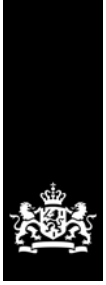


National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Probit function technical support document

Date: 6 June 2017
Document id: 20170606-propyleneimine-INTERIM
Status: interim
Author: Marc Ruijten
on behalf of RIVM

substance name	CAS number
Propyleneimine	**75-55-8**

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.

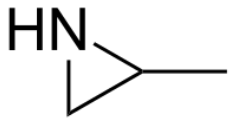
This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have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

1. Substance identification



CAS-number:	75-55-8
IUPAC name:	propyleneimine
Synonyms:	2-methylaziridine, 2-propyleneimine, methylethyleneimine
Molecular formula:	C_3H_7N, structural formula:
Molecular weight:	57.1 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	63°C (at 101.3 kPa)
Vapour pressure:	22 kPa (at 20°C)
Saturated vapor conc:	220000 ppm = 523 g/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.421 ppm (at 20°C and 101.3 kPa)
	1 ppm = 2.375 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H300-310-318-3330-350

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

Special considerations: the toxicity of propyleneimine is thought to be qualitatively similar but less potent than that of ethyleneimine, for which more acute lethality data are available. The description of toxic effects below was taken from the ethyleneimine document. The LT_{50} value in rats for 500 ppm exposure was 226 min. for propyleneimine and about 15 minutes for ethyleneimine, which gives an indication of relative potency.

Acute effects: Ethyleneimine is a very reactive direct acting alkylating agent. It's 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 exhibit 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 tract results in mucus secretion, upper airway and/or pulmonary oedema and laryngospasm. The resulting hypoxemia will cause CNS and cardiovascular (myocardial ischemia) effects. 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 ethylenimine has produced skin sensitization, slow healing dermatitis, rapidly reversible irritation to the eyes and respiratory tract, and blistering, reddening, and edema of the scrotum.

IARC classified propyleneimine as possibly carcinogenic to humans (Group 2B).

3. Human toxicity data

No informative reports on human toxicity following acute inhalation exposure were identified in which details about both 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 propyleneimine, covering references before and including 1995.

[1] References for mechanism of action and toxicological effects following acute exposure.

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 2 studies were identified -with 4 datasets for 2 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 2 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 propyleneimine are provided in Figure 1.

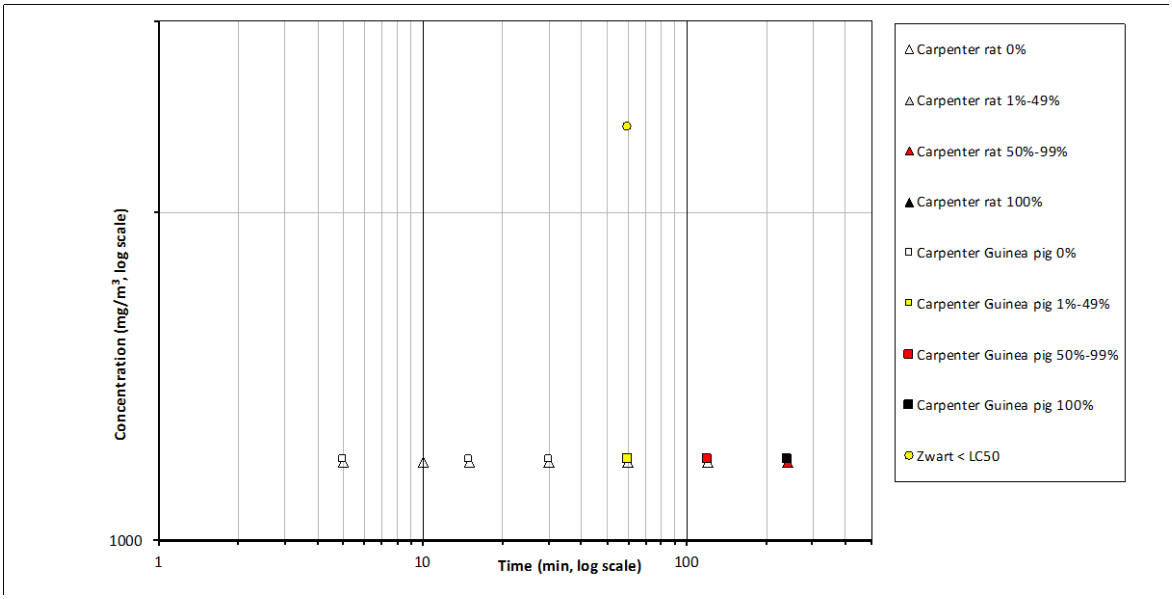


Figure 1 *All available acute lethality data for propyleneimine. The 1-hour LC_{50} determined by Zwart was > 2400 mg/m³.*

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

The only data available to derive a probit function were from two datasets with B1 quality. Probit functions have been calculated and reported in Appendix 1 for study B1.1. The results of the calculations are presented in the Table below. The scaled 30-minute values were calculated with the n-value of 1.056 derived for *ethyleneimine*, a structural analogue chemical 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

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

Study ID	Species	Probit (C in mg/m^3, t in min)	LT_{50} (min) at tested exposure concentration (95% C.I.)	LC_{50}, 30 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
B1.1	Rat	*1188-mg/m^3 - LT_{50}*	226 @ *1188 mg/m^3* (cfd-i not assessable)	*8041*	N/A
B1.1	Guinea pig	*1188-mg/m^3 - LT_{50}*	98 @ *1188 mg/m^3* (62.2 – 161)	*3645*	N/A

The data of the study B1.1 with rats and Guinea pigs are presented graphically below.

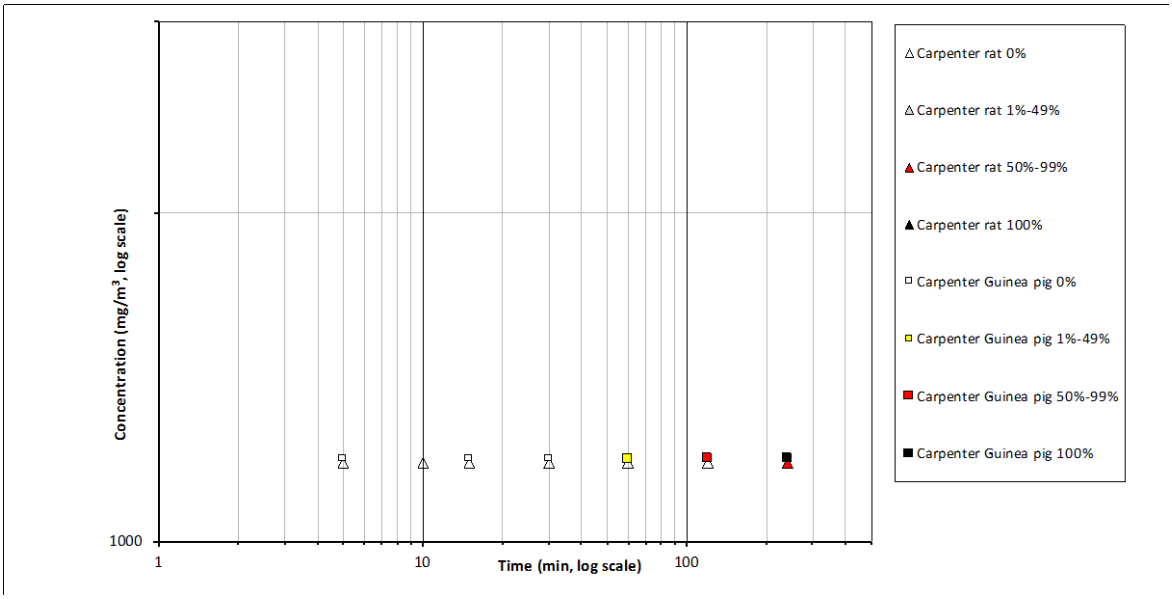


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of propyleneimine.*

Based on the equal reliability of the LT_{50} values, the guinea pig and rat data from study B1.1 were selected for the final dataset for the derivation of the animal probit function.

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

Study ID	Species	Probit (C in mg/m^3, t in min)	LT_{50} at tested exposure concentration (min) 95% C.I.	LC_{50}, 30 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
B1.1	Rat	*1188-mg/m^3 - LT_{50}*	226 – *1188 mg/m^3* (cfd-i not assessable)	*8041*	N/A
B1.1	Guinea pig	*1188-mg/m^3 - LT_{50}*	98 – *1188 mg/m* (62.2 – 161)	*3645*	N/A

The data of the selected dataset are presented graphically below.

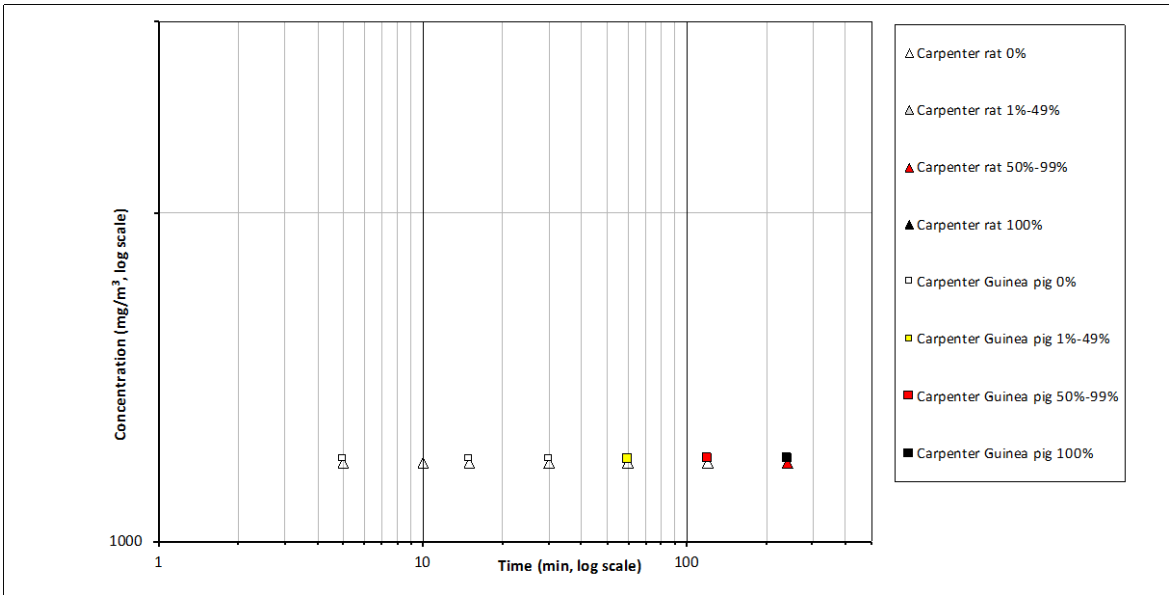


Figure 3 Final data selected for derivation of the animal probit function of propyleneimine (identical to figure 2).

6. Derivation of the human probit function

To derive the human probit function the Guinea pig and rat data from study B1.1 have been used to derive a point of departure. The reason was that these were the only data that allowed for the calculation of a confidence interval of the LT_{50} value.

As a point of departure for deriving the human probit function, the geometric mean LT_{50}-value was calculated from the LT_{50} values of the rat and guinea pig datasets of study B1.1.

The geometric mean 1188 mg/m^3 LT_{50} value was 148.8 minutes, which will be treated as a 148.8-minute geometric mean LC_{50} value of 1188 mg/m^3. The n-value derived for the *structurally analogue chemical ethylene imine* was 1.056.

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 rational for deviating from default factor of 3.
Nominal concentration	1	Liquid was evaporated, tested concentration ≤ 25% saturated vapor concentration.
Adequacy of database:	1	One limited B-study with 2 datasets for 2 species allowing calculation of an LT_{50} available. Rat data from Zwart and Carpenter suggest that the rat LC_{50} may be higher than the guinea pig LC_{50} by at least a factor 2, much the same as the same authors found for ethylene imine. The sparse dataset is supported by a larger dataset for ethylene imine.

The estimated human equivalent 148.8-minute LC_{50} value is 1188 / 3 = **396 mg/m^3**.

The experimentally determined n-value for the structurally analogue compound *ethyleneimine* was **1.056**. 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 98 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1.894 \times \ln (396^{1.056} \times 148.8)$ resulting in the a-value of **-16.44**.

$Pr = -16.4 + 1.89 \times \ln (C^{1.06} \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically weak basis. The probit function is based on 2 studies on propylene imine *and ethylene imine* in the rat and Guinea pig with B quality (without information on possible sex differences), with a total of 76 animals exposed to propylene imine.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 286 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 196 mg/m^3.

Table 4 *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	376	196
1% lethality, this probit	550	286
AEGL-3 (2010, final)	120	54
ERPG-3 (N/A)		N/A
LBW (2016)	120	67

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are higher. The AEGL derivation included a modifying factor of 2 due to the limited database. The LBW was updated to reflect the current AEGL values in 2016.

The LC-values are 5 times higher than those for ethylene imine, which fits the observations by Carpenter (study B1.1).

Appendix 1 Animal experimental research

Study ID: B1.1

Author, year:	***Carpenter 1948***
Substance:	Propylene imine
Species, strain, sex:	male Wistar albino rats and male and female guinea pigs
Number/sex/conc. group:	5-6 / exposure duration, with a total of 7 groups
Age and weight:	age unspecified, rat weighed mostly 90-120 grams, range 60-180 grams, guinea pig weighed 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	*N/A (whole body)*
Pressure distribution	*Not specified*
Homogeneity of test atmosphere in 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, and left in the chamber until concentration returned to zero.*
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	*Not appropriate*
Assessment of Reliability	***B1*** *Study has not been described (and probably performed) consistent with current standards, and only nominal (or even target) exposure levels were provided.*

[2] The concentration as presented in the publication is 500 ppm.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Exposed	Fatal
Rat	1188	N/A	5	6	0
Rat	1188	N/A	10	6	0
Rat	1188		15	6	0
Rat	1188		30	5	0
Rat	1188		60	6	0
Rat	1188		120	6	0
Rat	1188		240	6	5
Guinea pig	1188	N/A	5	6	0
Guinea pig	1188		15	6	0
Guinea pig	1188		30	6	0
Guinea pig	1188		60	6	1
Guinea pig	1188		120	5	3
Guinea pig	1188		240	6	6

Probit function

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

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

with t for time in minutes.

Species	A	b	n-value
Rat	*-78.0*	*15.3*	N/A
Guinea pig	*-55.3*	*2.30*	N/A

The probit functions presented above have been calculated without the 5- and 10-minute exposure data, that may need to be adjusted for short exposure duration respective to the chamber equilibration time. The calculated results including the 5- and 10-minute data differed only marginally.

The study did not provide information to assess a sex difference in the response to inhalation of propylene imine.

Species	Concentration (mg/m^3)	LT_{50} (mg/m^3) 95%-C.I.
Rat	1188	226 (data did not allow to calculate cdf-i)
Guinea pig	1188	98.0 (62.2 – 161)

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

The authors made the assessment that the mode of toxicity of propylene imine was similar to that of ethylene imine, but that propylene imine is 4-8 times less toxic than ethylene imine.

Study ID: B2.1 – ethylene imine

Author, year: Carpenter 1948
Substance: ***ethylene imine***
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[3]) 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

[3] 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

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

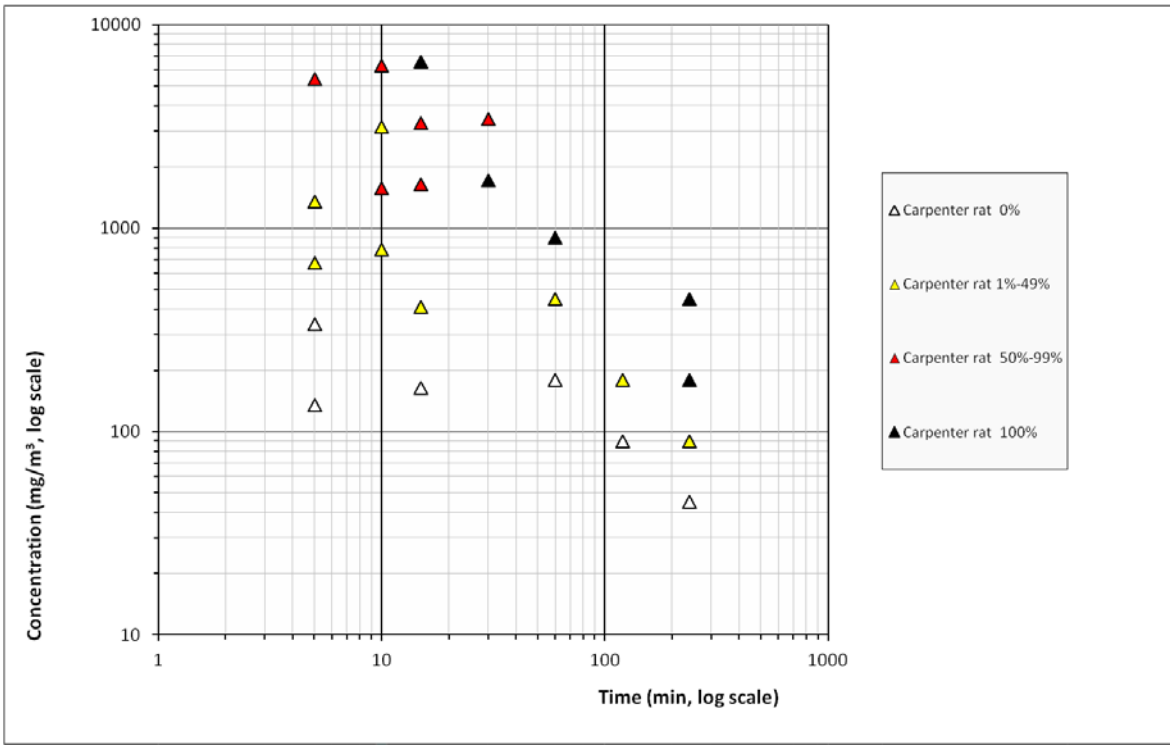
with C for concentration in mg/m^3, t for time in minutes.

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 - ethylene imine

Author, year: Carpenter 1948
Substance: ***ethylene imine***
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[4]) 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

[4] 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

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

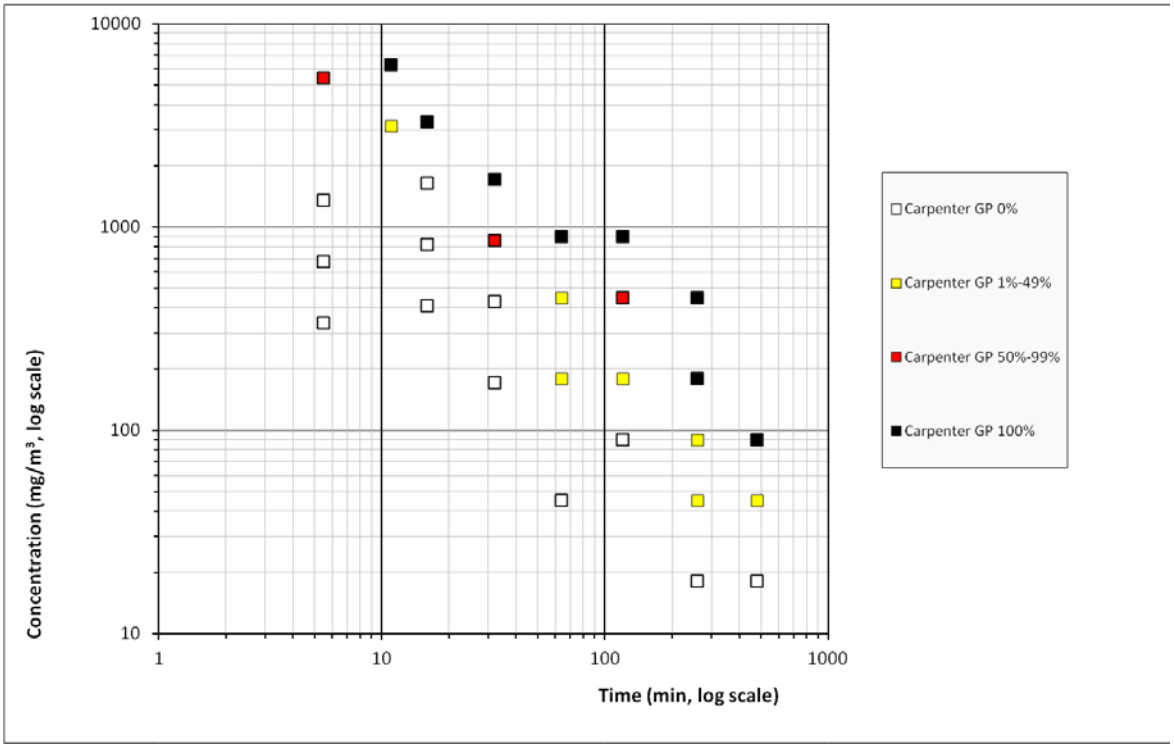
with C for concentration in mg/m^3, t for time in minutes.

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.



Study ID: C studies

Zwart (1989) performed 2 studies in male and female Wistar derived rats. In the range-finding study 1 animal / sex was exposed nose-only to 2400 mg/m^3 for 7.5, 15, 30, 60 or 120 minutes. Male animals exposed for 30 and 120 minutes died with red discoloured lungs. Subsequently 5 animals / sex were exposed to 2400 mg/m^3 for 1 hour. One female rat died during the 14 day observation period. It was concluded that the 1-hour LC_{50} of propyleneimine was higher than 2400 mg/m^3.
The study has a GLP statement and was performed according to OECD guideline 403.

Appendix 2 Reference list

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