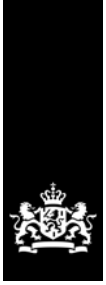


National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Probit function technical support document

Date: 6 June 2017
Document id: 20170606-Ethyleneimine-interim
Status: interim
Author: Marc Ruijten, CrisisTox Consult
commissioned by RIVM

substance name	CAS number
Ethyleneimine	**151-56-4**

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.

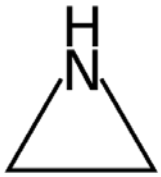
This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have 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 Ethyleneimine

1. Substance identification



CAS-number:	151-56-4
IUPAC name:	Ethyleneimine
Synonyms:	Aziridine
Molecular formula:	$(CH_2)_2$-NH, structural formula:
Molecular weight:	43.1 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	55°C (at 101.3 kPa)
Vapour pressure:	21.5 kPa (at 20°C)
Saturated vapor conc:	215000 ppm = 385.5 g/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.558 ppm (at 20°C and 101.3 kPa)
	1 ppm = 1.793 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H: 300+310-314-330-340-350

2. Mechanism of action and toxicological effects following acute exposure

Acute effects: Ethyleneimine is a very reactive direct acting alkylating agent. Its mode of action is similar to that of nitrogen and sulfur mustards.
Signs of toxicity in animals include eye irritation, respiratory tract irritation, respiratory difficulty and prostration; mice also exhibited complete loss of muscular coordination and convulsions. Congestion occurs in lungs and all internal organs, as well as damage to the kidney tubules, and albuminuria in rats and guinea pigs. 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 (myocardial ischemia) effects. The main cause for lethality is probably when the respiratory damage proceeds to inflammation, degeneration and necrosis of affected tissue, atelectasis, emphysema and finally death. Failure of other internal organs may also play a role.
Long-term effects: Occupational exposure to ethyleneimine has produced skin sensitization, slow healing dermatitis, rapidly reversible irritation to the eyes and respiratory tract, and blistering, reddening, and edema of the scrotum (Carpenter 1948).
IARC classified ethyleneimine as possibly carcinogenic to humans (Group 2B).

3. Human toxicity data

No informative reports on the 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.

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 ethyleneimine, 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:
   - Ethyleneimine 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 2 studies were identified -with 3 datasets for 3 species- with data on lethality following acute inhalation exposure. All 3 datasets were assigned status B for human probit function derivation.

Sensory irritation

No studies were identified in which sensory irritation for ethyleneimine was studied.

5. Probit functions from individual studies

All available acute lethality data on ethyleneimine are provided in Figure 1.

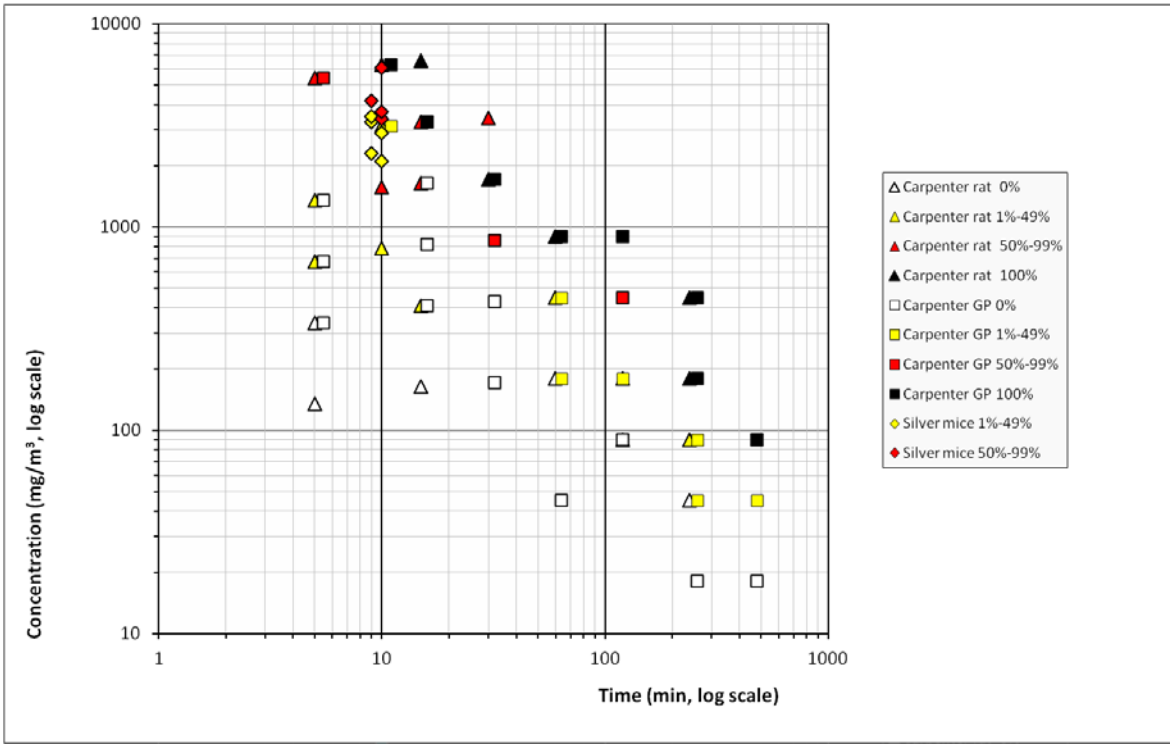


Figure 1 *All available acute lethality data for ethyleneimine*

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

Since studies with A quality were not identified, the probit function was derived using data from the studies with B2 and B1 quality listed in Table 1 below. The details of the calculation of the probit functions are presented in Appendix 1.

To enable intra-species pooling, LC_{50}-value of the B1-study was scaled using the average (rats and guinea pigs) n-value of 1.056 with the following formula:

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

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

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

Study ID	Species	Probit (C in mg/m³, t in min)	LC_{50} at tested exposure duration (mg/m³) 95% C.I.	LC_{50}, 30 minutes (mg/m³) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
B1.1	Mice		3870 - *10 min* (3528 – 4390)	*1367*	NA
B2.1	Rat	-5.08 + 1.08×lnC + 0.88×lnt		718 (575 – 893)	1.223 (1.01-1.44)
B2.2	Guinea pig	-17.5 + 2.25×lnC + 2.65×lnt		978 (809 – 1186)	0.889 (0.80–0.98)

The data of the 3 B studies with rats, guinea pigs and mice are presented graphically in figure 2 below (identical to figure 1).

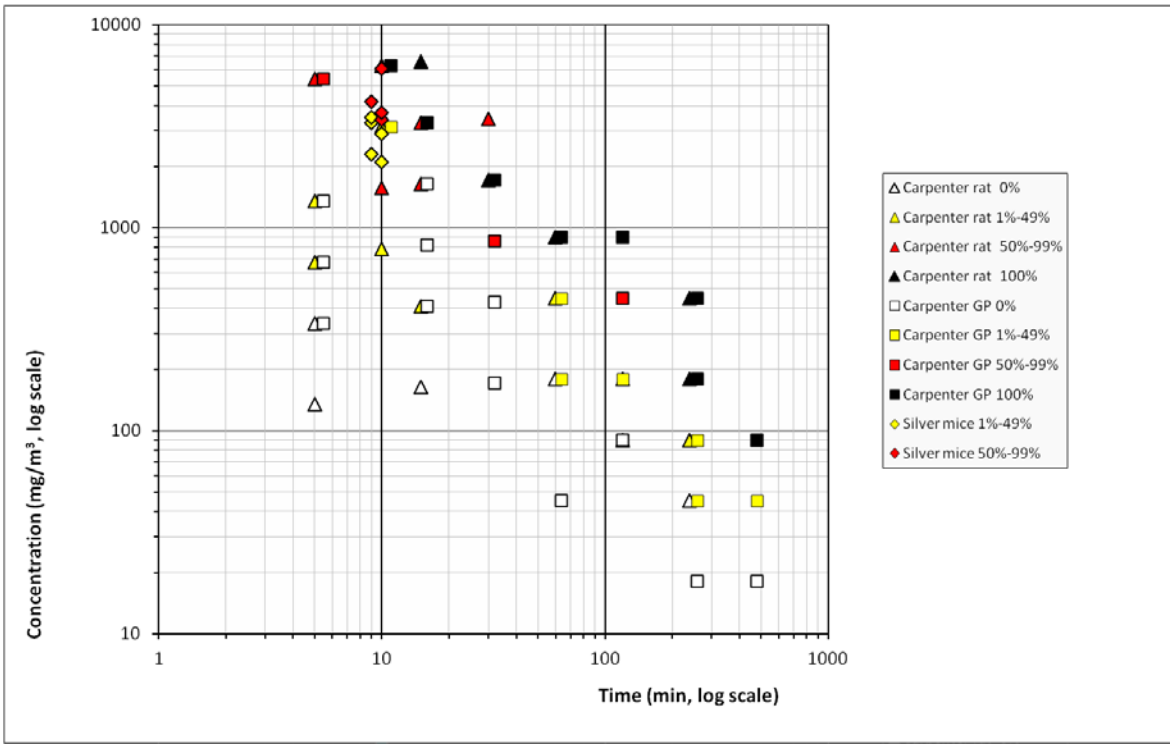


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of ethyleneimine (identical to figure 1)*

Based on visual inspection, criteria outlined in the guideline and comparison of 30-minute LC_{50} values, the data from studies B1.1, B2.1 and B2.2 were selected for the final dataset for the derivation of the animal probit function (Table 2, identical to Table 1 for the present data set). None of the data fit the requirements for derivation of a probit very well. The exposure duration of the B1 study is shorter than desired, and the Carpenter study (B2.1 and B2.2) does not meet current quality standards. Nevertheless, the 30-minute LC_{50} values differ by no more than a factor of 2. This is

considered to be a reasonably narrow range, particularly for a dataset that includes 3 animal species and 2 inhalation laboratories. It might have been chosen to leave the B2 studies out of the derivation, however, in this case they were included because they were considered equally appropriate as the 10-min B1 study.

The final data eligible for calculating the animal probit function contains 3 datasets from 3 studies and includes data from 3 animal species.

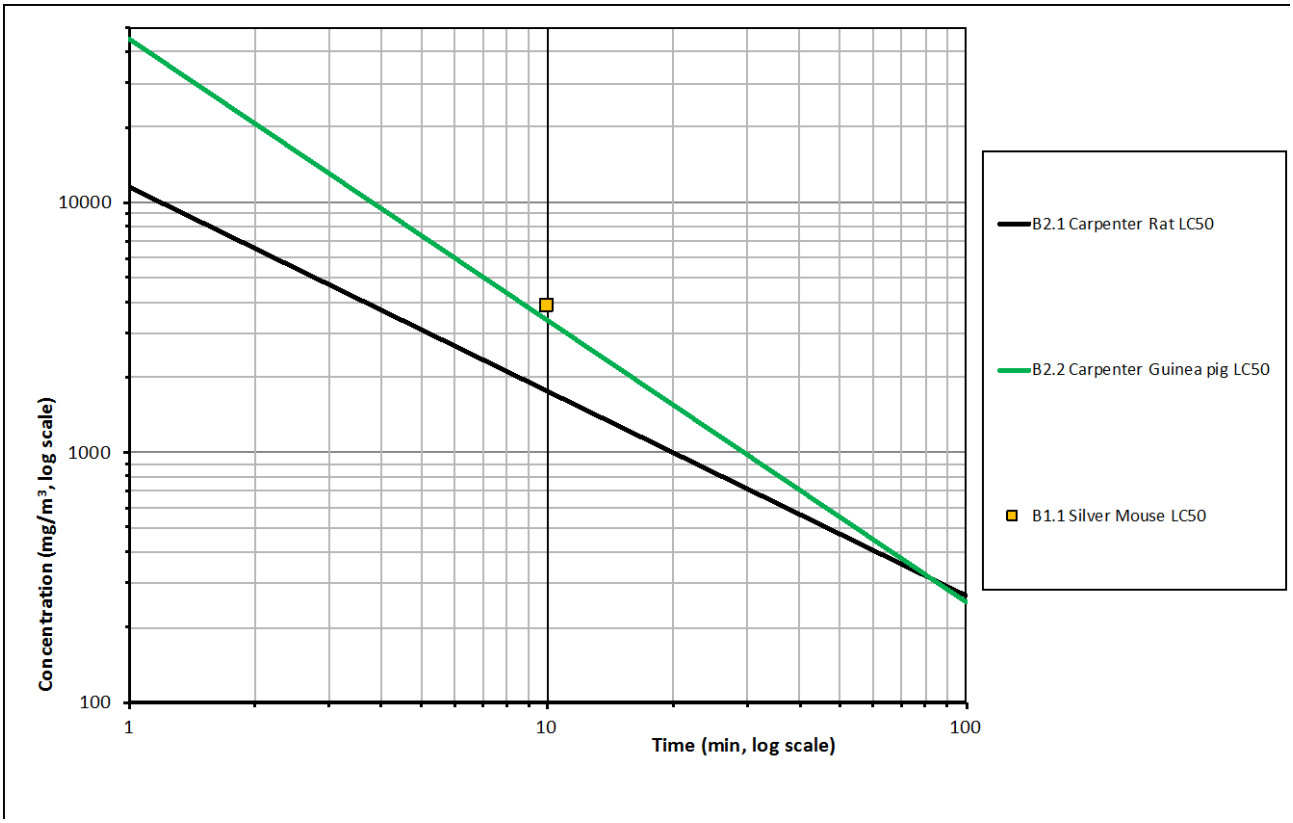


Figure 3 *LC_{50} values of* B1.1, B2.1 and B2.2 *datasets for* ethyleneimine*, over time where available.*

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

Study ID	Species	Probit (C in mg/m³, t in min)	LC_{50} at tested exposure duration (mg/m³) 95% C.I.	LC_{50}, 30 minutes (mg/m³) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
B1.1	Mice		3870 - *10 min* (3528 – 4390)	*1367*	NA
B2.1	Rat	-5.08 + 1.08×lnC + 0.88×lnt		718 (575 – 893)	1.223 (1.01-1.44)
B2.2	Guinea pig	-17.5 + 2.25×lnC + 2.65×lnt		978 (809 – 1186)	0.889 (0.80–0.98)

The data of the selected datasets are presented graphically in figure 4 below (identical to figure 1 for the present data set).

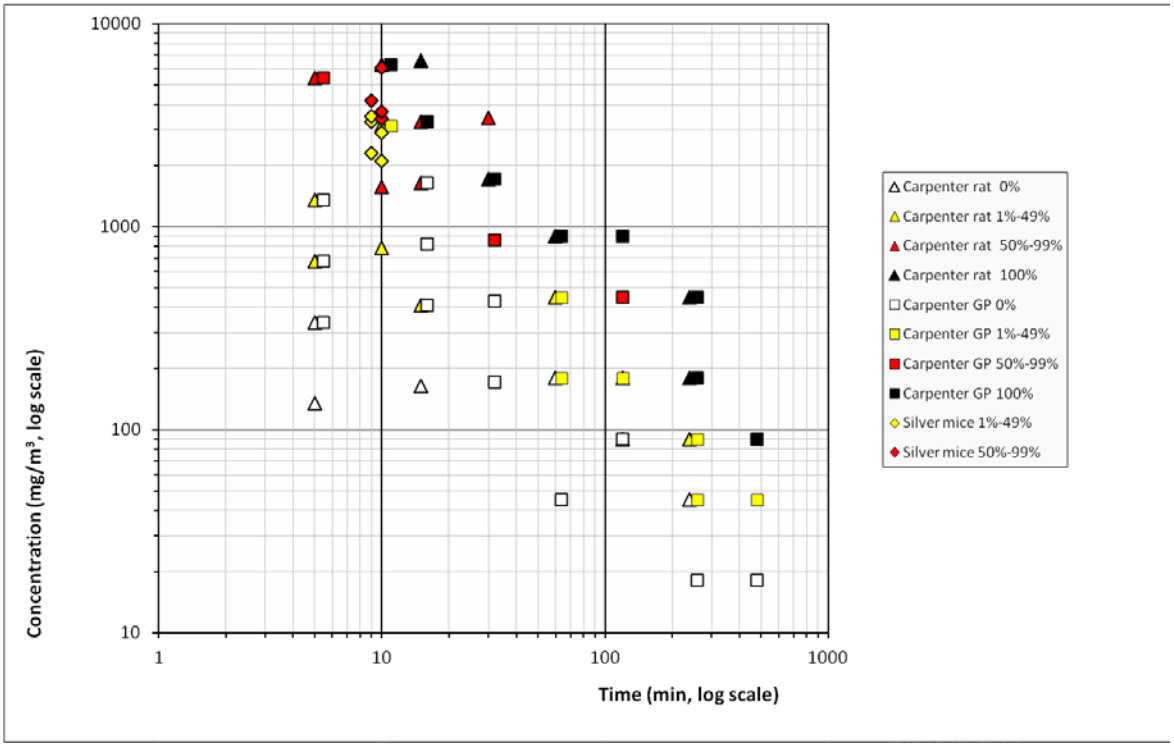


Figure 3 *Final data selected for derivation of the animal probit function of ethyleneimine (identical to figure 1).*

6. Derivation of the human probit function

As a point of departure for deriving the human probit function, the geometric mean LC_{50}-value was calculated from the LC_{50} values of studies B1.1 (Silver), B2.1 and B2.2 (Carpenter), and the arithmetic mean n-value was calculated from studies B2.1 and B2.2 (Carpenter).

First, the arithmetic mean n-value across species was calculated to be 1.056.

Second, the LC_{50}-values of the B1- and B2-studies were calculated for a common exposure duration of 30 minutes. To include the B1-study into intra-species pooling, the LC_{50}-value of the B1-study was scaled using the average (rats and guinea pigs) n-value of 1.056 with the following formula:

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

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

Finally, a geometric mean overall LC_{50}-value was calculated from the available LC_{50} values (1 per species). The overall formula for the geometric mean of time-scaled 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 30-minute weighted geometric mean LC_{50} value was 986.5 mg/m^3, the weighted arithmetic mean n-value was 1.056.

The human equivalent LC_{50} was calculated by applying the following assessment factors:

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	While the main target organ appears to be the respiratory system, other systemic (renal) effects cannot be excluded. Therefore, an AF = 1 seems inappropriate.
Nominal concentration	1	Nominal concentrations were reported below 25% of the Saturated Vapor Concentration.
Adequacy of database:	1	Even though the studies were old, 2 C×t datasets and one 10-min LC_{50} value were available.

The estimated human equivalent 30-minute weighted LC_{50} value is 986.5 / 3 = **329 mg/m^3**.

The n-value was **1.056**, determined as the weighted geometric mean n-value of datasets B2.1 and B2.2. Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1.894**.

The human probit function is then calculated on the human equivalent 10 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1.894 \times \ln(329^{1.056} \times 30)$ resulting in the a-value of **-13.03**.

$Pr = -13.0 + 1.89 \times \ln(C^{1.06} \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically acceptable basis. The probit function is based on 3 studies in rats, guinea pigs and mice with B1 and B2 quality, involving 170 rats, 191 guinea pigs and 180 mice. Exposure levels ranged from 45 – 7200 mg/m^3 for exposure durations ranging from 5 – 480 minutes.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 52 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 36 mg/m^3.

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
0.1% lethality, this probit	69	36
1% lethality, this probit	101	52
AEGL-3 (2008, final)	34	18
ERPG-3 (NA)		NA
LBW (2015)	27	15

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are higher. The most probable rationale is the inclusion of guinea pig and mouse data in the calculation of this probit function. The AEGL values were solely based on the rat data, the LBW was derived from the AEGL values. In addition, AEGL values have been set with higher assessment factors to allow a wider margin of safety for susceptible individuals.

Appendix 1 Animal experimental research

Study ID: B1.1

Author, year: Silver 1948
Substance: ethyleneimine
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. Condensation unlikely given the high vapour pressure.*
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	2.1×10^3	10	20	3
	2.3×10^3	10	20	3
	2.9×10^3	10	20	7
	3.3×10^3	10	20	3
	3.4×10^3	10	20	10
	3.5×10^3	10	20	4
	3.7×10^3	10	20	9
	4.2×10^3	10	20	13
	6.1×10^3	10	20	18

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*	*-12.9*	*2.17*	*N/A*

Duration (min)	*LC_{50} (mg/m^3) 95%-C.I.* ***Sex unknown***
10	3870 (3528 - 4390)

No graphical overview of the data is presented.

Study ID: B2.1

Author, year: Carpenter 1948
Substance: ethyleneimine
Species, strain, sex: male Wistar albino rats
Number/sex/conc. group: 5-12 /concentration-time combination (mostly 6), with a total of 30 groups
Age and weight: age unspecified, weight mostly 90-120 grams, range 60-180 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. Many details missing. Exposure was in a 10 ℓ chamber, which is very small for 6 animals.*
Stability of test compound in test atmosphere	*No mention of stability issues. Condensation unlikely given the high vapour pressure.*
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 at breathing zone of animals	*The compound was delivered into an evaporator by a syringe driven by a synchronous electric motor.*
Number of air changes per hour	*8 ℓ/min in a 10 ℓ chamber which equals 48 Air Changes/h.*
Equilibration time (t95)	*Calculated t95 = 3.75 min*
Start of exposure relative to equilibration	*Not described, but probably at start of concentration build-up*
Actual concentration measurement	*No chamber concentration measured, only nominal (or maybe even target[1]) concentrations reported.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*
Assessment of Reliability	***B2*** *Study has not been described (and probably performed) consistent with current standards, and only nominal (or even target) exposure levels were provided.*

Short-term exposure data

In this study animals have probably been placed in the exposure chamber before equilibrium of the test atmosphere has been reached, and retracted from the chamber at the designated exposure duration. While the procedure was not described in detail, there is no mention of equilibration or insertion of animals after equilibrium was reached. Rather, the authors indicated that they only had a limited supply of test

[1] The concentrations as presented in the publication are 4000, 2000, 1000, 500, 250, 100, 50 and 25 ppm.

material and tried to be as efficient with the material as possible, which explained the small inhalation chamber size. Therefore, the concentrations of all exposure durations less than 3 × T95 (i.e. 11.25 min) have been adjusted. All calculations have been performed with the adjusted concentrations.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Reported	Adjusted		Exposed	fatal
Rat	7172	5412	5	6	4
	7172	6276	10	6	5
	7172		15	6	6
	3586	3138	10	6	1
	3586		15	6	5
	3586		30	6	5
	1793	1353	5	5	1
	1793	1569	10	6	4
	1793		15	6	5
	1793		30	6	6
	897	678	5	6	1
	897	785	10	6	2
	897		15	6	3
	897		30	6	5
	897		60	6	6
	448	338	5	6	0
	448		15	6	1
	448		60	6	2
	448		120	6	3
	448		240	6	6
	179	135	5	6	0
	179		15	6	0
	179		60	6	0
	179		120	6	1
	179		240	6	6
	90		120	6	0
	90		240	5	2
	90		480	6	5
	45		240	6	0
	45		480	6	1

Probit function

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

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

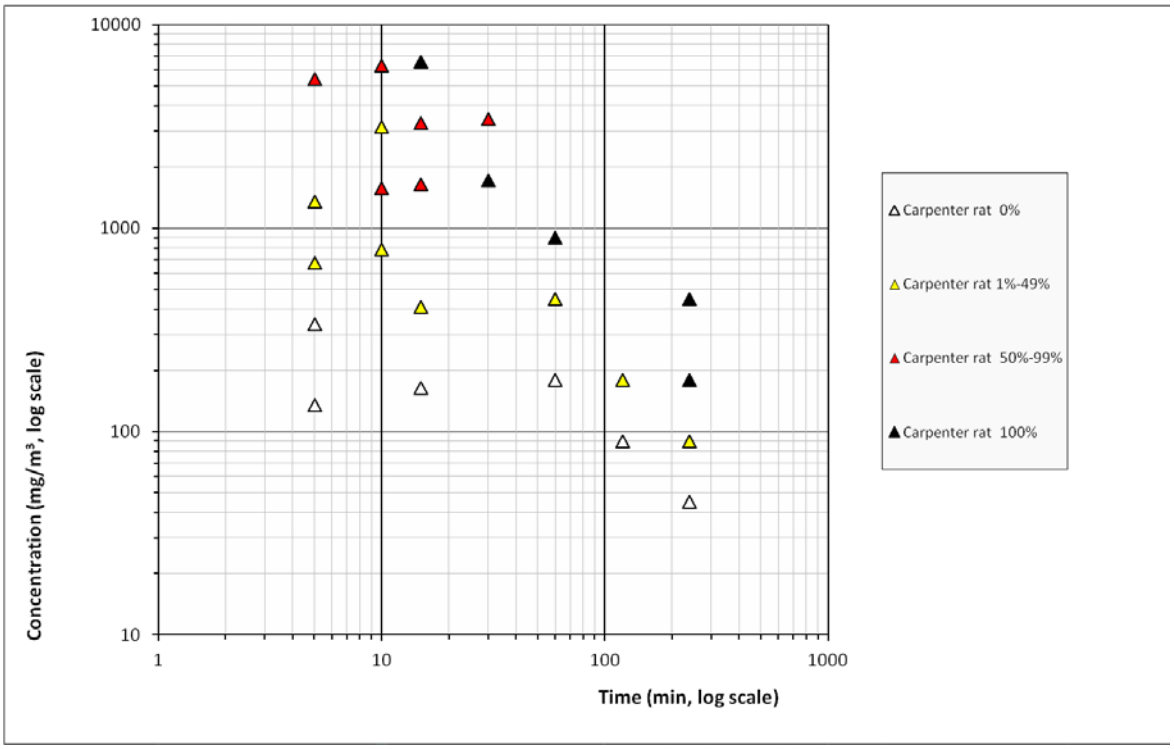
with C for concentration in mg/m^3, t for time in minutes.

Probit function	Species	a	b	c	n-value
Male rat all data	Rat	-5.08	1.08	0.88	1.22 (1.01 - 1.44)

For reason of comparison with the previous methodology the probit function and LC_{50} values for all data and a subselection of data > 5 min are both provided. In both cases adjusted concentrations have been used. For all further calculations the parameter estimates from the model including all data has been used.

Duration (min)	LC_{50} (mg/m^3) 95%-C.I.
10	1762 (1298 - 2379)
30	718 (575 – 893)
60	407 (326 – 507)

A graphical overview of the data is presented below. Each concentration-time combination (with 6 male animals) represents one point in the plot.



Study ID: B2.2

Author, year: Carpenter 1948
Substance: ethyleneimine
Species, strain, sex: guinea pigs, male and female
Number/sex/conc. group: 6 animals 'of mixed sex'
Age and weight: age unspecified, weight mostly 250-300 grams, range 210-390 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. Many details missing. Exposure was in a 10 ℓ chamber, which is very small for 6 animals.*
Stability of test compound in test atmosphere	*No mention of stability issues. Condensation unlikely given the high vapour pressure.*
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 at breathing zone of animals	*The compound was delivered into an evaporator by a syringe driven by a synchronous electric motor.*
Number of air changes per hour	*8 ℓ/min in a 10 ℓ chamber which equals 48 Air Changes/h.*
Study carried out according to GLP	*GLP did not exist at the time*
Equilibration time (t95)	*Calculated t95 = 3.75 min*
Start of exposure relative to equilibration	*Not described, but probably at start of concentration build-up*
Actual concentration measurement	*No chamber concentration measured, only nominal (or maybe even target[2]) concentrations reported.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*
Assessment of Reliability	***B2*** *Study has not been described (and probably performed) consistent with current standards, and only nominal (or even target) exposure levels were provided.*

Short-term exposure data

In this study animals have probably been placed in the exposure chamber before equilibrium of the test atmosphere has been reached, and retracted from the chamber at the designated exposure duration. While the procedure was not described in detail, there is no mention of equilibration or insertion of animals after equilibrium was reached. Rather, the authors indicated that they only had a limited supply of test

[2] The concentrations as presented in the publication are 4000, 2000, 1000, 500, 250, 100, 50 and 25 ppm.

material and tried to be as efficient with the material as possible, which explained the small inhalation chamber size. Therefore, the concentrations of all exposure durations less than 3 × T95 (i.e. 11.25 min) have been adjusted. All calculations have been performed with the adjusted concentrations.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Reported	Adjusted		Exposed	fatal
Guinea pig	7172	5412	5	6	4
	7172	6276	10	6	6
	3586	3138	10	12	1
	3586		15	6	6
	1793	1353	5	6	0
	1793		15	6	0
	1793		30	6	6
	897	678	5	6	0
	897		15	6	0
	897		30	6	5
	897		60	6	6
	897		120	6	6
	448	338	5	6	0
	448		15	6	0
	448		30	6	0
	448		60	6	2
	448		120	6	5
	448		240	6	6
	179		30	6	0
	179		60	6	1
	179		120	6	1
	179		240	6	6
	90		120	6	0
	90		240	5	2
	90		480	6	6
	45		60	12	0
	45		240	5	2
	45		480	6	2
	18		240	6	0
	18		480	6	0

Probit function

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

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

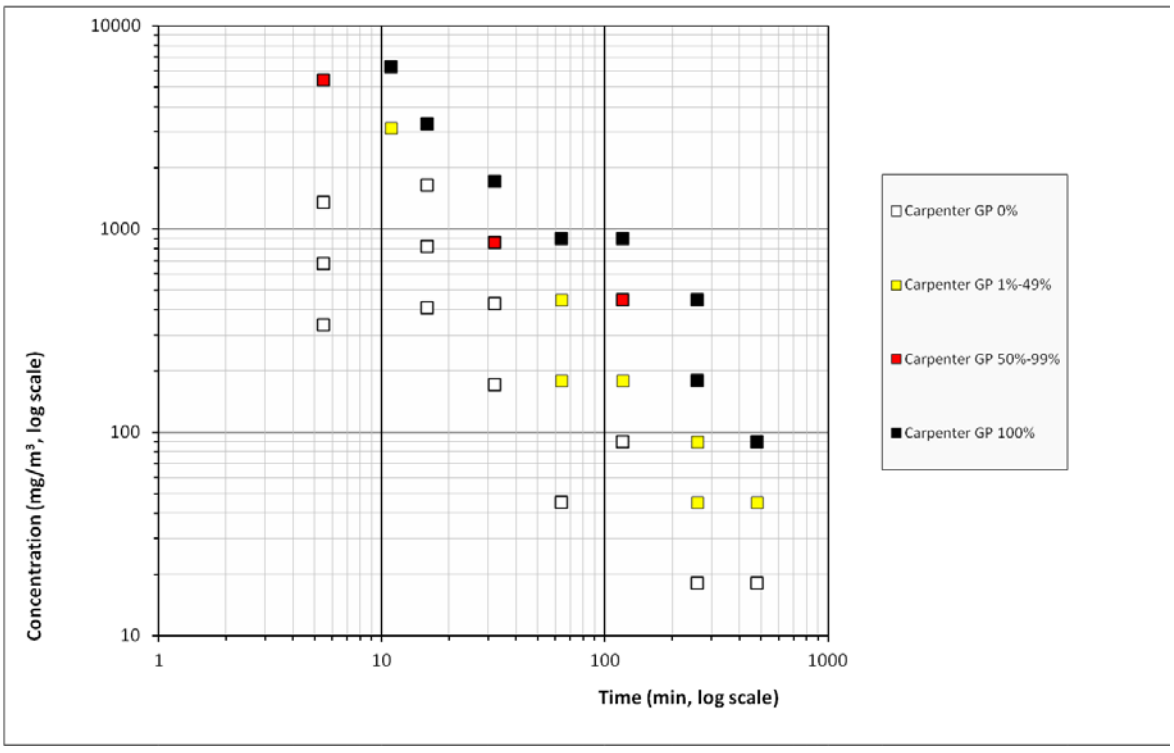
with C for concentration in mg/m^3, t for time in minutes.

Probit function	Species	a	b	C	n-value
All data	Guinea pig	-17.5	2.10	2.37	0.89 (0.80 – 0.98)

In all calculations adjusted concentrations have been used. The number of males and females in each exposure group was not reported, nor was the mortality by sex. Therefore, these data do not allow to estimate sex specific mortality.

Duration (min)	LC_{50} (mg/m^3) 95%-C.I.
10	3365 (2589 - 4431)
30	978 (809 – 1186)
60	448 (372 – 540)

A graphical overview of the data is presented below. Each concentration-time combination (with 6 animals, mixed male and female) represents one point in the plot.



Appendix 2 Reference list

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