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

Probit function technical support document

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| substance name | CAS number |
|---|---|
| **Ethylene oxide** | **75-21-8** |

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 ethylene oxide

## 1. Substance identification

| | |
|---|---|
| CAS-number: | 75-21-8 |
| IUPAC name: | oxirane |
| Synonyms: | 1,2-epoxyethane |
| Molecular formula: | $C_2H_4O$ |
| Molecular weight: | 44.1 g/mol |
| Physical state: | gas (at 20°C and 101.3 kPa) |
| Boiling point: | 11°C (at 101.3 kPa) |
| Vapour pressure: | 150 kPa (at 20°C) |
| Saturated vapor conc: | N/A |
| Conversion factor: | 1 mg/m$^3$ = 0.545 ppm (at 20°C and 101.3 kPa) |
| | 1 ppm = 1.834 mg/m$^3$ (at 20°C and 101.3 kPa) |
| Labelling: | H315-319-331-335-340-350 |

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

**Acute effects**: Ethylene oxide is a direct-acting alkylating agent; it alkylates DNA and proteins. Ethylene oxide is also a mild primary irritant and a central nervous system depressant. The main target organs and tissues for inhalation exposure to ethylene oxide are therefore the mucous membranes, the respiratory tract and the central nervous system. Symptoms observed upon inhalation exposure are signs of irritation, nasal discharge and lacrimation and adverse effects on the respiratory tract. Pulmonary effects include decreased respiration, congestion, haemorrhage and pulmonary edema. Neurological effects include hypoactivity, ataxia, and tremors. Ethylene oxide is rapidly absorbed from the respiratory tract upon inhalation exposure. Lethality appears to be due to respiratory failure, probably involving nervous system toxicity rather than respiratory tract irritation.
**Long-term effects:** Most effects observed after acute exposure are reversible, including effects on the nervous system. Chronic exposure exacerbates the peripheral nerve damage. Human studies have provided evidence suggestive of reproductive toxicity, some evidence of an association between exposure to ethylene oxide and genetic damage to somatic cells, and limited evidence of carcinogenicity.

## 3. Human toxicity data

No informative reports on 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.
In humans, ethylene oxide affects the eyes, respiratory tract, central and peripheral nervous system, gastro-intestinal tract, hematopoietic system, and possibly the reproductive system and foetus. No ethylene oxide-related fatalities in humans are reported. Several human case reports on ethylene oxide exposure were available in literature, though no precise information on the exposure concentration and period is available.
A nurse was acutely exposed to ethylene oxide while disposing an ampule that accidentally dropped. Acute exposure to a calculated concentration of at least 500 ppm (917 mg/m$^3$) (based on the release of 17 g ethylene oxide in a sterilizer bag) for 2 to 3 min resulted in immediate symptoms of intoxication including repeated

[1] AEGL (2010)

episodes of nausea, stomach spasms, paleness, light-headedness, short periods of unconsciousness, convulsions and apnoea. The exposure was estimated by the authors to be even considerably higher than the calculated 917 mg/m$^3$ (not further specified). Signs and symptoms of peripheral neuropathy were reported in four workers where a sterilizer leaked during a 2-month period. The ethylene oxide level during this period was estimated to exceed 700 ppm (1284 mg/m$^3$). Other case studies reported effects such as irritation of mucous membranes, effects on the GI-tract, haemolysis and neurological effects (dizziness, speech difficulty, incoordination, muscular numbness, tingling, cramps and weakness) at levels above the odour threshold (i.e. 260 ppm = 477 mg/m$^3$) (AEGL, 2010).
A study performed in workers incidentally exposed to ethylene oxide peak levels (as measured by haemoglobin adduct levels) showed that exposure to concentrations up to 785 mg/m$^3$ (8h TWA) did not cause any permanent mutational/cytogenetic damage in their lymphocytes. Haemoglobin adduct levels (2-hydroxyethyl adducts to the N-terminal valine residues (N-2-hydroxyethylvaline) in haemoglobin) were very high in workers accidentally exposed to ethylene oxide (approximately one month after the incident) when compared to adduct levels in workers chronically exposed to ethylene oxide for <5 or >15 years or human subjects from a control group. Genetic tests (including tests for *hprt* mutants, micronuclei and SCE in lymphocytes) were performed on blood samples collected 89-180 days after the incidental exposure. One of the exposed workers reported sickness and vomiting following the accident. The presence of other (acute) symptoms (such as mucous membrane and respiratory irritation) was not reported (Tates *et al.*, 1995).

# 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 ethylene oxide, 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 6 studies were identified -with 8 datasets for 4 species- with data on lethality following acute inhalation exposure. No datasets were assigned status A for deriving the human probit function, 5 datasets were assigned status B1 and 3 were assessed to be unfit (status C) for human probit function derivation.

## Sensory irritation

No relevant studies on sensory irritation were found.

# 5. Probit functions from individual studies

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

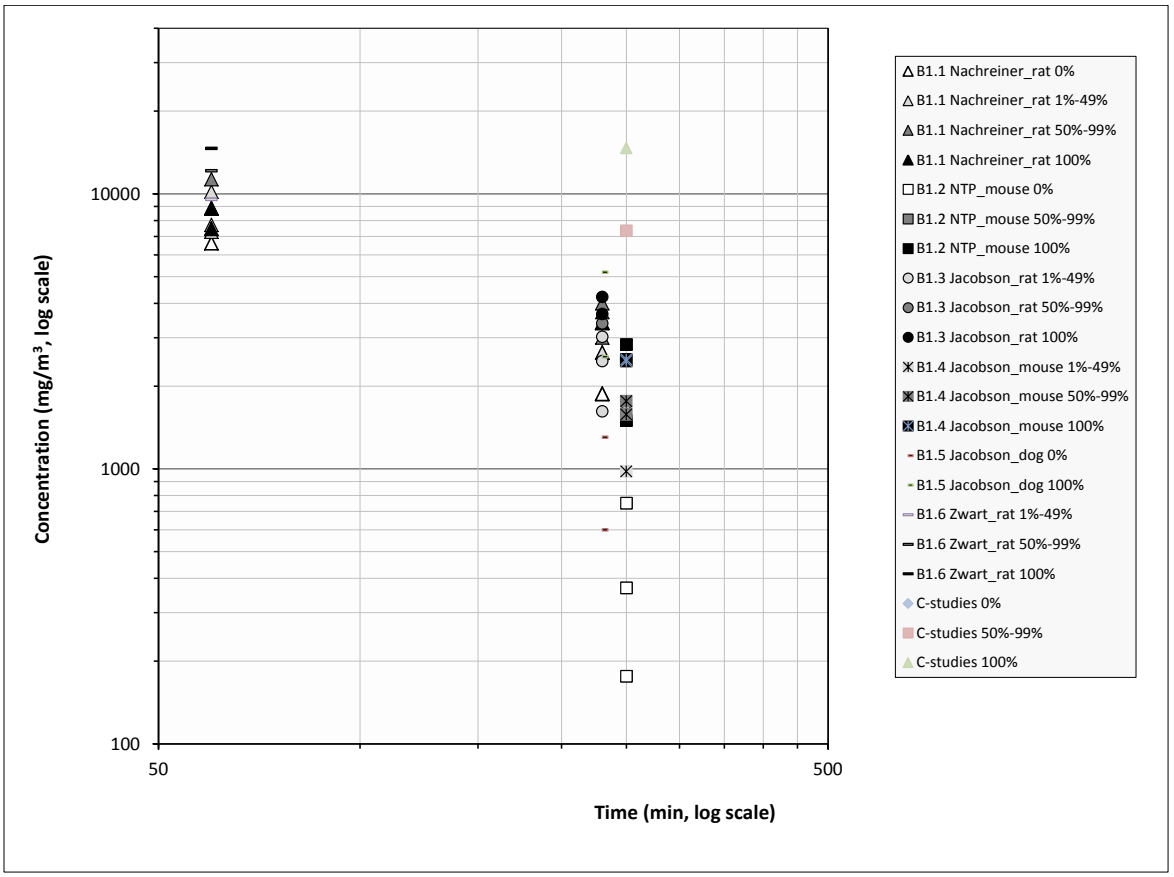



***Figure 1*** *All available acute lethality data for ethylene oxide.*

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

It was not possible to derive a probit function for ethylene oxide based on studies with A quality as these were not available. Therefore, the probit function was derived using data from the studies with B1 quality, none of which enabled to produce a concentration-time-lethality relationship.

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 1.

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

| **Study ID** | **Species** | **Probit (C in mg/m³, t in min)** | **$LC_{50}$, at tested concentration (mg/m³) 95% C.I.** | **$LC_{50}$, 60 minutes (mg/m³) 95% C.I.** *(underline italic for scaled values)* |
|---|---|---|---|---|
| B1.1 | Rat | *60-min $LC_{50}$* | | 9166 (C.I. could not be calculated)[C]<br>10730 (9958-11670)[M]<br>7459 (7072-7922)[F] |
| | | *240-min $LC_{50}$* | 3207 (C.I. could not be calculated)[C]<br>3683 (3450-3912)[M]<br>2817 (2605-3029)[F] | *6414* *<br><br>*7366* *<br>*5634* * |

| Study ID | Species | Probit (C in mg/m³, t in min) | $LC_{50}$, at tested concentration (mg/m³) 95% C.I. | $LC_{50}$, 60 minutes (mg/m³) 95% C.I. *(underline italic for scaled values)* |
|---|---|---|---|---|
| B1.2 | Mouse | *240-min $LC_{50}$* | 1322 (C.I. could not be calculated) | *2644* * |
| B1.3 | Rat | *240-min $LC_{50}$* | 2603 (C.I. could not be calculated) | *5206* * |
| B1.4 | Mouse | *240-min $LC_{50}$* | 1547 (1327-1720) | *3094* * |
| B1.5 | Dog | *240-min $LC_{50}$* | 1824 (C.I. could not be calculated) | *3648* * |
| B1.6 | Rat | *60-min $LC_{50}$* | | 10730 (9573 - 11750) |

[C] combined analysis males and females (i.e. without sex as covariate)
[M] $LC_{50}$-value for male based on analysis with sex as covariate
[F] $LC_{50}$-value for female based on analysis with sex as covariate
[*] $LC_{50}$-values were scaled with the default n-value of 2

The data of the rat studies B1.1, B1.3 and B1.6, mouse studies B1.2 and B1.4 and dog study B1.5 are presented graphically below.

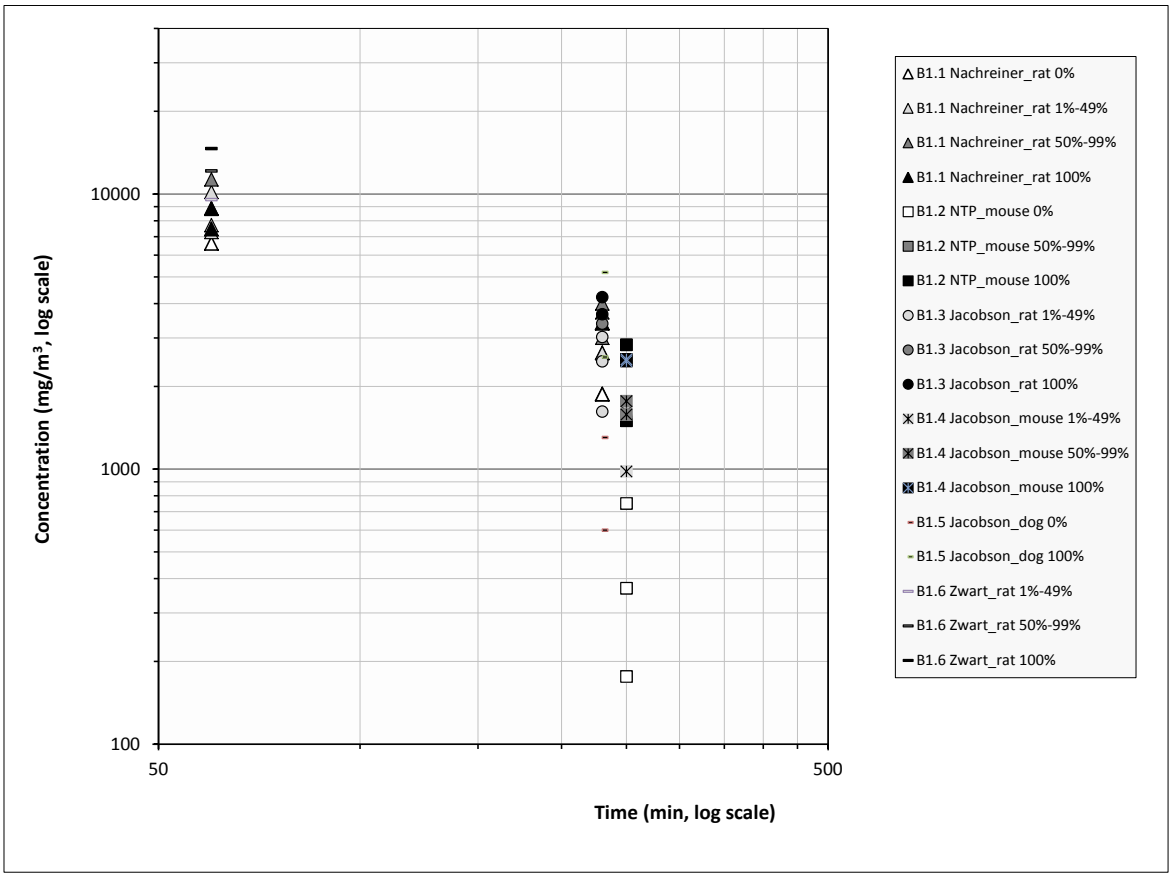



***Figure 2*** *Data selected for the initial analysis for the derivation of the animal probit function of ethylene oxide.*

Based on criteria outlined in the guideline, the 60-min dataset of the rat study B1.1 and rat study B1.6 were selected for the final dataset for the derivation of the animal probit function. The 240-min dataset of rat study B1.1 and the data of rat study B1.3, mouse studies B1.2 and B1.4 and dog study B1.5 (all including an exposure duration of 240 min) were not selected for the final dataset.
According to the methodology, studies with an exposure duration closer to the target of 30-60 min are preferred. An extrapolation from an exposure duration of 240 min to

a 30-60 min value, which, in absence of a substance-specific n-value, should in this case be applied with the default n-value of 2, is believed to be too uncertain. Therefore, preference was given to the 60-min dataset of rat study B1.1 and rat study B1.6.
The 240-min dataset of rat study B1.1 and the data of rat study B1.3, mouse studies B1.2 and B1.4 and dog study B1.5 do however provide support for selecting the 60-min dataset of rat study B1.1 and B1.6.

Figure 3 provides an overview of $LC_{50}$ value for the selected studies in the final analysis. The data that were selected for final analysis of the animal probit function are presented in Table 2 and Figure 4.

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

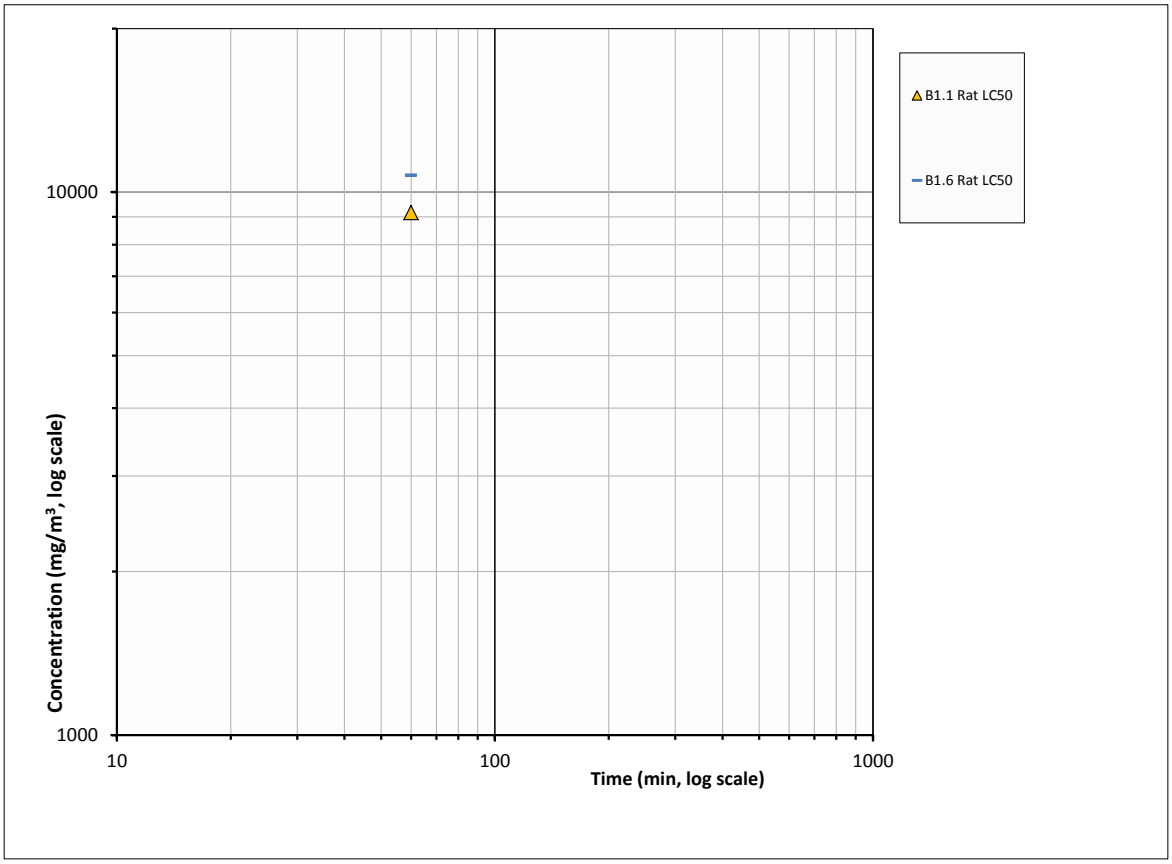



***Figure 3*** *$LC_{50}$ value of the 60-min B1.1 dataset and study B1.6 for ethylene oxide.*

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

| Study ID | Species | Probit (C in mg/m³, t in min) | $LC_{50}$, at tested concentration (mg/m³) 95% C.I. | $LC_{50}$, 60 minutes (mg/m³) 95% C.I. *(underline italic for scaled values)* |
|---|---|---|---|---|
| B1.1 | Rat | *60-min $LC_{50}$* | | 9166 (C.I. could not be calculated)[C]<br>10730 (9958-11670)[M]<br>7459 (7072-7922)[F] |
| B1.6 | Rat | *60-min $LC_{50}$* | | 10730 (9573 - 11750) |

[C] combined analysis males and females (i.e. without sex as covariate)
[M] $LC_{50}$-value for male based on analysis with sex as covariate
[F] $LC_{50}$-value for female based on analysis with sex as covariate

The data of the selected datasets are presented graphically below.

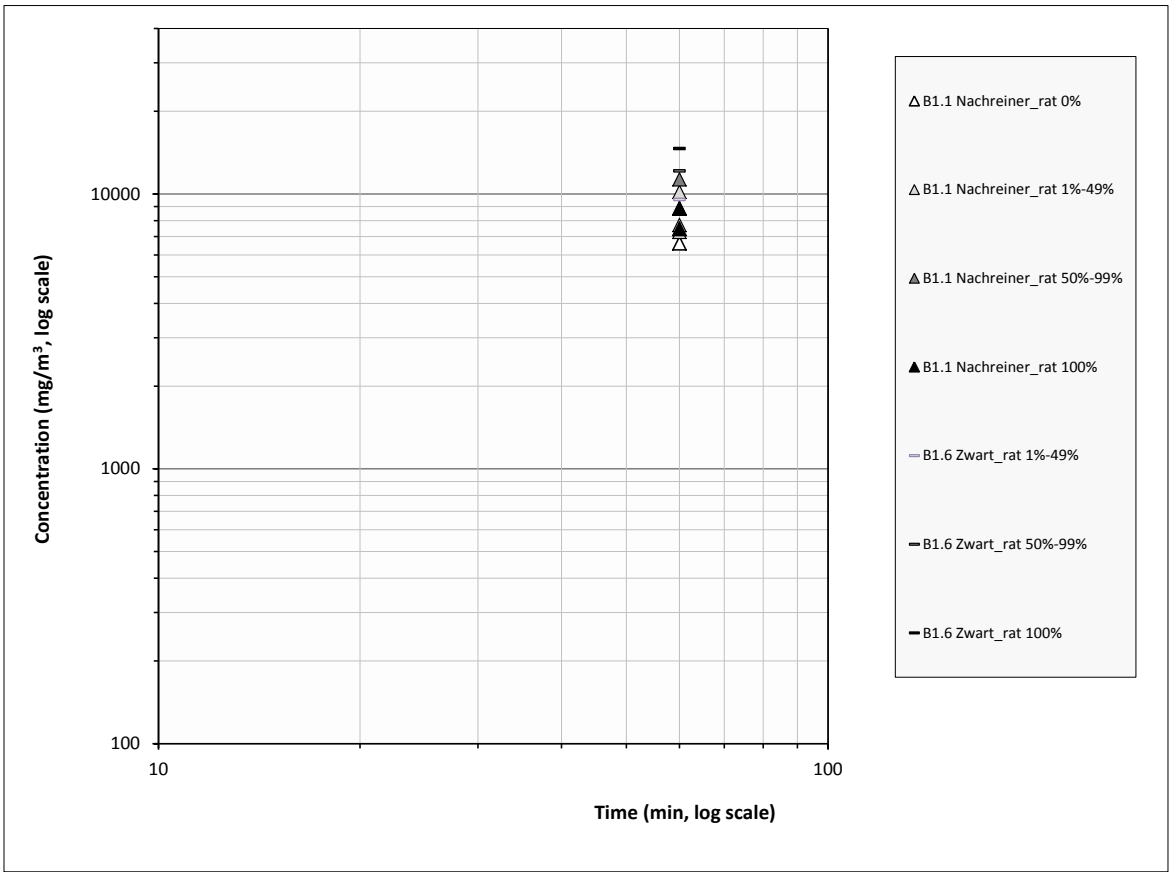



**Figure 4** Final data selected for derivation of the animal probit function of ethylene oxide. Note that only the 60-min dataset of study B1.1 was included.

# 6. Derivation of the human probit function

To derive the human probit function the results from the 60-min dataset of rat study B1.1 and the dataset of rat study B1.6 have been used to derive a point of departure as outlined above.

The rat geometric mean $LC_{50}$-value was calculated from all available 60-min $LC_{50}$ values of studies B1.1 and B1.6. The rat 60-min $LC_{50}$-value was 9917 mg/m$^3$. The formula for the geometric mean of the $LC_{50}$-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 60-minute geometric mean animal $LC_{50}$ value of 9917 mg/m$^3$.

Application of an overall assessment factor of 3 (determined by an interspecies factor of 3) would result in a 60-min $LC_{01}$ of 1031 mg/m$^3$, which is in conflict with human data. A study performed in workers incidentally exposed to ethylene oxide peak levels showed that exposure to concentrations ranging from 53 mg/m$^3$ up to 785 mg/m$^3$ (8h TWA) did not lead to health effects (as measured by *hprt* mutants, micronuclei and SCE in lymphocytes) (Tates *et al*., 1995). The presence of other effects (such as respiratory irritation) was not described.

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: | 1 | Default value of 3 was reduced to 1, see text above |
| Nominal concentration | 1 | B1-studies with analytically determined concentrations |
| Adequacy of database: | 1 | Two B1-datasets supported by five 240-min datasets. |

The estimated human equivalent 60-minute $LC_{50}$ value is 9917 / 1 = **9917 mg/m$^3$**.

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

The human probit function is then calculated on the human equivalent 60 min $LC_{50}$ using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1.0 \times \ln(9917^{2.0} \times 60)$ resulting in the a-value of **-17.50**.

**$Pr = -17.5 + 1 \times \ln(C^2 \times t)$ with C in mg/m$^3$ and t in min.**

The derived human probit function has a scientifically sound basis. The probit function is based on two studies in the rat with B1 quality, including in total 11 C x t combinations and lethality in the range of 0-100%.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 2117 mg/m$^3$ and the calculated human 60 min $LC_1$ (Pr = 2.67) is 3096 mg/m$^3$.

***Table 4*** *LC-values calculated with the derived probit function compared with existing acute inhalation exposure guidelines.*

| Estimated level | 30 min (mg/m$^3$) | 60 min (mg/m$^3$) |
|---|---|---|
| 0.1% lethality, this probit | 2994 | 2117 |
| 1% lethality, this probit | 4378 | 3096 |
| AEGL-3[2] (2010, final) | 660 | 367 |
| ERPG-3[2] (2014) | - | 917 |
| LBW (2015) | 2200 | 1200 |

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

[2] 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:*** ***Nachreiner (1991) / Nachreiner (1992) / Snellings et al. (2011)****

Substance: Ethylene oxide
Species, strain, sex: Rat, Sprague-Dawley, male/female
Number/sex/conc. group: 5 males and/or females/group
Age and weight: males: 51-60 days, females: 57-61 days (mean/group)
males: 220-285 g, females: 180-200 g (mean/group)
Observation period: 14 days

* Nachreiner (1991) and Nachreiner (1992) are inhalation experiments with ethylene oxide with a 4-hour and 1-hour exposure duration, respectively. The experiments were performed with the same type of animals consecutively at the same laboratory, however reported separately. In 2011, parts of the results of the two experiments were published in the public literature (Snellings *et al*., 2011).

**Evaluation of study quality**

| **Criteria** | ***Comment*** |
|---|---|
| Study carried out according to GLP | yes |
| 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 | No information available |
| Use of vehicle (other than air) | No |
| Whole body / nose-only (incl. head/nose-only) exposure | Whole body |
| Type of restrainer | N/A |
| Pressure distribution | *1-hour exposure*: The inhalation chamber (120 L) was under positive pressure (and placed in a negative pressure containment system for safety)<br>*4-hour exposure*: The inhalation chamber (1300 L) was under negative pressure |
| Homogeneity of test atmosphere in breathing zone of animals | *1-hour exposure*: Ethylene oxide was generated using a cylinder with liquid ethylene oxide. The vapour was regulated through stainless steel tubing and then metered through a calibrated flow meter, diluted with filtered-room air before being carried into the exposure chamber.<br>*4-hour exposure*: Ethylene vapour was generated using a cylinder with liquid ethylene oxide maintained at 35°C in a water bath. The resulting ethylene oxide vapour was regulated using stainless steel tubing and delivered through a flowmeter into a mixing chamber with filtered-room air before being carried into the inhalation chamber. |

| | |
|---|---|
| Number of air changes per hour | *1-hour exposure*: 15 air changes per hour (airflow rate: 30 L/min)<br>*4-hour exposure*: 14 air changes per hour (airflow rate 300 L/min) |
| Equilibration time (t95) | *1-hour exposure*: 12 min, however see below<br>*4-hour exposure*: 13 min |
| Start of exposure relative to equilibration | *1-hour exposure*: it is stated by the authors that "because of the short exposure duration, the test vapor atmosphere was generated in the chamber before placement of the animals in the chamber. After maintaining the desired ethylene oxide target exposure concentration, the animals were quickly inserted into the chamber by a sliding draw mechanism, and at the end of the one hour, they were quickly removed."<br>*4-hour exposure*: Not specified. |
| Actual concentration measurement | Analysis of exposure chamber atmosphere (1-hour exposure: every 15 minutes, 4-hour exposure: every 30 minutes) performed using a gas chromatograph equipped with a flame ionization detector |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | N/A |
| | |
| Assessment of Reliability | **B1**<br>Well performed study. Limited to two exposure durations. |

**Results**

| Species | Concentration ($mg/m^3$) | | Exposure duration (min) | Lethality | |
|---|---|---|---|---|---|
| | Measured | Adjusted | | Male | Female |
| | | | | Dead/tested | |
| | 6619 | - | 60 | - | 0/5 * |
| | 7274 | - | 60 | - | 2/5 |
| | 7453 | - | 60 | - | 5/5 * |
| | 7706 | - | 60 | - | 1/5 |
| | 8853 | - | 60 | 0/5 | 5/5 |
| | 10171 | - | 60 | 1/5 | - |
| | 11299 | - | 60 | 4/5 | - |
| | 1873 | - | 240 | 0/5 * | 0/5 * |
| | 2646 | - | 240 | 0/5 * | 1/5 |
| | 3002 | - | 240 | - | 4/5 |
| | 3393 | - | 240 | 0/5 | 5/5 |
| | 3716 | - | 240 | 4/5 | - |
| | 4002 | - | 240 | 4/5 | - |

* data not presented in Snellings *et al*. 2011

**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 = male, 1 = female).

Probit functions have been derived for the 60 minute exposure data, the 240 minute exposure data, and data of both exposure durations combined.

**60 minute exposure duration**

| Probit function | Species | a | b | c | d | n-value |
|---|---|---|---|---|---|---|
| Sex as variable | *Rat* | *-121* | *13.6* | - | *4.95* | - |
| Sexes combined | *Rat* | *-8.62* | *1.49* | - | - | - |

| *Duration (minutes)* | *$LC_{50}$ ($mg/m^3$) 95%-C.I.* ***Male*** | *$LC_{50}$ ($mg/m^3$) 95%-C.I.* ***Female*** | *$LC_{50}$ ($mg/m^3$) 95%-C.I.* ***Combined*** |
|---|---|---|---|
| 60 | 10730 (9958-11670) | 7459 (7072-7922) | 9166 (C.I. could not be calculated) |

**240 minute exposure duration**

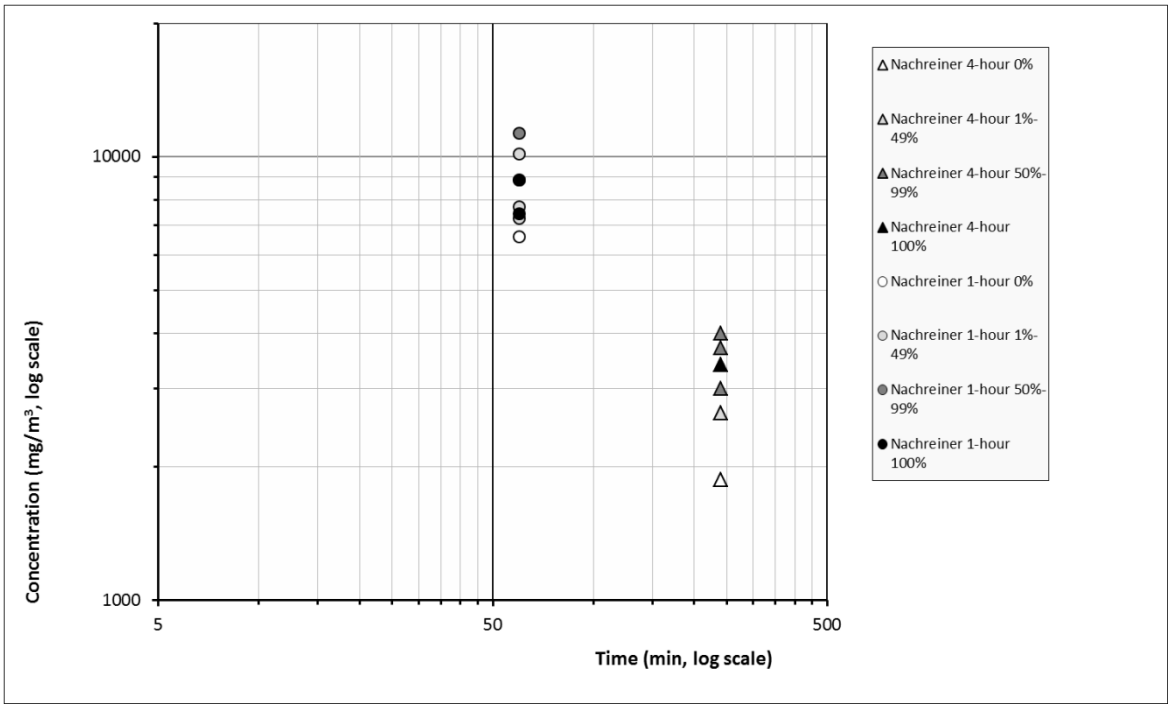
| Probit function | Species | a | b | c | d | n-value |
|---|---|---|---|---|---|---|
| Sex as variable | *Rat* | *-119* | *15.1* | - | *4.04* | - |
| Sexes combined | *Rat* | *-33.0* | *4.71* | - | - | - |

| *Duration (minutes)* | *$LC_{50}$ ($mg/m^3$) 95%-C.I.* ***Male*** | *$LC_{50}$ ($mg/m^3$) 95%-C.I.* ***Female*** | *$LC_{50}$ ($mg/m^3$) 95%-C.I.* ***Combined*** |
|---|---|---|---|
| 240 | 3683 (3450-3912) | 2817 (2605-3029) | 3207 (C.I. could not be calculated) |

As only data of two exposure durations are available, an n-value will not be accepted (*an n-value can be estimated mathematically from a study with two exposure durations, the Probit Expert Panel will only accept n-values derived from at least 3 exposure durations). For this reason, data from both exposure durations were analysed separately to derive the animal probit function and $LC_{50}$ values.

The $LC_{50}$ values for both sexes did not differ by more than a factor of 2. This does not support the proposition that sex differences exist in the lethal response. For this reason the data from both sexes were pooled and analysed to derive the animal probit function.

A graphical overview of the data is presented below. Each concentration-time combination represents one point in the plot.


Concentration (mg/m³, log scale)
10000
1000
5
50
500
Time (min, log scale)
Nachreiner 4-hour 0%
Nachreiner 4-hour 1%-49%
Nachreiner 4-hour 50%-99%
Nachreiner 4-hour 100%
Nachreiner 1-hour 0%
Nachreiner 1-hour 1%-49%
Nachreiner 1-hour 50%-99%
Nachreiner 1-hour 100%

1
2

# Study ID: B1.2

***Author, year:*** ***NTP, 1987***

Substance: Ethylene oxide

Species, strain, sex: Mouse, B6C3F$_1$-strain, male + female

Number/sex/conc. group: 5

Age and weight: 8-10 weeks old

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) | No statement of compliance with OECD guideline 403 provided |
| Stability of test compound in test atmosphere | Samples of ethylene oxide exposure chamber atmospheres were examined for the occurrence of potential degradation products, specifically ethylene glycol and acetaldehyde. No evidence for any degradation product was detected. |
| Use of vehicle (other than air) | No |
| Whole body / nose-only (incl. head/nose-only) exposure | Whole body |
| Type of restrainer | N/A |
| Pressure distribution | No information |
| Homogeneity of test atmosphere in breathing zone of animals | Ethylene oxide vapour was generated using a cylinder with liquefied ethylene oxide in a 55°C water bath. Ethylene oxide vapour was routed through a manifold to dual gas metering valves that controlled the gas flow to each chamber. After passing through the metering valves, ethylene oxide was mixed with air and entered the exposure chamber. |
| Number of air changes per hour | The chamber air was changed at 10 times/h during exposure. |
| Equilibration time (t95) | Insufficient information to calculate t95 |
| Start of exposure relative to equilibration | No information |
| Actual concentration measurement | Two methods were used for analysis of inhalation chamber atmosphere: photo ionization detector or gas chromatograph equipped with flame ionization detector |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | N/A |
| | |
| Assessment of Reliability | **B1**<br>Well performed study. Limited to one exposure duration. |

**Results**

| Species | Concentration ($mg/m^3$) | | Exposure duration (min) | Lethality | |
|---|---|---|---|---|---|
| | Measured | Adjusted | | Male | Female |
| | | | | Dead/tested | |
| mouse | 176 | - | 240 | 0/5 | 0/5 |
| | 369 | - | 240 | 0/5 | 0/5 |
| | 750 | - | 240 | 0/5 | 0/5 |
| | 1497 | - | 240 | 5/5 | 4/5 |
| | 2828 | - | 240 | 5/5 | 5/5 |

**Probit function**

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

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

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

| Probit function | Species | a | b | d | n-value |
|---|---|---|---|---|---|
| Sex as variable | Mouse | -68.5 | 10.5 | -2.81 | - |
| Sexes combined | Mouse | -68.9 | 10.3 | - | - |

The $LC_{50}$ values for both sexes did not differ by more than a factor of 2. This does not support the proposition that sex differences exist in the lethal response. For this reason the data from both sexes were pooled and analysed to derive the animal probit function.

| Duration (min.) | $LC_{50}$ ($mg/m^3$) 95%-C.I. Male | $LC_{50}$ ($mg/m^3$) 95%-C.I. Female | $LC_{50}$ ($mg/m^3$) 95%-C.I. Combined |
|---|---|---|---|
| 240 | 1059 (a C.I. could not be derived) | 1382 (a C.I. could not be derived) * | 1322 (a C.I. could not be derived) |

* NTP (1987) calculated a 4-hour $LC_{50}$ of 1210 $mg/m^3$ (95%-CI: 934-1570 $mg/m^3$)

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

A graphical overview of the data is not presented.

# Study ID: B1.3

***Author, year:*** ***Jacobson et al., 1956***
Substance: Ethylene oxide
Species, strain, sex: rat, unknown strain, male
Number/sex/conc. group: 10
Age and weight: no information available
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 | No information available |
| Use of vehicle (other than air) | No |
| Whole body / nose-only (incl. head/nose-only) exposure | Whole body |
| Type of restrainer | N/A |
| Pressure distribution | No information available |
| Homogeneity of test atmosphere in breathing zone of animals | Ethylene oxide air concentrations were established and maintained in the exposure chambers (0.4 $m^3$ for rodents) by passing the gas at a controlled rate through a flowmeter into a mixing bowl, where it was diluted with room air. |
| Number of air changes per hour | No information available |
| Equilibration time (t95) | Insufficient information to calculate t95 |
| Start of exposure relative to equilibration | No information available |
| Actual concentration measurement | Ethylene oxide atmospheres were analysed using a colorimetric procedure in which ethylene oxide was collected in a solution of 60% $CaCl_2$ and 0.1 N HCl or a 50% solution of $MgBr_2$ and 0.1 N $H_2SO_4$ and titrated with NaOH. Both methods gave similar results. |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | N/A |
| | |
| Assessment of Reliability | **B1**<br>Well performed study, though some details on study characteristics missing. Limited to one exposure duration. |

**Results**

| Species | Concentration ($mg/m^3$) | | Exposure duration (min) | Lethality |
|---|---|---|---|---|
| | Measured | Adjusted | | Male |
| | | | | Dead/tested |
| Rat | 1618 | - | 240 | 2/10 |

| | | | | |
|---|---|---|---|---|
| | 2463 | - | 240 | 2/10 |
| | 3022 | - | 240 | 4/10 |
| | 3380 | - | 240 | 9/10 |
| | 3653 | - | 240 | 10/10 |
| | 4215 | - | 240 | 10/10 |

**Probit function**

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

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

with C for concentration in $mg/m^3$.

| Probit function | Species | a | b | n-value |
|---|---|---|---|---|
| | Rat | -20.6 | 3.25 | - |

| Duration (min.) | LC50 ($mg/m^3$) 95%-C.I.; as calculated by TSD author | LC50 ($mg/m^3$) 95%-C.I.; as presented by Jacobson *et al*. |
|---|---|---|
| 240 | 2603 (a CI could not be calculated) | 2678 (1137-4677) |

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

A graphical overview of the data is not presented.

# Study ID: B1.4

***Author, year:*** ***Jacobson et al., 1956***
Substance: Ethylene oxide
Species, strain, sex: mouse, unknown strain, male
Number/sex/conc. group: 10
Age and weight: no information available
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 | No information available |
| Use of vehicle (other than air) | No |
| Whole body / nose-only (incl. head/nose-only) exposure | Whole body |
| Type of restrainer | N/A |
| Pressure distribution | No information available |
| Homogeneity of test atmosphere in breathing zone of animals | Ethylene oxide air concentrations were established and maintained in the exposure chambers (0.4 $m^3$ for rodents) by passing the gas at a controlled rate through a flowmeter into a mixing bowl, where it was diluted with room air before entering the chamber. |
| Number of air changes per hour | No information available |
| Equilibration time (t95) | Insufficient information to calculate t95 |
| Start of exposure relative to equilibration | No information available |
| Actual concentration measurement | Ethylene oxide atmospheres were analysed using a colorimetric procedure in which ethylene oxide was collected in a solution of 60% $CaCl_2$ and 0.1 N HCl or a 50% solution of $MgBr_2$ and 0.1 N $H_2SO_4$ and titrated with NaOH. Both methods gave similar results. |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | N/A |
| | |
| Assessment of Reliability | **B1**<br>Well performed study, though some details on study characteristics missing. Limited to one exposure duration. |

**Results**

| Species | Concentration ($mg/m^3$) | | Exposure duration (min) | Lethality |
|---|---|---|---|---|
| | Measured | Adjusted | | Female |
| | | | | Dead/tested |
| Mouse | 978 | - | 240 | 1/10 |

| | | | | |
|---|---|---|---|---|
| | 1577 | - | 240 | 6/10 |
| | 1618 | - | 240 | 3/10 |
| | 1761 | - | 240 | 7/10 |
| | 2463 | - | 240 | 10/10 |
| | 2503 | - | 240 | 10/10 |

**Probit function**

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

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

with C for concentration in $mg/m^3$.

| Probit function | Species | a | b | n-value |
|---|---|---|---|---|
| | Mouse | -23.0 | 3.82 | - |

| Duration (min.) | LC50 ($mg/m^3$) 95%-C.I.; as calculated by TSD author | LC50 ($mg/m^3$) 95%-C.I.; as presented by Jacobson *et al*. |
|---|---|---|
| 240 | 1547 (1327-1720) | 1531 (1143-1907) |

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

A graphical overview of the data is not presented.

# Study ID: B1.5

***Author, year:*** ***Jacobson et al., 1956***
Substance: Ethylene oxide
Species, strain, sex: dog, beagle, male
Number/sex/conc. group: 3
Age and weight: no information available
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 | No information available |
| Use of vehicle (other than air) | No |
| Whole body / nose-only (incl. head/nose-only) exposure | Whole body |
| Type of restrainer | N/A |
| Pressure distribution | No information available |
| Homogeneity of test atmosphere in breathing zone of animals | Ethylene oxide air concentrations were established and maintained in the exposure chambers (0.7 $m^3$ for dogs) by passing the gas at a controlled rate through a flowmeter into a mixing bowl, where it was diluted with room air. |
| Number of air changes per hour | No information available |
| Equilibration time (t95) | Insufficient information to calculate t95 |
| Start of exposure relative to equilibration | No information available |
| Actual concentration measurement | Ethylene oxide atmospheres were analysed using a colorimetric procedure in which ethylene oxide was collected in a solution of 60% $CaCl_2$ and 0.1 N HCl or a 50% solution of $MgBr_2$ and 0.1 N $H_2SO_4$ and titrated with NaOH. Both methods gave similar results. |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | N/A |
| | |
| Assessment of Reliability | **B1**<br>Well performed study, though some details on study characteristics missing. Limited to one exposure duration. |

**Results**

| Species | Concentration ($mg/m^3$) | | Exposure duration (min) | Lethality |
|---|---|---|---|---|
| | Measured | Adjusted | | Male |
| | | | | Dead/tested |
| Dog | 600 | - | 240 | 0/3 |
| | 1302 | - | 240 | 0/3 |

| | | | | |
|---|---|---|---|---|
| | 2555 | - | 240 | 3/3 |
| | 5190 | - | 240 | 3/3 |

**Probit function**

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

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

with C for concentration in $mg/m^3$.

| Probit function | Species | a | b | n-value |
|---|---|---|---|---|
| | Dog | -73.8 | 10.5 | - |

| Duration (min.) | LC50 ($mg/m^3$) 95%-C.I.; as calculated by TSD author | LC50 ($mg/m^3$) 95%-C.I.; as presented by Jacobson *et al*. |
|---|---|---|
| 240 | 1824 (a CI could not be calculated) | 1761 (a CI could not be calculated) |

For B1-studies: No C × t probit function could be calculated from these data alone.

A graphical overview of the data is not presented.

# Study ID: B1.6

***Author, year:*** ***Zwart, 1986***

Substance: ethylene oxide
Species, strain, sex: rat, Bor: WISW=Cpb:WU, male/female
Number/sex/conc. group: 5/sex/conc. group
Age and weight: age not specified, weight: 140 g (m) and 116 g (f)
Observation period: 14 days

**Evaluation of study quality**

| **Criteria** | ***Comment*** |
|---|---|
| Study carried out according to GLP | yes |
| 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 | No information |
| Use of vehicle (other than air) | No |
| Whole body / nose-only (incl. head/nose-only) exposure | Whole body (in horizontally placed glass tubes) |
| Type of restrainer | N/A |
| Pressure distribution | No information |
| Homogeneity of test atmosphere in breathing zone of animals | An ethylene oxide test atmosphere was generated by mixing an adjustable flow of ethylene oxide with the airflow before entering the exposure chamber |
| Number of air changes per hour | The capacity of the inhalation chamber is 15 L. The exposure chamber was ventilated with 1200 L air/hour (20 L/min), resulting in 80 air changes per hour |
| Equilibration time (t95) | 2.25 min |
| Start of exposure relative to equilibration | No information |
| Actual concentration measurement | The concentration of ethylene oxide in the test atmosphere was determined by IR analysis in a sample drawn continuously from the test tube |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | N/A |
| | |
| Assessment of Reliability | **B1**<br>Well-performed study; Limited to one exposure duration. |

**Results**

| Species | Concentration ($mg/m^3$) | | Exposure duration (min) | Lethality |
|---|---|---|---|---|
| | Measured | Adjusted | | Male + female* |
| | | | | Dead/tested |
| Rat | $9.55 \times 10^3$ | N/A | 60 | 2/10 |
| Rat | $12.14 \times 10^3$ | N/A | 60 | 8/10 |
| Rat | $14.64 \times 10^3$ | N/A | 60 | 10/10 |

* Lethality data presented for male and female animals combined

**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 |
|---|---|---|---|---|
| | Rat | -65.3 | 7.57 | - |

| Duration (min.) | $LC_{50}$ ($mg/m^3$) 95%-C.I. Male+female | $LC_{50}$ ($mg/m^3$) 95%-C.I. Male+female (as reported by study author) |
|---|---|---|
| 60 | 10730 (9573 - 11750) | 10950 (9510-12170) |

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

## Study ID: C studies

Carpenter *et al*. (1949) reported an acute inhalation study with male or female Sherman rats exposed to ethylene oxide. A 4-hour exposure to 4000 ppm (7336 mg/m$^3$; nominal concentration) was reported including a 14-day post-exposure period. However, no precise lethality data were presented. At this exposure, lethality was between approximately 50% (i.e. 33% (2/6) and 67% (4/6)).

Waite *et al.* (1930) exposed guinea pigs to ethylene oxide vapour. The exposure concentrations were 0.025, 0.05, 0.13, 0.3, 0.7, 1.4-2.5, 4, 5.1, 6.3-6.4, 8.5 vol% (250, 500, 1300, 3000, 7000, 14000-25000, 40000, 51000, 63000-64000, 85000 ppm corresponding to 459, 917, 2385, 5503, 12841, 25682-45860, 73376, 93554, 115567-117401, 155923 mg/m$^3$). Exposure durations varied from 1 to 480 minutes. One to four guinea pigs were used for each exposure.
Deaths occurred during exposure to 155923 mg/m$^3$ ethylene oxide for 33 min. Deaths also occurred within 24 h after exposure to concentrations of 115567-117401 mg/m$^3$ for 10 or 20 min, 45860 mg/m$^3$ for 60 min, 25682 mg/m$^3$ for 60 or 107 min, 12841 mg/m$^3$ for 150 min, and 5503 mg/m$^3$ for 330 min. Deaths occurred between 1 and 8 days in groups exposed to 93554 mg/m$^3$ for 6 min, 73376 mg/m$^3$ for 20 min, 25682 mg/m$^3$ for 20 min, 12841 mg/m$^3$ for 60 min, 5503 mg/m$^3$ for 190 min, and 2385 mg/m$^3$ for 480 min. No deaths occurred in the groups exposed to 25682 mg/m$^3$ for 10 min, 12841 mg/m$^3$ for 20 min, 5503 mg/m$^3$ for 70 min, 2385 mg/m$^3$ for up to 290 min, and 459 mg/m$^3$ or 917 mg/m$^3$ for 480 min (lethality data taken from AEGL (2010), however, these data could not be retrieved quantitatively by TSD author from the original publication).

Weil *et al.* (1963) exposed rats to ethylene oxide for four hours by inhalation. Limited details on study characteristics are available. Concentrations recorded were nominal and not analytically verified. Exposure to a nominal concentration of 4000 ppm (7336 mg/m$^3$) for 4 hours resulted in mortality rate of 0/6. Exposure for 4 hours to 8000 ppm (14672 mg/m3) resulted in mortality rate of 6/6.

# Appendix 2 Reference list

AEGL, National Research Council. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Volume 9. Washington, DC. The National Academies Press, 2010.

Carpenter CP, Smyth HF, Pozzani UC, “The assay of acute vapor toxicity, and the grading and interpretation of results on 96 chemical compounds,” The Journal of Industrial Hygiene and Toxicology, vol. 31, no. 6, pp. 343–349, 1949.

Chemiekaarten, 32e editie, Den Haag. TNO/SDU uitgevers 2017

ERPG. Emergency Response Planning Guideline; Ethylene Oxide. American Industrial Hygiene Association, 2005.

Jacobson, K.H., E.B. Hackley, and L. Feinsliver. 1956. The toxicity of inhaled ethylene oxide and propylene oxide vapors. AMA Arch. Ind. Health 13(3):237-244 (a: rat, b: mouse, c: dog).

Nachreiner, DJ. (1991). Ethylene oxide: Acute vapor inhalation toxicity test in rats (Four-hour test). Project ID 54-76. Bushy Run Research Center, Export, PA.

Nachreiner, DJ. (1992). Ethylene oxide: Acute vapor inhalation toxicity testing according to D.O.T. regulations (One-hour test). Project ID 54-593. Bushy Run Research Center, Export, PA.

NTP (National Toxicology Program), 1987. Toxicology and Carcinogenesis Studies of Ethylene Oxide (CAS No. 75-21-8) in B6C3F1 Mice (Inhalation Studies). NTP TR 326. NIH 88-2582. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program, Research Triangle Park, NC.

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

Ruijten M.W.M.M., J.H.E. Arts, P.J. Boogaard, P.M.J. Bos, H. Muijser, A. Wijbenga. Methods for the derivation of probit functions to predict acute lethality following inhalation of toxic substances. RIVM report 2015-0102. Bilthoven, RIVM, 2015.

Snellings, W.M., Nachreiner D.J., Pottenger L.H., 2011. Ethylene oxide: acute four-hour and one-hour inhalation toxicity testing in rats. Journal of Toxicology 2011.

Tates A.D., Boogaard P.J., Darroudi F., Natarajan A.T., Caubo M.E., van Sittert N.J., 1995. Biological effect monitoring in industrial workers following incidental exposure to high concentrations of ethylene oxide. Mutation Research 329, 63-77.

Zwart (1986). Acute (one-hour) inhalation toxicity study of ethylene oxide in rats. Civo institutes TNO. Report No. V86.548/260831.

Waite CP, Patty FA, Yang WP, 1930. Acute response of guinea pigs to vapors of some new commercial organic compounds. IV. Ethylene oxide. Public Health Rep. 45(32):1832-1844.

Weil CS, Condra N, Haun C, Streigel JA, 1963. Experimental Carcinogenicity and Acute Toxicity of Representative Epoxides. Am. Ind. Hyg. Assoc. J. 24:305-325.