16
 17

18 Based on criteria outlined in the guideline the data from studies B1.1 and B1.2 were
 19 selected for the final dataset for the derivation of the animal probit function. These
 20 were the only eligible data. The data that were selected for final analysis of the
 21 animal probit function are presented in Table 2 and Figure 3.
 22

1 The final data eligible for calculating the animal probit function contains 2 datasets
 2 from 2 studies and includes data from 2 animal species.

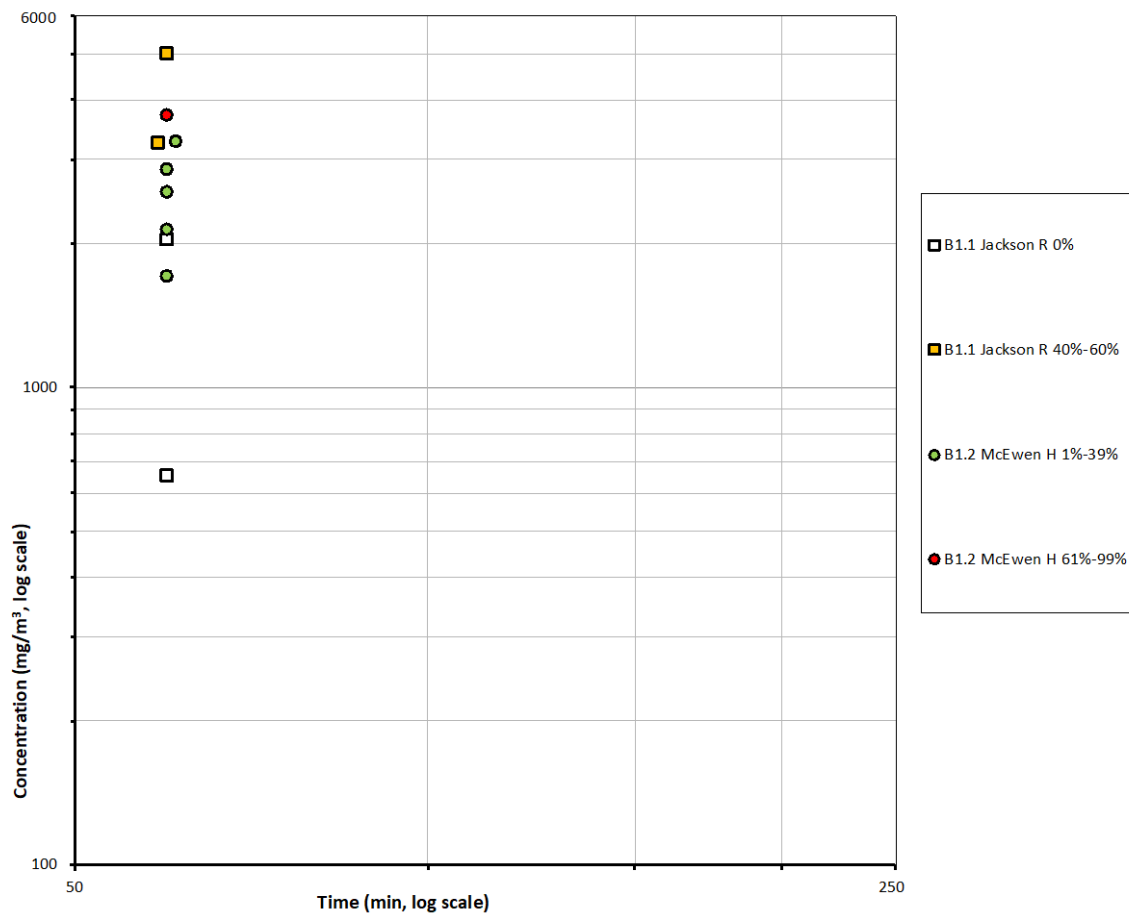
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5 **Table 2** Data selected for the derivation of the animal probit function of hydrazine.

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

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 9

The data of the selected datasets are presented graphically below.



10
 11
 12
 13
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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.

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 16
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The LC₅₀-values of both B1-studies were available for a common exposure duration of 60 minutes with a rat LC₅₀ of 4610 mg/m³ and a hamster LC₅₀ of 3185 mg/m³.

1 A geometric mean overall LC₅₀-value was calculated from the 2 available LC₅₀ values.
 2 The formula for the geometric mean of LC₅₀-values between species is as follows:

3

$$4 \quad \overline{LC}_{50} = \left[\prod_{j=1}^s LC_{50,j} \right]^{(1/s)}$$

5

6 With \overline{LC}_{50} = geometric mean LC₅₀-value across species
 7 LC_{50,j} = LC₅₀-value of species j.
 8 s = number of species for which LC₅₀-values are pooled (j= 1...s).

9

10 No reliable experimentally determined n-value was available, so the default n-value of
 11 2.0 was used.

12

13 The Point of Departure for the human probit function is a 60-minute geometric mean
 14 animal LC₅₀ value of 3640 mg/m³ and a default n-value of 2.

15

16 The human equivalent LC₅₀ was calculated by applying the following assessment
 17 factors:

18

19 **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.

20

21 The estimated human equivalent 60-minute LC₅₀ value is 3640 / 3 = **1213 mg/m³**.

22

23 No reliable experimentally determined n-value was available, so the default n-value of
 24 **2** was used. Assuming a regression coefficient (b×n) of 2 for the slope of the curve,
 25 the b-value can be calculated as 2 / n = **1**.

26

27 The human probit function is then calculated on the human equivalent 60 min LC₅₀
 28 using the above parameters to solve the following equation to obtain the a-value (the
 29 intercept): $5 = a + 1 \times \ln(1213^2 \times 60)$ resulting in the a-value of **-13.297**.

30

31 **Pr = -13.3 + 1 × ln (C² × t) with C in mg/m³ and t in min.**

32

33 The derived human probit function has a scientifically acceptable basis. The probit
 34 function is based on 2 studies in the rat and hamster with B1 quality, 100 animals, an
 35 exposure duration of 60 minutes and a response rate from 0%-90%.

36

37 The calculated human 60 min LC_{0.1} (Pr = 1.91) calculated with this probit equation is
 38 259 mg/m³ and the calculated human 60 min LC₁ (Pr = 2.67) is 379 mg/m³.

39

40

1 **Table 4** *LC-values calculated with the derived probit function compared with*
2 *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

3
4 Compared with equivalent (inter)national guideline levels as presented in the table
5 above, the lethal levels derived with this probit function are higher.
6
7

¹ AEGL and ERPG values were converted from ppm to mg/m³ with the conversion factor calculated in section 1. Therefore, the AEGL and ERPG values in mg/m³ 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.

1 **Results**

Species	Concentration (mg/m ³)	MMAD ± GSD (µm)	% respirable (<6 µm)	Exposure duration (min)	Lethality	
					Male	Female
Rat	Hydrazine					
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

2
3 All lethality occurred within 3 days after exposure.

4
5 The nominal-to-analytical ratio was about 3, except for group 2 (where it equalled 8).
6 Due to the deviating aerosol generation system, nominal-to-analytical ratio and
7 MMAD, the data from group 2 were not included in the calculations.

8
9 **Probit function**

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

$$12 \quad Pr = a + b \times \ln C + d \times S$$

13 with C for concentration in mg/m³ and S for sex (0 = female, 1 = male).
14
15
16

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

17
18 The LC₅₀ values for both sexes did not differ by more than a factor of 2. This does not
19 support the proposition that sex differences exist in the lethal response. For this
20 reason, the data from both sexes were pooled and analysed to derive the animal
21 probit function.
22
23

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)

24
25 The authors calculated the LC₅₀ values as follows: 5.8 mg/l (males), 3.4 mg/l
26 (females), 4.2 mg/l (sexes combined). No C × t probit function could be calculated
27 from these data alone.
28
29
30

² For this group a different aerosol generator was used.

1 **Study ID: B1.2**

2

3 **Author, year:** **McEwen 1975**

4 Substance: Hydrazine

5 Species, strain, sex: Male golden Syrian hamsters

6 Number/sex/conc. group: 10 / group

7 Age and weight: not specified

8 Observation period: 14 days

9

10 **Evaluation of study quality**

Criteria	Comment
Study carried out according to GLP	<i>GLP did not exist at the time</i>
Study carried out according to OECD 403 guideline(s)	<i>OECD guideline 403 did not exist at the time</i>
Stability of test compound in test atmosphere	<i>Stable</i>
Use of vehicle (other than air)	<i>Air</i>
Whole body / nose-only (incl. head/nose-only) exposure	<i>Whole-body</i>
Type of restrainer	<i>N/A, whole-body</i>
Pressure distribution	<i>No information</i>
Homogeneity of test atmosphere in breathing zone of animals	<i>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.</i>
Number of air changes per hour	<i>1-2 cubic feet / min in a 3.5 cubic feet chamber, therefore 17-34 air changes/hour.</i>
Equilibration time (t95)	<i>Calculated t95 = 5-10.5 minutes</i>
Start of exposure relative to equilibration	<i>After complete equilibration of the chamber atmosphere, the animals were quickly introduced into the chamber with a sliding cage drawer.</i>
Actual concentration measurement	<i>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.</i>
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	<i>No mention of aerosol</i>
Assessment of Reliability	B1 <i>Well conducted study. Only 1 exposure duration.</i>

11

12

13

1 **Results**

Species	Concentration (mg/m ³)		Exposure duration (min)	Lethality males
	Measured	Adjusted		Dead/tested
Hamster	1709		60	2/10
	2136		60	2/10
	2564		60	3/10
	2857		60	3/10
	3271		60	3/10
	3699		60	9/10

2

3 **Probit function**

4 The probit function and associated LC-values have been calculated using the

5 DoseResp program (Wil ten Berge, 2016) as

6
$$Pr = a + b \times \ln C$$

7 with C for concentration in mg/m³.

8

Probit function	Species	a	b
	Hamster	-10.7	1.95

9

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

11

12

Duration (min.)	LC ₅₀ (mg/m ³) 95%-C.I. Male
60	3185 (2676 - 5336)

13

14

15

16

1 **Study ID: C studies**

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

14
15 Jacobson (1955) Groups of 10 male rats and 10 female mice (strain, age and weight
16 unspecified) were exposed to hydrazine for 4 hours (probably whole-body) and
17 observed for 14 days. The authors reported LC₅₀ values of 750 mg/m³ for rats and
18 330 mg/m³ for mice. Lethality percentages have to be assessed from a graph which
19 also includes datapoints for a number of methylated hydrazines.

20
21 Latendresse (1995) exposed rats (5/sex) and hamsters (10 males) to 1000 mg/m³ for
22 1 hour as part of a study to determine the oncogenic potential of hydrazine. All
23 animals survived the exposure and the observation period that followed (duration
24 unknown).

Appendix 2 Reference list

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