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

Probit function technical support document

Date: 15 March 2018
Document id: 20180315-acrolein-interim
Status: interim
Author: Dr Ir M. Ruijten
On behalf of RIVM
E-mail response to: safeti-nl@rivm.nl

substance name	CAS number
Acrolein	**107-02-8**

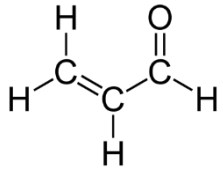
This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

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/en/Topics/P/Probit_functions

Technical support document Acrolein



1. Substance identification

CAS-number:	107-02-8
IUPAC name:	acrylaldehyde
Synonyms:	acrolein, 2-propenal, prop-2-enal, AC
Molecular formula:	C_3H_4O
Molecular weight:	56.1 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	53°C (at 101.3 kPa)
Vapour pressure:	29.5 kPa (at 20°C)
Saturated vapor conc:	291214 ppm = 679580 mg/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.429 ppm (at 20°C and 101.3 kPa)
	1 ppm = 2.334 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	Human H300-311-314-330

2. Mechanism of action and toxicological effects following acute exposure

Acute effects: The main target organs and tissues for inhalation exposure to acrolein are the cornea, conjunctiva, skin and respiratory tract. Acrolein dissolves in the mucous membranes of the respiratory tract and eyes and produces tissue damage and necrosis. The health endpoints are all related to the irritant and corrosive properties of acrolein. Symptoms of high exposure are laboured breathing, secretions from nose, mouth and eyes and prostration.

Damage occurs in the respiratory system, particularly the upper respiratory tract resulting in mucus secretion, upper airway and/or pulmonary oedema and laryngospasm. The resulting hypoxemia will cause CNS and cardiovascular (myocardial ischemia) effects. Lethality results when the respiratory damage proceeds to inflammation, degeneration and necrosis of affected tissue, atelectasis, emphysema and finally death (AHLS 2014, NRC 2004).

Long-term effects: Chronic exposure produces essentially the same type of health effects. Reactive Airways Dysfunction Syndrome, an acquired asthma-like condition has been described to develop after single exposure to a high concentration of acrolein. Symptoms occur within minutes to hours after the initial exposure and may persist as non-specific bronchial hyperresponsiveness for months to years.

3. Human toxicity data

Yant (1930) exposed human volunteers to 0.6-1.0% acrolein in methyl chloride, to test the suitability of acrolein as a warning agent for detecting leakage of methyl chloride from refrigerators. The warning response was tested in a gas chamber with 28 m^3 volume, which was equilibrated before the subjects entered. Some volunteers were acquiainted with the nature of the experiments before exposure, others were not. The authors state that “individual results were in close practical agreement with the avarege” as presented. A 2.3 mg/m^3 exposure produced moderate nasal irritation and practically intolerable eye irritation with lacrimation after 5 minutes, and 13 mg/m^3 did the same in 1 minute. A 4-minute exposure, which was built up in the chamber with the subjects present to 4.2 mg/m^3 in 3 minutes, produced profuse lacrimation and intolerable eye irritation.

Weber-Tschopp *et al* (1977) carried out 3 experiments with healthy students, and measured the response with questionnaires, eye blinking and respiratory rates. The following result were obtained:

1. 53 subjects were exposed in groups of 3 to a concentration increasing from 0 to 1.4 mg/m^3 in 35 min, followed by 5 min to 1.4 mg/m^3.

2. 5 consecutive 1.5 min exposures of 42 subjects in groups of 4 to 0, 0.35, 0,70, 1,05 and 1.4 mg/m^3 with 8 minutes between exposures.

The eye-irritation index increased to strong for the continuous exposure and 'little' for the discontinuous exposure. The nose irritation index indicated less nose- than eye irritation (up to little-average).

3. A 60-min continuous exposure of 46 subjects to 0.70 mg/m^3. This produced 'average' eye irritation, 'little' node irritation and 'no-little skin irritation in the neck.

No serious chemical-related injury was reported.

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 acrolein, 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 12 studies were identified -with 16 datasets for 6 species- with data on lethality following acute inhalation exposure. No datasets were assigned status A for deriving the human probit function, 6 datasets were assigned status B and 10 were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

A total of 5 studies were identified in which sensory irritation was studied. In these studies, the following RD_{50} values were observed:

Table 1 *Sensory irritation data for acrolein*

Species/strain	RD_{50} (mg/m^3) 95& CI	Exposure duration (min)	Author/year
Swiss mice	2.96 ^{NS}	10	ECHA 2017
SW Mice	3.5 ^{P} (in a 'typical recording') (2.6-4.9)	10	Kane 1977
B6C3F1 mice	1.41 ^{P} (1.16-1.73)	10	Steinhagen 1984
SW mice	1.03 ^{C,P} (0.70-1.52)	10	Steinhagen 1984
Rat	14 ^{NS} (3.5-18.1)	10	Babiuk 1985
Rat	21.5 ^{F,P} (15.2-32.0)	30	Cassee 1993

P: a plateau was reached, C: continuously decreasing response, F: fading of response during exposure, NS: not specified if a plateau in response was reached.

ECHA reference seems to be from a 1980 Haskell report with RD50 = 1.27 ppm

5. Probit functions from individual studies

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

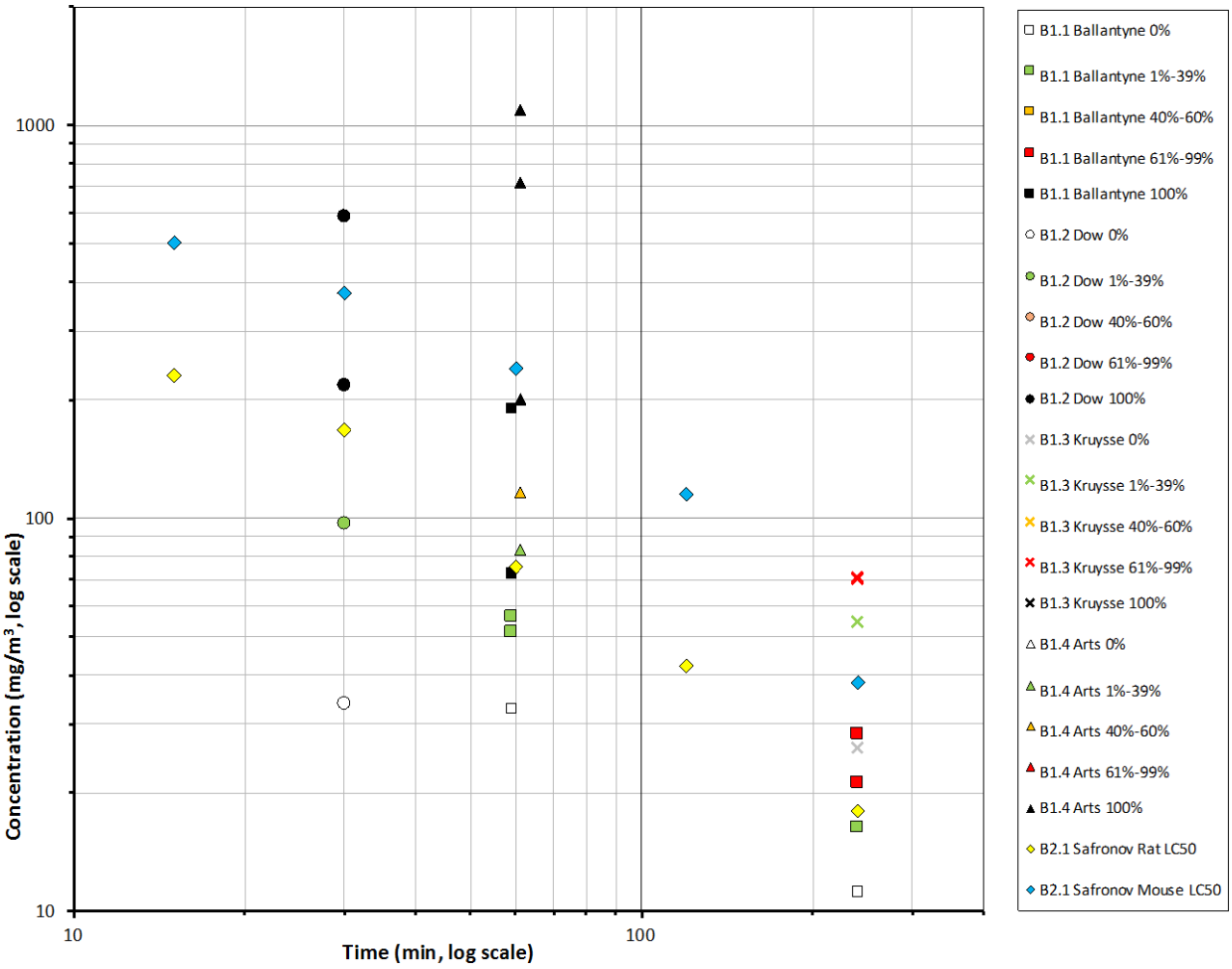


Figure 1 *All available acute lethality data for acrolein.*

The data that were selected for initial analysis of the animal probit function are presented in Table 2 and Figure 2. All B1 studies were selected for the initial analysis of the animal probit function for acrolein. Study B2.1 was used to derive an n-value.

To enable intra-species pooling, LC_{50}-values from study B1.1, B1.3 and B1.4 were scaled using the average rat n-value for acrolein from study B2.1 of 1.069 for extrapolation of rat data, and the average n-value of 1.082 (rat and mouse) for extrapolation of hamster data 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 = rat or average rat/mouse n-value

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 2 *Data selected for initial analysis of the animal probit function of acrolein.*

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. *(scaled values)*	n-value 95% C.I.
B1.1	Rat	*60-min LC_{50}*	58.5 (55.2 – 63.4)	*111.9*	N/A
B1.1	Rat	*240-min LC_{50}*	19.7 (16.7 – 23.6)	*137.8*	N/A
B1.2	Rat	*30-min LC_{50}*		107.1 (unable to calculate cfd-i)	N/A
B1.3	Hamster	*240-min LC_{50}*	60.1 (46.2 – 66.2)	*411.1*	
B1.4	Rat	*60-min LC_{50}*	121.0 (101.7 – 177.4)	*231.4*	N/A
B2.1	Rat	*30-min LC_{50}*		168 (112 - 252)	1.069
B2.1	Mouse	*30-min LC_{50}*		375 (250 - 562)	1.094

The data of the all B1 studies with rats are presented graphically below.

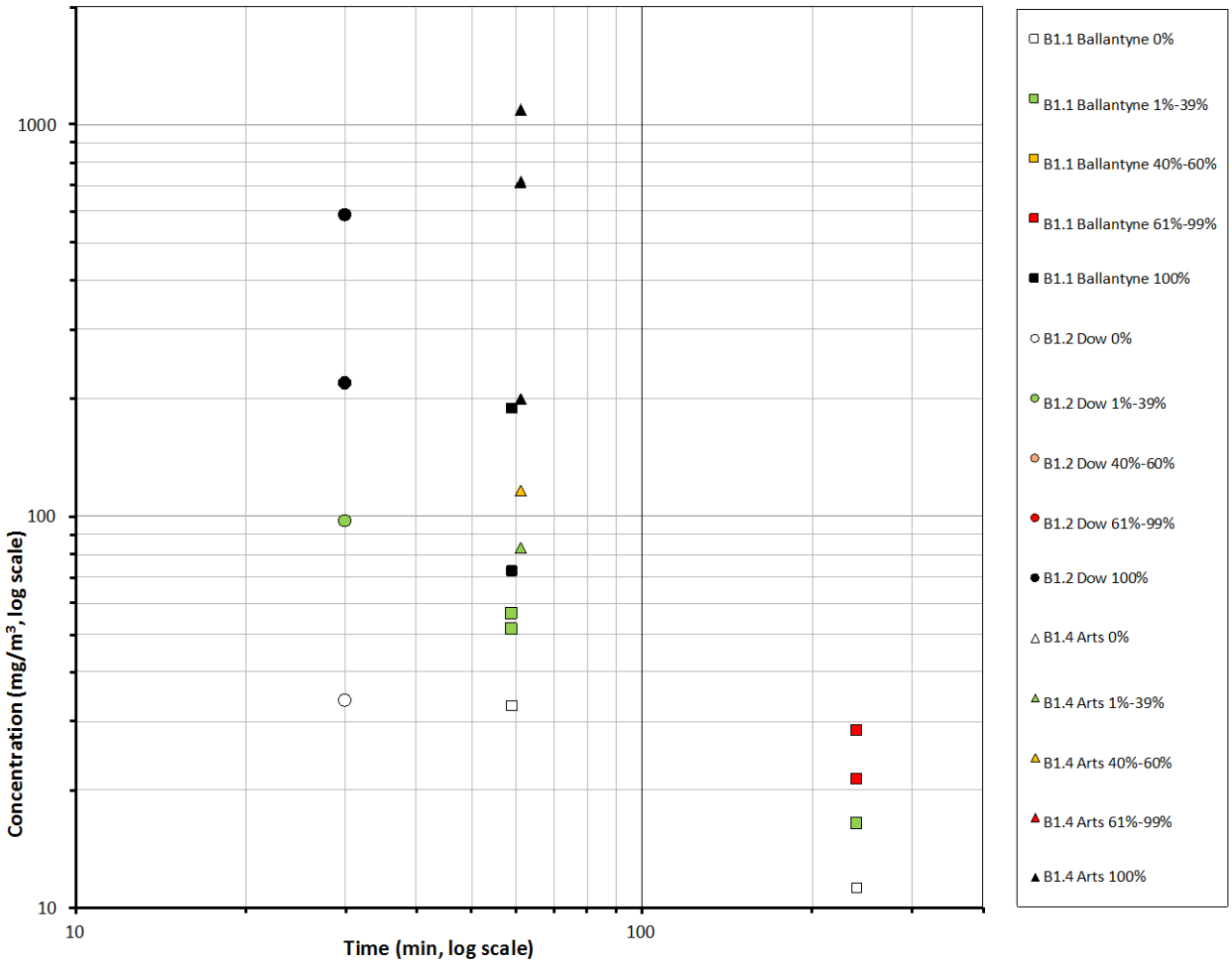


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of acrolein.*

Based on criteria outlined in the guideline the data from studies B1.1 (60-min data only), B1.2 and B1.4 were selected for the final dataset for the derivation of the animal LC_{50} value. The 240-min data from studies B1.1 and B1.3 were not included in the final analysis because of the uncertainty involved with extrapolation of the 240-min data from study B1.3 to the target 30-min exposure duration. The data that were selected for final analysis of the animal probit function are presented in Table 3 and Figure 3. Study B2.1 was used to calculate acrolein-specific rat and mouse n-values. The final data eligible for calculating the LC_{50} for the animal probit function contains 3 datasets from 3 studies and includes data from 1 animal species.

Table 3 *Data selected for the derivation of the animal probit function of acrolein.*

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. *(scaled values)*	n-value 95% C.I.
B1.1	Rat	*60-min LC_{50}*	58.5 (55.2 – 63.4)	*111.9*	N/A
B1.2	Rat	*30-min LC_{50}*		107.1 (unable to calculate cfd-i)	N/A
B1.4	Rat	*60-min LC_{50}*	121.0 (101.7 – 177.4)	*231.4*	N/A

The data of the selected datasets are presented graphically below.

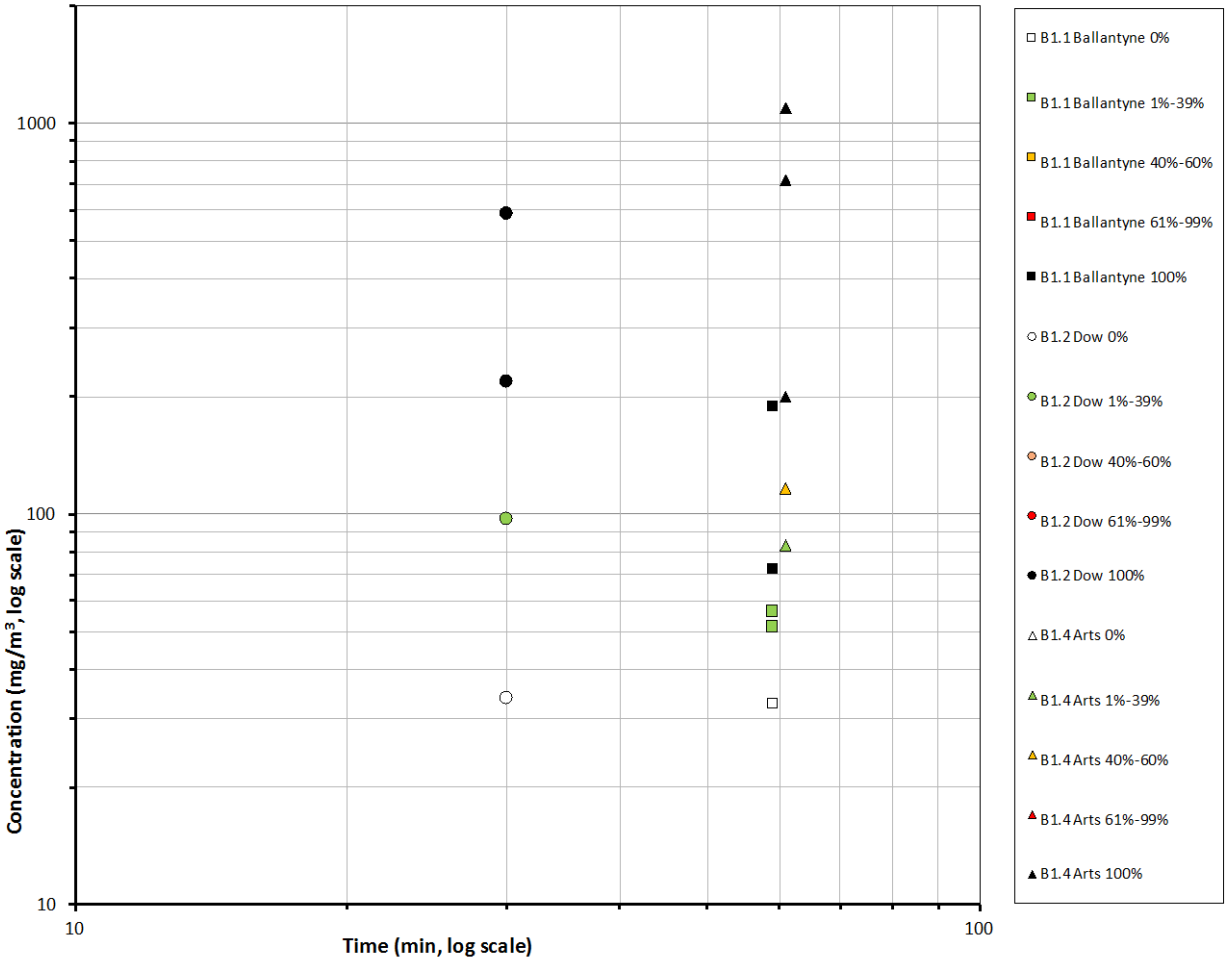


Figure 3 Final data selected for derivation of the animal probit function of acrolein.

6. Derivation of the human probit function

To derive the human probit function the results from studies B1.1, B1.2 and B1.4 (and B2.1 for derivation of the n-value) have been used to derive a point of departure as outlined above.

First, the arithmetic mean n-value was calculated from the rat and mouse datasets in study B2.1. These values are almost identical, and match the n-value derived from study B1.1 (albeit with only 2 exposure durations) very closely.

The species-specific n-values from study B2.1 were 1.069 for the rat and 1.094 for the mouse. The arithmetic mean n-value across species is the simple arithmetic mean of the species-specific mean n-values, without weight and was calculated to be 1.082.

Second, the LC_{50}-values of all applicable B1-studies were calculated for a common exposure duration of 30 minutes. To enable this intra-species pooling, the LC_{50}-value of rat studies B1.1 and B1.4 were scaled using the rat specific n-value of 1.069 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 30-min exposure duration for intra-species pooling
t_{test} = tested exposure duration
n = rat specific n-value of 1.069

Finally, the rat geometric mean LC_{50}-value was calculated from the available (time-scaled) LC_{50} values of studies B1.1, B1.2 and B1.4. The geometric mean rat LC_{50}-value was 140.5 mg/m^3. The formula for the geometric mean of time-scaled LC_{50}-values from 1 species is as follows:

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

With $\overline{LC_{50}}$ = geometric mean LC_{50}-value
$LC_{50,i}$ = LC_{50}-value of study i.
m = number of observations on LC_{50}-values (i=1…m).

The Point of Departure for the human probit function is a 30-minute geometric mean rat LC_{50} value of 140.5 mg/m^3 and an arithmetic mean n-value of 1.082.

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

Table 4 *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	2	The rat appears to be the most susceptible animal species. The interspecies extrapolation factor was lowered based on the human and baboon data.

Assessment factor for:	Factor	Rationale
Nominal concentration	1	Analytically determined concentrations in studies B1.1 and B1.2
Adequacy of database:	1	Reasonable database with 3 rat B1 studies and a supporting B2 study in rats and mice.

The estimated human equivalent 30-minute LC_{50} value is 140.5 / 2 = **70.2 mg/m^3**.

The experimentally determined average n-value from rats and mice was **1.082**. Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1.849**.

The human probit function is then calculated on the human equivalent 30 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1.849 \times \ln (36.5^{1.082} \times 30)$ resulting in the a-value of **-9.794**.

$Pr = -9.79 + 1.85 \times \ln (C^{1.08} \times t)$ 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 4 studies in the rat and mouse with B quality, with 188 rats in B1 studies and approx. 500 animals in the B2 study, with exposure durations ranging from 15-240 minutes and concentrations ranging from 11-8220 mg/m^3.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 6.4 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 9.3 mg/m^3.

Table 5 *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	12.1	6.4
1% lethality, this probit	17.7	9.3
AEGL-3[1] (2010, final)	5.8	3.3
ERPG-3[1] (2017)		3.5
LBW (2015)	5.8	3.3

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are approximately identical (slightly higher).
In studies with human volunteers (Weber-Tschopp 1977 and Yant 1930) severe irritation was experienced by healthy test subjects exposed to up to 4 mg/m^3 for a few minutes, and slight to moderate irritation following a 60-min exposure to 0.7 mg/m^3 or a 40-min exposure to 1.4 mg/m^3.
Single baboons survived 5-min exposures to up to 1178 mg/m^3 (follow-up was incompletely documented); the 5-min LC_1 calculated with this probit function is 62 mg/m^3.

[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: ***Ballantyne et al 1989***
Substance: Acrolein
Species, strain, sex: male and female Sprague-Dawley rats
Number/sex/conc. group: 5/sex/group
Age and weight: 57-60 days for 1-hr study, 72-93 days for 4-hr study, 172-248 gr for females, 232-323 gr for males
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*No 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	*No issues reported*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole-body, individually housed in 4-hr exposure, 5 by sex per cage for 1-hr exposure*
Type of restrainer	*N/A*
Pressure distribution	*Not specified.*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere was generated by metering liquid from a syringe pump into a heated glass evaporator (in some cases, glass beads were added to increase the surface area for evaporation). The resultant vapor was carried to the exposure chamber by an airstream through the evaporator.*
Number of air changes per hour	*50-52 l/min through a 120 liter chamber for 1-hr exposure (25-26 ACH).* *200 l/min through a 900 liter chamber for 4-hr exposure (13.3 ACH), except 21.2 mg/m^3 group (100 l/min, 6.7 ACH).*
Equilibration time (t95)	*7.2 min for 1-hr exposure, 13.5 / 27 min for 4-hr exposure*
Start of exposure relative to equilibration	*After equilibration of the chamber atmosphere*
Actual concentration measurement	*4-6 air samples for 1-hr exposure, 10 samples for 4-hr exposure at an unspecified location. Analysis: GC/FID.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Not applicable*
Assessment of Reliability	***B1*** *Well conducted study with only 2 exposure durations.*

Results

Species	Concentration (mg/m^3)	Exposure duration (min)	Lethality	
	Measured		Male	Female
Rat	189.1	60	5/5	5/5
	72.4	60	5/5	5/5
	56.0	60	2/5	1/5
	51.3	60	0/5	1/5
	32.7	60	0/5	0/5
	28.2	240	5/5	3/5
	21.2	240	3/5	4/5
	16.3	240	3/5	0/5
	11.2	240	0/5	0/5

Necropsy of animals that died revealed perinasal and perioral encrustation, mottled discoloration of the lungs and liver, clear fluid in the trachea and thoracic cavity, gas-filled stomach and intestine, and opaque and cloudy corneas. Histological features in the lungs included congestion and intra-alveolar hemorrhage, fibrin deposition in the smaller airways and necrosis and exfoliation of bronchiolar epithelium. Time of death was 3 hr – 6 days, with the exception of 1 lethality on day 13.

Probit function

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

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

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

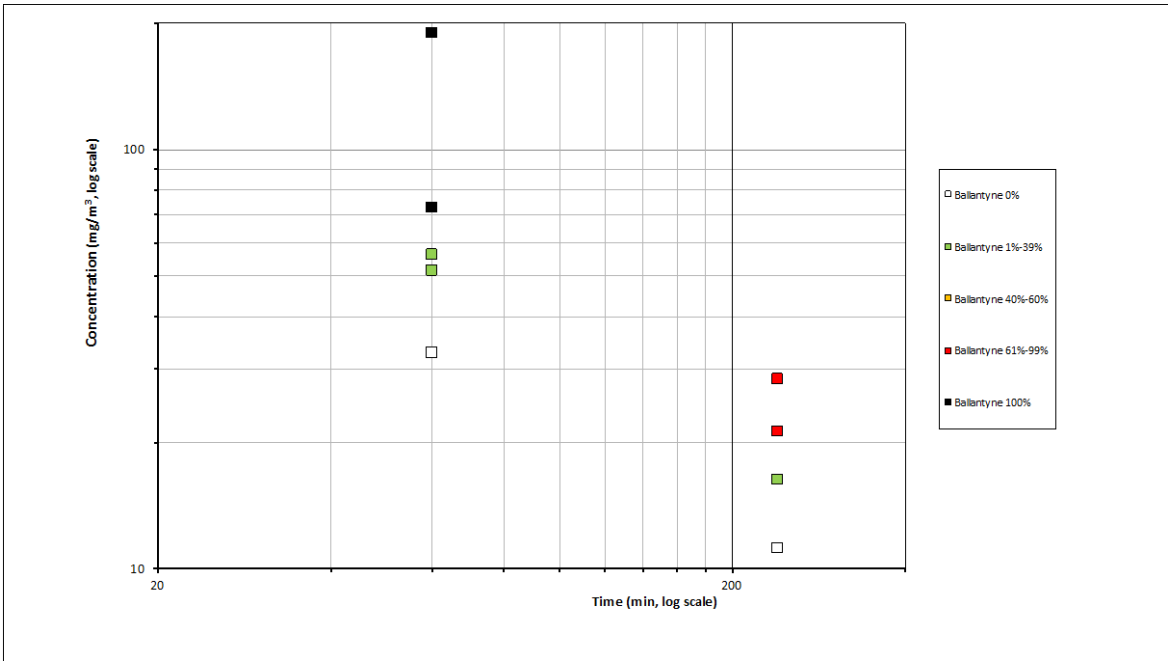
Probit function	Species	a	b	c	d	n-value
Sex as variable	Rat	-29.5	4.64	3.71	0.53	1.250 (1.073 - 1.427)
Sexes combined	Rat	-27.5	4.40	3.55		1.247 (1.065 - 1.429)

The 60-min LC_{50} values from the C×t analysis with all data were 56.8 mg/m^3 for males and 63.7 mg/m^3 for females (65 and 60 mg/m^3 resp. according to the authors). 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 analyzed to derive the animal probit function.

While the study was also analyzed with the full dataset (which enables calculation of an n-value, albeit based on only 2 exposure durations), for the purpose of probit derivation this study will be treated as a 60-min LC_{50} study. The 240-min LC50 value, calculated only with the 240-min data is 19.7 (16.7 – 23.6) mg/m^3.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combine, all data	LC_{50} (mg/m^3) 95%-C.I. Combined, 60 min data only
10	254 (187 - 359)	NA
30	105 (87.7 – 129)	NA
60	60.3 (53.8 – 68.6)	58.5 (55.2 – 63.4)

A graphical overview of the data is presented below. Each concentration-time combination (with 5 male and 5 female rats) represents one point in the plot.


Concentration (mg/m3, log scale)
100
10
20
200
Time (min, log scale)
Ballantyne 0%
Ballantyne 1%-39%
Ballantyne 40%-60%
Ballantyne 61%-99%
Ballantyne 100%

Study ID: B1.2

Author, year: ***Dow Chemical, 1976***
Substance: Acrolein
Species, strain, sex: Male Sprague-Dawley rats
Number/sex/conc. group: 7 / conc. group
Age and weight: 300-325 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*
Stability of test compound in test atmosphere	*Initially, it was found extremely difficult to attain a steady-state concentration of acrolein, possibly due to wall or animal fur loss. For this reason, the chamber was pre-conditioned and vented afterwards until the concentration of acrolein in air was below the detection limit of 5 mg/m^3.*
Use of vehicle (other than air)	*air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole-body*
Type of restrainer	*N/A*
Pressure distribution	*Chamber was held 'under slight negative pressure', not further specified.*
Homogeneity of test atmosphere in breathing zone of animals	*Acrolein was evaporated.*
Number of air changes per hour	*Airflow of 75 l/min through a 160 liter chamber, equals 28.1 ACH.*
Equilibration time (t95)	*Calculated t95 = 6.4 minutes*
Start of exposure relative to equilibration	*15 min. before concentration build-up but after chamber conditioning. Forced ventilation immediately after exposure.*
Actual concentration measurement	*Approx. every 3 minutes, analyzed with gas chromatography.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Well performed study with only 1 exposure duration.*

After cessation of the exposure the chamber was vented as quickly as possible. The authors state that >90% of the acrolein was gone in 1 minute, and acrolein fell below measurable levels within 2 minutes.

Since the exposure duration > 3×t95, no concentration adjustments were made even though the animals were in the exposure chamber during concentration build-up.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Dead	tested
Rat	33.8		30	0	7
	96.8		30	1	7
	218		30	7	7
	586		30	7	7

The fatality following exposure to 96.8 mg/m^3 occurred after 72 hours, at 218 mg/m^3 after 48-72 hours and at 586 mg/m^3 within 24 hours. Gross pathology of deceased animals revealed upper respiratory congestion, lung congestion and haemorrhage, liver and kidney congestion. Surviving rats in the 96.8 mg/m^3 group showed upper respiratory congestion only, no visible lesions in the 33.8 mg/m^3 group.

Probit function

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

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

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

Probit function	Species	a	b	n-value
Male rats	Rat	-44.2	10.5	N/A

Since the experiment was performed with male rats only, sex differences in response to acrolein inhalation could not be ascertained.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Male
30	107.1 (unable to calculate cfd-i)

Since partial mortality was observed in only 1 concentration, the reliability of the LC_{50} value is considered to be limited. The authors assessed that the LC_{50} value was 'about 60 ppm' (140 mg/m^3).

Study ID: B1.3

Author, year: ***Kruysse 1971***
Substance: acrolein
Species, strain, sex: Male and female Syrian Golden Hamsters
Number/sex/conc. group: 5/sex/concentration group
Age and weight: Young adults, males 115 gram, females 125 gram
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	*Acrolein was freshly generated at TNO's section of organic synthesis due to instability of the substance*
Use of vehicle (other than air)	*Nitrogen*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body, individually housed in a glass cylinder*
Type of restrainer	*N/A*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*A nitrogen stream of 50 ml/min was saturated with acrolein at room temp by means of a glass saturating column, filled with Chromosorb W, to which the acrolein was added at intervals. This small saturated nitrogen flow was mixed with filtered and dried air from the compressed air line in order to provide a dosing flow. Part of the latter was mixed at different concentrations with another air flow which was fed into two glass inhalation cylinders at a rate of 4 l/min. per cylinder with 5 animals.*
Number of air changes per hour	*Flow of 0.8 l/min/animal*
Equilibration time (t95)	*Insufficient information to calculate*
Start of exposure relative to equilibration	*Not specified*
Actual concentration measurement	*Samples were taken 'from within the cylinder' and analyzed with gas chromatography.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Missing information on a number of study quality criteria*

All deaths occurred between 24 hours and 12 days after exposure.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Hamster	26.1		240	0/5	0/5
	54.4		240	1/5	2/5
	70.0		240	4/5	4/5
	70.9		240	4/5	4/5

Probit function

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

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

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

Probit function	Species	a	b	d	n-value
Sex as variable	Hamster	-16.64	5.31	-0.21	N/A
Sexes combined	Hamster	-16.61	5.28		N/A

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 analyzed 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
240	61.32 (46.35 – 71.37)	58.90 (41.85 – 68.00)	60.1 (46.2 – 66.2)

The results for males and females were derived from the analysis with sex as covariate.

Study ID: B1.4

Author, year: ***Arts 1987***
Substance: acrolein
Species, strain, sex: Male and female SPF/Wistar rats
Number/sex/conc. group: 5/sex/concentration group
Age and weight: Adults, males 225 gram, females 168 gram
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)	*Compliance with OECD guideline 403 not specifically mentioned, but many of the conditions appear to be met.*
Stability of test compound in test atmosphere	*No stability issues mentioned (despite such issues in the 1971 TNO study)*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body, individually housed in a glass cylinder*
Type of restrainer	*N/A*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*An adjustable airflow was bubbled through the heated acrolein. The resulting acrolein-in-air mixture was then mixed with the main airflow before entering the inhalation chamber.*
Number of air changes per hour	*1.0-1.4 m^3/hr through a 15-liter chamber (66.7-93.3 ACH).*
Equilibration time (t95)	*Insufficient information to calculate*
Start of exposure relative to equilibration	*Not specified*
Actual concentration measurement	*Samples were taken 'from within the cylinder' and analyzed with gas chromatography.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Missing information on a number of study quality criteria*

All deaths occurred between 24 hours and 12 days after exposure.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Rat	83		60	1/5	0/5
	116		60	4/5	2/5
	235		60	5/5	5/5
	712		60	5/5	5/5
	1094		60	5/5	5/5
	4220		60	5/5	5/5
	8220		60	5/5	5/5

Probit function

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

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

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

Probit function	Species	a	b	d	n-value
Sex as variable	Rat	-21.59	5.53	1.24	N/A
Sexes combined	Rat	-12.85	3.72		N/A

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 analyzed 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	98.1 (73.2 – 131.5)	122.8 (122.8 – 232.9)	121.0 (101.7 – 177.4)

The results for males and females were derived from the analysis with pooled sexes. The author calculated an LC_{50} value of 109.7 mg/m^3 (109.2 - 110.2) with an earlier version of the DoseResp software. Deleting the data from exposure levels higher than 712 mg/m^3 from the analysis produced the exact same calculated combined sex LC_{50} value.

Study ID: B2.1

Author, year: ***Safronov 1993***
Substance: Acrolein
Species, strain, sex: Albino male rats, albino male mice
Number/sex/conc. group: 6 rats/group, 10 mice/group
Age and weight: Rats 150-200 gr, mice 18-20 gr.
Observation period: 2 weeks

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*No GLP statement provided*
Study carried out according to OECD 403 guideline(s)	*No statement of compliance with OECD guideline 403 provided*
Stability of test compound in test atmosphere	*Not specified*
Use of vehicle (other than air)	*Not specified*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*Not specified*
Homogeneity of test atmosphere in breathing zone of animals	*Not specified, other than that 'exposure was performed by a dynamic method'.*
Number of air changes per hour	*Not specified*
Equilibration time (t95)	*No data available to calculate t95*
Start of exposure relative to equilibration	*Not specified*
Actual concentration measurement	*"Concentration in the chamber was estimated using the gas chromatography method"*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B2*** *Few study details are available. LC_{50} values and confidence intervals are provided for 5 exposure durations. The method of probit-analysis was used for LC_{50} calculation.*

Results

Exposure duration (min)	LC_{50} (mg/m^3) 95%-C.I.	LC_{50} (mg/m^3) 95%-C.I.
	Mouse	Rat
15	500 (350 – 690)	230 (164 - 322)
30	375 (250 – 562)	168 (112 - 252)
60	240 (117 – 336)	75 (54 - 106)
120	115 (92-143)	42 (34-53)
240	38 (31 – 45)	18 (11 - 29)

Probit function

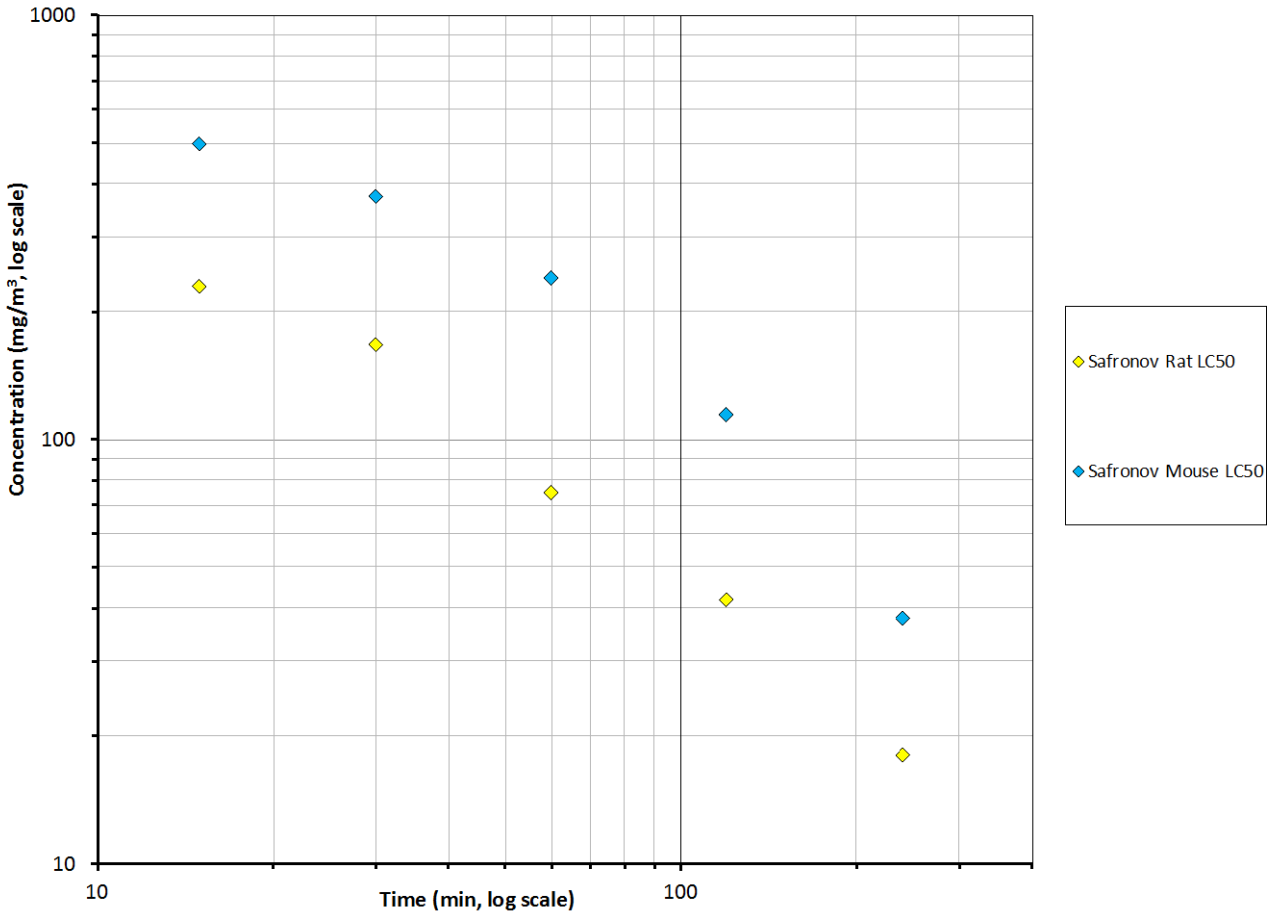
No C × t probit function could be calculated from these data alone. However, as LC_{50} values were provided for different exposure durations an n-value could be calculated using the following formula (AEGL SOP):

$$-n = \frac{N\sum(\log t)^2 - (\sum \log t)^2}{N\sum(\log t)(\log C) - (\sum \log t)(\sum \log C)}$$

The calculated n-value was 1.069 for rats and 1.094 for mice.

These values were calculated taking into account all available LC_{50} values, even though the calculated n-value seems to be sensitive to selection of data.
For mice, the n-value is almost exactly 2 when based on the 15-min to 60–min LC_{50} values; including the data from longer exposure durations decreases the calculated n-value.
For rats, a similar trend is observed, albeit less pronounced.

A graphical overview of the LC_{50} values is presented below.



Study ID: C studies

Crane *et al* (1986) studied the ability of acrolein as a smoke constituent to influence escape time in aircraft fires. Sprague-Dawley rats were exposed on a treadmill to static concentrations until incapacitation. After incapacitation occurred, the treadmill was stopped and animals were further exposed until death. The exposure concentration was generated by evaporating acrolein from a petri dish in the enclosed cage; exposure was initiated when all of the liquid had evaporated. The exposure level was measured with a GC every 9-10 minutes. Time until incapacitation and death are summarized in the figure next to the text. NB: exposure concentrations are in parts per thousand. 10 ppt equals 23,340 mg/m^3.

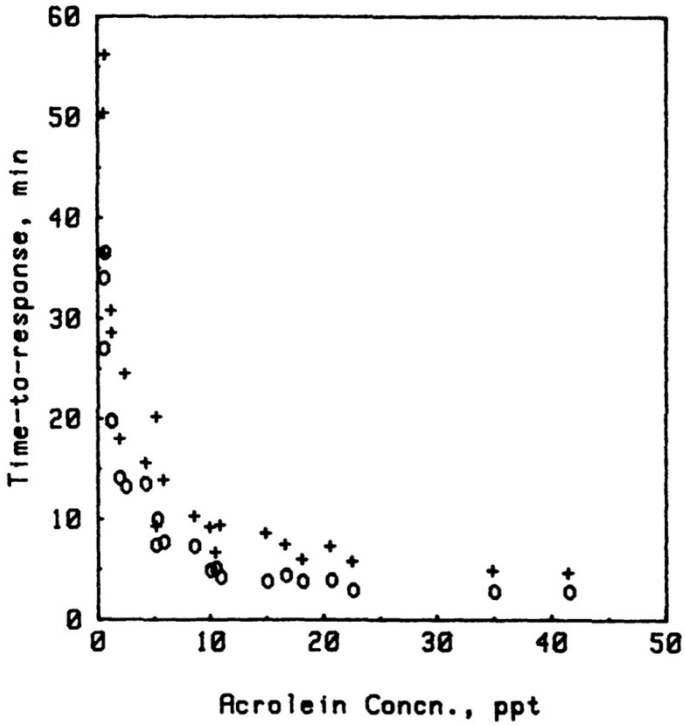


Figure 2. Times-to-incapacitation (O) and times-to-death (+) plotted against the average chamber concentration of acrolein. Each point represents one rat, exposed individually; N=22.

Skog (1950) reported a 30-min LC_{50} value of 0.3 mg/l (300 mg/m^3) in rats (whole-body exposed). Concentrations were determined nominally, observation period 3 weeks.

As the result of exposure to acrolein during an escape-performance test, 1/1 male baboon died 1.5 h after a 5-minute exposure to 6478 mg/m^3 acrolein and 1/1 died 24 h after exposure to 2392 mg/m^3 (Kaplan 1987). Both animals developed severe respiratory effects and died from pulmonary edema. The highest non-lethal exposure was 1178 mg/m^3 (2 animals); the observation period was not specified, but possibly indefinite; part of the animals were exposed to multiple concentrations.

Male Dunkin-Hartley guinea pigs (number per group not specified) were exposed to acrolein at 0 or 3.7 mg/m^3 (generated as an aerosol) for 7.5 h on each of two consecutive days (Turner et al. 1993, as described in NRC 2010). There were no deaths in the control group, and 14% of the acrolein-exposed animals died.

In another study, tracheotomized guinea pigs died 6 minutes into an exposure to 3734 mg/m^3 (Davis et al. 1967, as described in NRC 2010).

Exposure of 20 hamsters, 12 rats and 4 rabbits per group for 6 h/d, 5 d/w for 13 weeks to 0, 0.93, 3.27 and 11.4 mg/m^3 produced only lethality in rats (3/12 animals per sex) at the 11.4 mg/m^3 exposure level (besides other non-lethal toxicity in all species). Rats appeared to be the most susceptible species, showing treatment-related abnormalities even at 0.93 mg/m^3 (Feron 1978).

Philippin (1970) reported a 6-hour LC50 of 154 mg/m^3 in albino mice. The observation period was only 24 hours post-exposure however, and in other studies significant mortality occurred beyond this point during follow-up.

Smyth (1956) reported that 4 hours of inhalation at 18.7 mg/m^3 (8 ppm) of acrolein killed one of six rats and that 37.3 mg/m^3 (16 ppm) killed all exposed animals.

Appendix 2 Reference list

Arts JHE. Acute (1-hour) inhalation toxicity study of acrolein in rats. Report nr V87.181/261344. Zeist, CIVO Institutes TNO, 1987.

Ballantyne B, DE Dodd, IM Pritts, DJ Nachreiner, EH Fowler. Acute Vapour Inhalation Toxicity of Acrolein and its Influence as a Trace Contaminant in 2-Methoxy-3,4-dihydro-2H-pyran. Human Toxicol. 1989;8:229-235.

Chemiekaarten. Ed 32. Den Haag. TNO/SDU uitgevers, 2017.

Crane CR, DC Sanders, BR Endecott, JK Abbott. Inhalation toxicology: VII. Times to Incapacitation and Death for Rats Exposed Continuously to Atmospheric Acrolein Vapor. FAA BCivil Aeromedical Institute, Report DOT/FAA/AM-86/5. Oklahoma City (May 1986).

Davis, TR, SP Battista, CJ Kensler. Mechanism of respiratory effects during exposure of guinea pigs to irritants. Arch. Environ. Health 1967;15(4):412-419.

Dow Chemical Co. Initial submission: a study of the inhalation toxicity of acrolein (final report) with cover letter dated 031892. Final report dated 12 Jan 1976. Doc ID 88-920001478S. Also listed under https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/13444/7/3/3/?documentUUID=51fce23f-1c39-4fa3-95bb-0d957697d103.

ECHA. Acrylalheyde – Registration dossier – ECHA. Retrieved 11/05/2017. https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/13444/7/13.

Feron, VJ, A Kruysse, HP Til, HR Immel. Repeated exposure to acrolein vapour: subacute studies in hamsters, rats and rabbits. Toxicol. 1978;9:47-57.

Kaplan H. Effects of irritant gases on avoidance/escape performance and respiratory response of the baboon. Toxicology 1987;47:165-179.

Kruysse A. Acute inhalation toxicity of acrolein in hamsters. Report R 3516. Zeist, TNO Central Institute for Nutrition and Food Research, 1971.

National Research Council (NRC). Acute Exposure Guideline Levels for Selected Airborne Chemicals. Volume 8. Washington, DC. The National Academies Press, 2010.

Philippin C, A Gilgen, E Grandjean. Etude toxicologique de l'acroléine chez le souris. Int Arch Arbeitsmed. 1970;26:281-305.

RIVM 2016. Interventiewaarden gevaarlijke stoffen. http://www.rivm.nl/rvs/Normen/Rampen_en_incidenten/Interventiewaarden.

Ruijten MWMM, JHE Arts, PJ Boogaard *et al*. Methods for the derivation of probit functions to predict acute lethality following inhalation of toxic substances. RIVM report 2015-0102. Bilthoven, RIVM, 2015.

Safronov GA, NS Nevmerzhitsky, LA Tiunov, LV Tiunova. Comparative acute inhalation toxicity of aliphatic aldehydes and ketones according to exposure time. Current Toxicology 1993;1:47-51.

Smyth, Jr HF. Hygienic Standards for Daily Inhalation. Am. Ind. Hyg. Assoc. Q. 1956;17:144.

Skog E. A Toxicological Investigation of Lower Aliphatic Aldehydes. I. Toxicity of Formaldehyde, Acetaldehyde, Propionaldehyde and Butyraldehyde; as well as of Acrolein and Crotonaldehyde. Acta Pharmacol. 1950;6:299-318.
Steinhagen, WH; CS Barrow. Sensory Irritation Structure-Activity Study of Inhaled Aldehydes in B6C3F1 and Swiss-Webster Mice. Toxicol. Appl. Pharmacol. 1984;72:495-503.

Turner, CR, RB Stow, SD Talerico, EP Christian, JC Williams. Protective role for neuropeptides in acute pulmonary response to acrolein in guinea pigs. J. Appl. Physiol. 1993;75:2456-2465.

Weber-Tschopp, A, T Fischer, R Gierer *et al*. Experimentelle Reizwirkung von Akrolein auf den Menschen. Int. Arch. Occup. Environ. Health 1977;40:117-130.

Yant WP, HK Schrenk, FA Patty *et al*. Acrolein as a warning agent for detecting leakage of methyl chloride from refrigirators. R.I. 3027. Report of investigation, Dept. of Commerce – Bureau of Mines. 13 pp. July 1930.