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

Probit function technical support document

Date: 4 May 2018
Document id: 20180504-Nitrogen dioxide-INTERIM
Status: interim
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| substance name | CAS number |
|---|---|
| **Nitrogen dioxide** | **10102-44-0** |

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 Nitrogen dioxide

## 1. Substance identification

| | |
|---|---|
| CAS-number: | 10102-44-0 |
| IUPAC name: | nitrogen dioxide |
| Synonyms: | nitrogen(IV) oxide |
| Molecular formula: | $NO_2$ |
| Molecular weight: | 46.0 g/mol |
| Physical state: | liquid (at 20°C and 101.3 kPa) |
| Boiling point: | 21°C (at 101.3 kPa) |
| Vapour pressure: | 96 kPa (at 20°C) |
| Saturated vapor conc: | 960000 ppm = 1836 g/m$^3$ (at 20°C) |
| Conversion factor: | 1 mg/m$^3$ = 0.523 ppm (at 20°C and 101.3 kPa) |
| | 1 ppm = 1.913 mg/m$^3$ (at 20°C and 101.3 kPa) |
| Labelling: | H314-H330 |

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

**Acute effects**: $NO_2$ is an irritant to the mucous membranes and might cause coughing and dyspnoea during exposure. After less severe exposure, symptoms might persist for several hours before subsiding. With more severe exposure, pulmonary oedema ensues with signs of chest pain, cough, dyspnoea, cyanosis and moist rales heard on auscultation. Lethality results from bronchospasm and pulmonary oedema in association with hypoxemia and respiratory acidosis, metabolic acidosis, shift of the oxyhaemoglobin dissociation curve to the left, and arterial hypotension. A characteristic of $NO_2$ intoxication after the acute phase is a period of apparent recovery followed by late-onset bronchiolar injury that manifests as bronchiolitis fibrosa obliterans as long-term effect.

**Long-term effects**: Epidemiology studies on the long-term effects of elevated concentrations of $NO_2$ are conflicting. It is likely that increases in respiratory illnesses are from $NO_2$ in combination with other pollutants and that short-term peak concentrations are more detrimental than chronic, low-level exposures.

## 3. Human toxicity data

According to AEGL (2012), exposure to $NO_2$ at >15 ppm (29 mg/m$^3$) causes immediate irritation with pulmonary oedema followed by a latent period of apparent recovery in healthy individuals. A second phase of symptoms can occur after several hours or days, which include fever with progressively more severe dyspnea, cyanosis, and cough, and inspiratory and expiratory rales. The concentration causing death in humans is approximately ≥150 ppm (≥287 mg/m$^3$), but no duration of exposure was presented.

Most case reports do not present information on exposure concentrations or durations; however, welders exposed to 30 and 90 ppm (57 and 172 mg/m$^3$) for 40 min experienced varying degrees of dyspnea, cough, headache, chest tightness, nausea, and cyanosis, and hospitalization was required for pulmonary edema at the higher concentration (AEGL, 2012).

In a human volunteer study, healthy adults (n=10-14/group) were exposed for 2h to 0.5-30 ppm (0.96-57 mg/m$^3$) nitrogen dioxide (Henschler et al., 1960). They reported that a 2-h exposure to $NO_2$ at 20 ppm (38 mg/m$^3$) did not cause any irritation when preceded by several exposures to lower concentrations during the preceding days; however, exposure at 30 ppm (57 mg/m$^3$) for 2h caused definite discomfort. Three

[1] AEGL (2012)

individuals exposed to $NO_2$ at 30 ppm (57 mg/m$^3$) for 2h perceived an intense odour on entering the chamber; odour detection quickly diminished and was completely absent after 25-40 min. One individual experienced a slight tickling of the nose and throat mucous membranes after 30 min, and the others after 40 min. All subjects experienced a burning sensation after 70 min and an increasingly severe cough for the next 10-20 min, but coughing decreased after 100 min. However, the burning sensation continued and moved into the lower sections of the airways and was finally felt deep in the chest. At that time, marked sputum secretion and dyspnea were noted. Toward the end of the exposure, the subjects reported the exposure conditions to be bothersome and barely tolerable. A sensation of pressure and increased sputum secretion continued for several hours after cessation of exposure.
The following effects were noted:
0.5 ppm (0.96 mg/m$^3$): metallic taste
1.5 ppm (2.9 mg/m$^3$): dryness of the throat
4 ppm (7.7 mg/m$^3$): sensation of constriction
25 ppm (48 mg/m$^3$): prickling of the nose
30 ppm (57 mg/m$^3$): burning sensation in nose and chest, cough, dyspnea, sputum production

# 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 nitrogen dioxide, 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 9 studies were identified -with 19 datasets for 6 species- with data on lethality following acute inhalation exposure. Five datasets were assigned status A for deriving the human probit function, no datasets were assigned status B and 14 were assessed to be unfit (status C) for human probit function derivation.

## Sensory irritation

No studies on sensory irritation were found.

# 5. Probit functions from individual studies

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

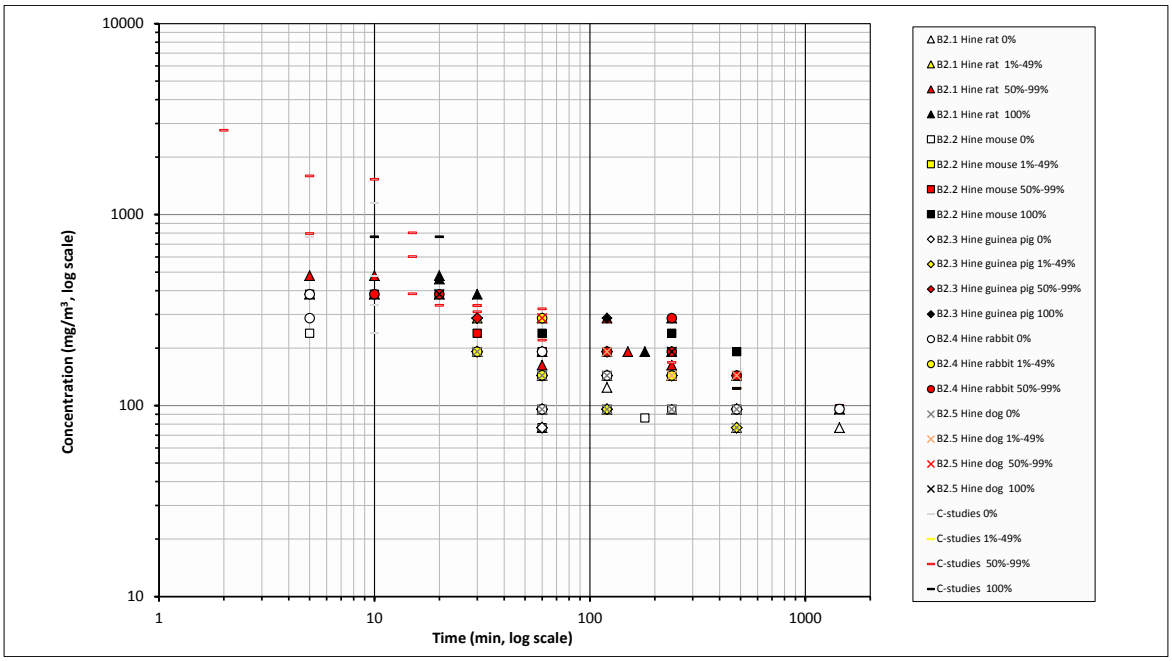



***Figure 1*** *All available acute lethality data for nitrogen dioxide.*

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

All B2 studies were selected for derivation of the animal probit function for nitrogen dioxide.

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 nitrogen dioxide.*

| Study ID | Species | Probit (C in mg/m$^3$, t in min) | $LC_{50}$, 30 minutes (mg/m$^3$) 95% C.I. *(underline italic for scaled values)* | n-value 95% C.I. |
|---|---|---|---|---|
| B2.1 | Rat | -20.5 + 3.97×lnc + 1.04×lnt | 254 (228-280) | 3.81 (3.10-4.52) |
| B2.2 | Mouse | -36.5 + 6.76×lnc + 1.53×lnt | 214 (201-227) | 4.41 (3.84-4.99) |
| B2.3 | Guinea pig | -11.2 + 2.64×lnc + 0.686×lnt | 186 (144-238) | 3.85 (2.05-5.66) |
| B2.4 | Rabbit | -10.3 + 2.23×lnc + 0.658×lnt | 355 (285-587) | 3.39 (2.10-4.68) |
| B2.5 | Dog | -28.9 + 5.31×lnc + 1.18×lnt | 278 (172-369) | 4.50 (1.83-7.16) |

The data of the rat B2.1, mouse B2.2, guinea pig B2.3, rabbit B2.4 and dog B2.5 studies are presented graphically below.

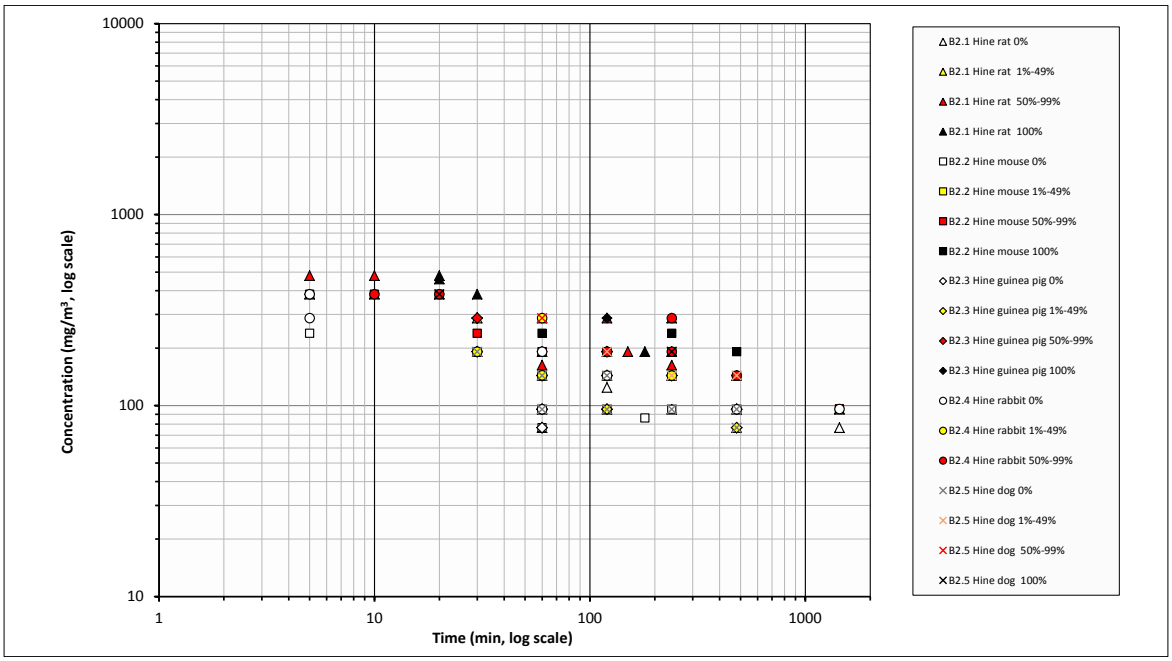



***Figure 2*** *Data selected for the initial analysis for the derivation of the animal probit function of nitrogen dioxide.*

Based on criteria outlined in the guideline the data from the rat B2.1, mouse B2.2, guinea pig B2.3, rabbit B2.4 and dog B2.5 studies were selected for the final dataset for the derivation of the animal probit function. The reason for including these studies is that the studies are considered to be of sufficient quality, including sufficient C x t combinations.

Figure 3 provides an overview of $LC_{50}$ values and $LC_{50}$-time relationships for all 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 5 datasets from one study and includes data from 5 animal species.

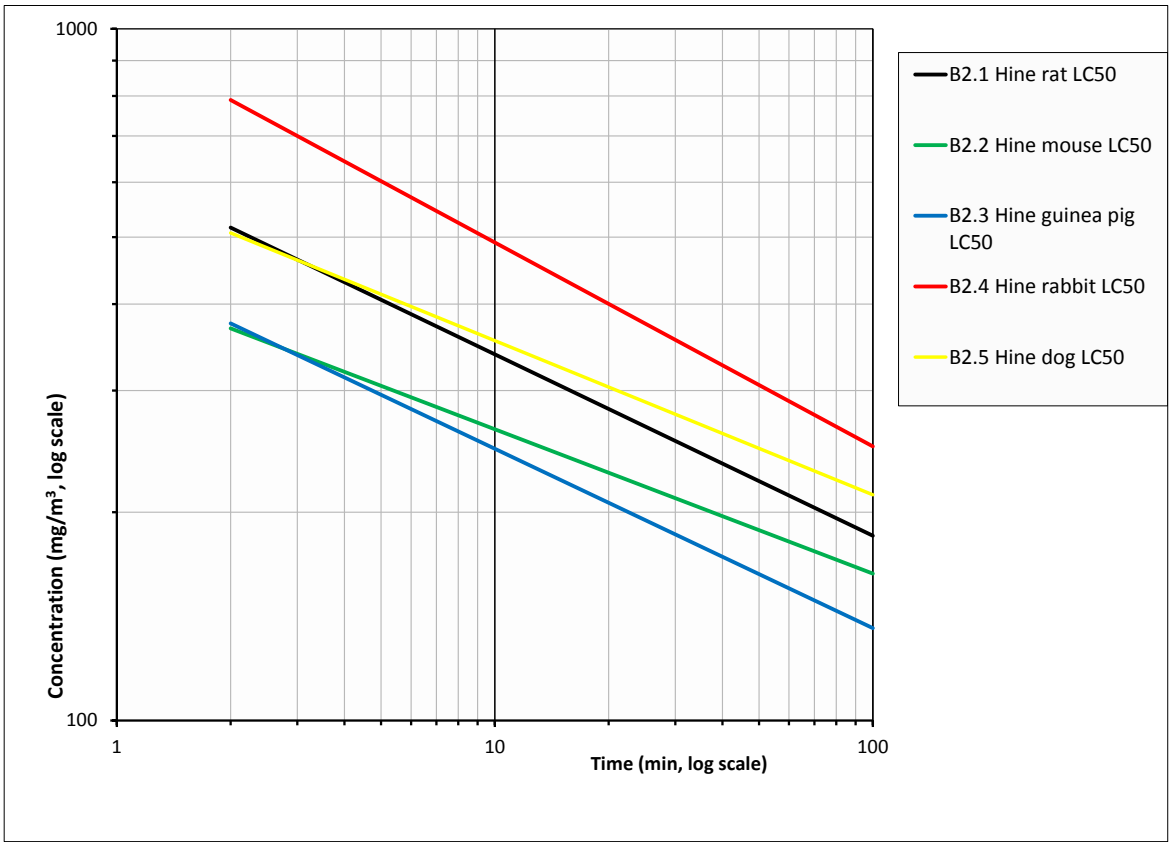



***Figure 3*** *$LC_{50}$ values of all B2 datasets for nitrogen dioxide, over time where available.*

***Table 2*** *Data selected for the derivation of the animal probit function of nitrogen dioxide (identical to table 1).*

| Study ID | Species | Probit (C in mg/m$^3$, t in min) | $LC_{50}$, 30 minutes (mg/m$^3$) 95% C.I. *(underline italic for scaled values)* | n-value 95% C.I. |
|---|---|---|---|---|
| B2.1 | Rat | -20.5 + 3.97×lnc + 1.04×lnt | 254 (228-280) | 3.81 (3.10-4.52) |
| B2.2 | Mouse | -36.5 + 6.76×lnc + 1.53×lnt | 214 (201-227) | 4.41 (3.84-4.99) |
| B2.3 | Guinea pig | -11.2 + 2.64×lnc + 0.686×lnt | 186 (144-238) | 3.85 (2.05-5.66) |
| B2.4 | Rabbit | -10.3 + 2.23×lnc + 0.658×lnt | 355 (285-587) | 3.39 (2.10-4.68) |
| B2.5 | Dog | -28.9 + 5.31×lnc + 1.18×lnt | 278 (172-369) | 4.50 (1.83-7.16) |

The data of the selected datasets are presented graphically below.

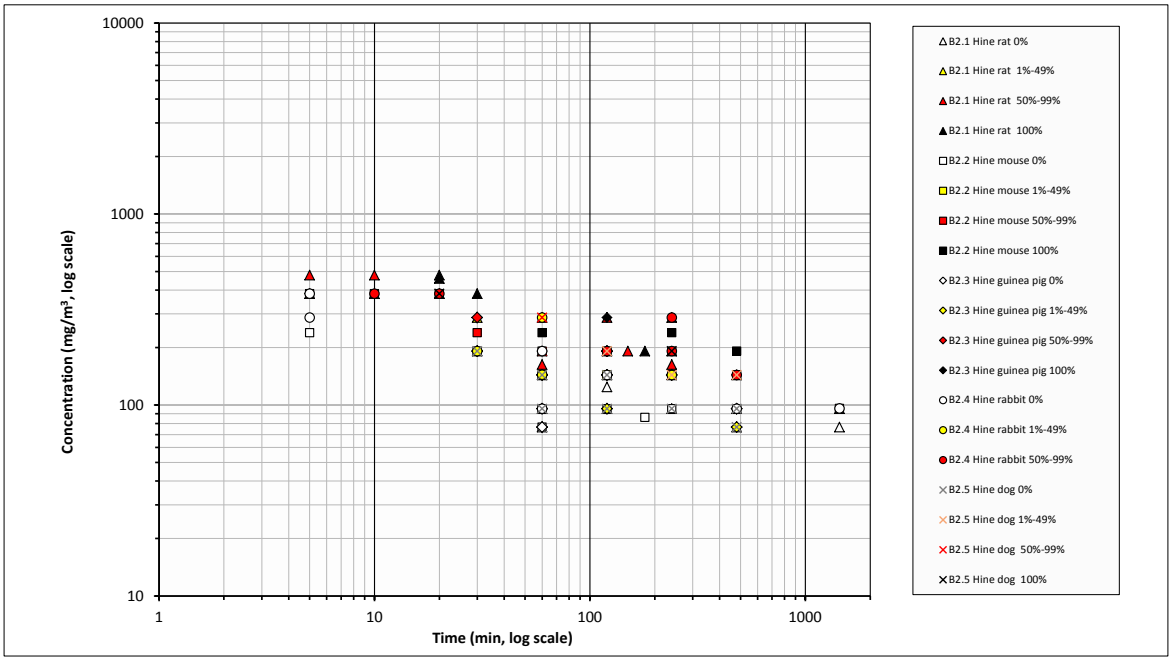



**Figure 4** Final data selected for derivation of the animal probit function of nitrogen dioxide (identical to figure 2).

## 6. Derivation of the human probit function

To derive the human probit function the results from the rat B2.1, mouse B2.2, guinea pig B2.3, rabbit B2.4 and dog B2.5 studies have been used to derive a point of departure as outlined above.

The arithmetic mean n-value was calculated from the rat B2.1, mouse B2.2, guinea pig B2.3, rabbit B2.4 and dog B2.5 studies. The species-specific n-value was 3.81 for the rat, 4.41 for the mouse, 3.85 for the guinea pig, 3.39 for the rabbit, and 4.50 for the dog. The arithmetic mean n-value across species is the arithmetic mean of the species-specific mean n-values, without weight and was calculated to be 3.99.

The species-specific $LC_{50}$-values, calculated for a common exposure duration of 30 minutes, were 254 mg/m$^3$ for the rat (study B2.1), 214 mg/m$^3$ for the mouse (study B2.2), 186 mg/m$^3$ for the guinea pig (study B2.3), 355 mg/m$^3$ for the rabbit (study B2.4) and 278 mg/m$^3$ for the dog (study B2.5). From these, a geometric mean overall $LC_{50}$-value of 251 mg/m$^3$ was calculated from the available $LC_{50}$ values (one per species) according to the general formula for the geometric mean of time-scaled $LC_{50}$-values below:

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

With $\overline{LC_{50}}$ = geometric mean $LC_{50}$-value across species
$LC_{50,j}$ = $LC_{50}$-value of species j.
s = number of species for which $LC_{50}$-values are pooled (j= 1…s).

Hence, the Point of Departure for the human probit function is a 30-minute geometric mean animal $LC_{50}$ value of 251 mg/m$^3$ and an arithmetic mean n-value of 3.99.

Application of an overall assessment factor of 3 (determined by an interspecies factor of 3) would result in a 60-min $LC_{01}$ of 22 mg/m$^3$ (corresponding probit function and resultant LC-values not shown), which is in conflict with human data. Henschler et al. (1960) performed several experiments on healthy, male volunteers. They reported that a 2-h exposure to $NO_2$ at 20 ppm (38 mg/m$^3$) did not cause any irritation when preceded by several exposures to lower concentrations during the preceding days. Exposure to 57 mg/m$^3$ for 2 h resulted in marked irritation, discomfort and respiratory effects (burning sensation in nose and chest, cough, dyspnoea, sputum production). See section 3 of this TSD for details on this human volunteer study. Therefore, the interspecies assessment factor is lowered to 1, resulting in LC-values that are less conflicting with these human data.

Further, species variability has been summarized by AEGL (2012) as follows:
"Several studies indicate that there is a size-dependent species sensitivity to $NO_2$; larger animals are apparently less sensitive than smaller animals. Dogs showed only mild signs of irritation at concentrations that caused pulmonary edema in rats (Carson et al. 1962). Dogs also survived exposures to $NO_2$ at 1,000 ppm *(1913 mg/m$^3$)* for 136 min and at 5,000 ppm *(9565 mg/m$^3$)* for up to 22 min (Greenbaum et al. 1967) and sheep survived exposure at 500 ppm *(957 mg/m$^3$)* for 15-20 min (Januszkiewicz and Mayorga 1994). In contrast, 15-min and 1-h $LC_{50}$ values in the rat were 201-420 *(385-803 mg/m$^3$)* and 115-168 ppm *(220-321 mg/m$^3$)*, respectively (Gray et al. 1954; Carson et al. 1962). On the basis of the available data, humans are not more sensitive than larger laboratory animals. For example, irritation was reported for humans exposed to $NO_2$ at 30 ppm *(57 mg/m$^3$)* for 2 h (Henschler et al. 1960), dogs exposed at 20 ppm *(38 mg/m$^3$)* for 24 h (Hine et al. 1970), and monkeys exposed at 35 ppm *(70 mg/m$^3$)* for 2 h (Henry et al. 1969).
Elsayed et al. (2002) examined species variability to $NO_2$ through dosimetry; the calculated total inspired dose from experimental measurements in rats and sheep was compared with the theoretical dose of an average human. Whether normalized for body weight, lung volume, or alveolar surface area, the total effective dose was greater in rats then sheep then humans. Taking physiologic and anatomical factors into consideration, rats had a much higher effective dose than the larger animals. The authors concluded that $NO_2$ toxicity is associated with inhaled-dose distribution per unit lung volume or lung surface rather than per unit body mass (Elsayed et al. 2002)."

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 | B2-studies with analytically determined concentrations; however, only target concentrations presented |
| Adequacy of database: | 1 | The database consists of one well-performed B2 study with 5 datasets |

The estimated human equivalent 30-minute $LC_{50}$ value is 251 / 1 = **251 mg/m$^3$**.

The experimentally determined n-value was **3.99** (arithmetic mean of the rat B2.1, mouse B2.2, guinea pig B2.3, rabbit B2.4 and dog B2.5 studies). Assuming a

regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **0.50**.

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): $5 = a + 0.50 \times \ln(251^{3.99} \times 30)$ resulting in the a-value of **-7.76**.

**$Pr = -7.76 + 0.50 \times \ln(C^{3.99} \times t)$ 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 five datasets (derived from one study) with B2 quality (rat, mouse, guinea pig, rabbit, dog), including in total 102 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 46 $mg/m^3$ and the calculated human 60 min $LC_1$ (Pr = 2.67) is 67 $mg/m^3$.

***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 | 54 | 46 |
| 1% lethality, this probit | 79 | 67 |
| AEGL-3[2] (2012, final) | 48 | 38 |
| ERPG-3[2] (2003) | - | 57 |
| LBW (2016) | 150 | 120 |

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are approximately identical, except for the LBW which is higher.
It is noted that the available animal data (including non-human primate data) indicate that the derived probit function may overestimate the human lethality.

[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: B2.1

***Author, year:*** ***Hine et al, 1970***

Substance: Nitrogen dioxide
Species, strain, sex: rat, Long-Evans, males
Number/sex/concentration group: variable number/group (range: 4-31)
Age and weight: No information available on age of the animals; 200±50 g
Observation period: varying from 30 to 360 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 | *The temperature of the hood (in which the delivery apparatus for the gas mixture was enclosed) was maintained at 30°C in order to prevent $NO_2$ in the reservoir from condensing. No further information provided.* |
| Use of vehicle (other than air) | *N/A* |
| Whole body / nose-only (incl. head/nose-only) exposure | *Whole body* |
| Type of restrainer | *N/A* |
| Pressure distribution | *To protect the experimenters from accidental leaks, the delivery apparatus was enclosed in a separately ventilated hood with a sliding glass front. The temperature of the hood was maintained at approximately 30°C in order to prevent the $NO_2$ in the reservoir from condensing, giving uneven concentrations or causing mechanical difficulties by plugging of the line.* |
| Homogeneity of test atmosphere in breathing zone of animals | *Test atmosphere was generated by mixing $NO_2$ with air drawn from the room. Adequate mixing of chamber air was obtained by baffle plates and circulating fans.* |
| Number of air changes per hour | *5-20 air changes/hour.*<br>*Exposures were carried out in chambers of 200, 1000 and 10,000 liter capacity; not specified which chamber volume was used for which species.*<br>*It is however stated that the animal volume to chamber volume ratio never exceeded 1:50.* |
| Equilibration time (t95) | *Insufficient information to calculate t95* |
| Start of exposure relative to equilibration | *No information* |

| | |
|---|---|
| Actual concentration measurement | *$NO_2$ was determined spectrophotometrically. Once an equilibrium state was reached the concentrations were monitored with a frequency commensurate with the duration of exposure, generally hourly.* |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | *N/A* |
| | |
| Assessment of Reliability | ***B2*** *Well-performed study, including multiple concentration-time combinations. However, only the target concentrations were presented.* |

Additional remark:
The authors state that "Death generally occurred within 2 to 8 hours after the time of gassing, and the majority of deaths occurred within 24 hours".

**Results**

| Species | Concentration ($mg/m^3$) | | Exposure duration (min) | Lethality |
|---|---|---|---|---|
| | Target | Adjusted | | Male |
| | | | | Dead/tested |
| Rat | 9.6 | N/A | No deaths, time increments up to and including 1440 min. However no information on total number of animals per group. In total 40 rats exposed. | |
| Rat | 19 | N/A | | |
| Rat | 38 | N/A | | |
| Rat | 77 | N/A | 60 | 0/6 |
| Rat | 77 | N/A | 480 | 0/6 |
| Rat | 77 | N/A | 1440 | 0/10 |
| Rat | 96 | N/A | 60 | 0/17 |
| Rat | 96 | N/A | 120 | 0/12 |
| Rat | 96 | N/A | 240 | 0/12 |
| Rat | 96 | N/A | 480 | 0/12 |
| Rat | 96 | N/A | 1440 | 3/10 |
| Rat | 124 | N/A | 120 | 0/9 |
| Rat | 144 | N/A | 60 | 3/31 |
| Rat | 144 | N/A | 120 | 1/12 |
| Rat | 144 | N/A | 240 | 7/12 |
| Rat | 144 | N/A | 480 | 12/12 |
| Rat | 163 | N/A | 60 | 6/12 |
| Rat | 163 | N/A | 240 | 5/10 |
| Rat | 191 | N/A | 30 | 0/5 |
| Rat | 191 | N/A | 60 | 3/5 |
| Rat | 191 | N/A | 120 | 8/8 |
| Rat | 191 | N/A | 150 | 9/11 |
| Rat | 191 | N/A | 180 | 10/10 |
| Rat | 191 | N/A | 240 | 29/29 |
| Rat | 287 | N/A | 30 | 2/10 |
| Rat | 287 | N/A | 60 | 10/13 |
| Rat | 287 | N/A | 120 | 10/12 |

| | | | | |
|---|---|---|---|---|
| Rat | 287 | N/A | 240 | 4/4 |
| Rat | 383 | N/A | 5 | 6/12 |
| Rat | 383 | N/A | 10 | 8/12 |
| Rat | 383 | N/A | 20 | 5/5 |
| Rat | 383 | N/A | 30 | 4/4 |
| Rat | 459 | N/A | 20 | 4/4 |
| Rat | 478 | N/A | 5 | 2/4 |
| Rat | 478 | N/A | 10 | 2/4 |
| Rat | 478 | N/A | 20 | 4/4 |

**Probit function**

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

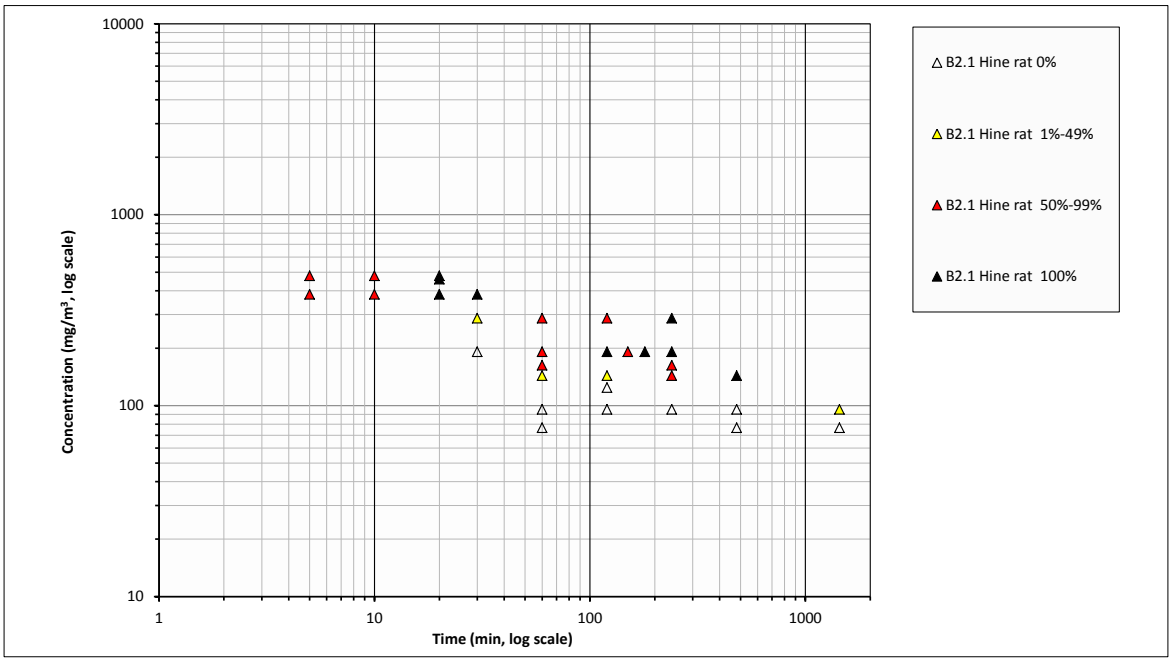
$Pr = a + b \times lnC + c \times lnt$

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

| Probit function | Species | a | b | c | n-value |
|---|---|---|---|---|---|
| | Rat | -20.5 | 3.97 | 1.04 | 3.810 (3.097-4.524) |

| Duration (min.) | $LC_{50}$ ($mg/m^3$) 95%-C.I. Male |
|---|---|
| 10 | 338 (291-390) |
| 30 | 254 (228-280) |
| 60 | 211 (194-229) |

A graphical overview of the data is presented below. Each concentration-time combination (with 4-31 male animals) represents one point in the plot.



# Study ID: B2.2

***Author, year:*** ***Hine et al, 1970***

Substance: Nitrogen dioxide
Species, strain, sex: mouse, Swiss-Webster, males
Number/sex/concentration group: variable number/group (range: 5-14)
Age and weight: age not specified ("young mice"); 20±3 g
Observation period: varying from 30 to 360 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 | *The temperature of the hood (in which the delivery apparatus for the gas mixture was enclosed) was maintained at 30°C in order to prevent $NO_2$ in the reservoir from condensing. No further information provided.* |
| Use of vehicle (other than air) | *N/A* |
| Whole body / nose-only (incl. head/nose-only) exposure | *Whole body* |
| Type of restrainer | *N/A* |
| Pressure distribution | *To protect the experimenters from accidental leaks, the delivery apparatus was enclosed in a separately ventilated hood with a sliding glass front. The temperature of the hood was maintained at approximately 30°C in order to prevent the $NO_2$ in the reservoir from condensing, giving uneven concentrations or causing mechanical difficulties by plugging of the line.* |
| Homogeneity of test atmosphere in breathing zone of animals | *Test atmosphere was generated by mixing $NO_2$ with air drawn from the room. Adequate mixing of chamber air was obtained by baffle plates and circulating fans.* |
| Number of air changes per hour | *5-20 air changes/hour.*<br>*Exposures were carried out in chambers of 200, 1000 and 10,000 liter capacity; not specified which chamber volume was used for which species.*<br>*It is however stated that the animal volume to chamber volume ratio never exceeded 1:50.* |
| Equilibration time (t95) | *Insufficient information to calculate t95* |
| Start of exposure relative to equilibration | *No information* |

| | |
|---|---|
| Actual concentration measurement | *$NO_2$ was determined spectrophotometrically. Once an equilibrium state was reached the concentrations were monitored with a frequency commensurate with the duration of exposure, generally hourly.* |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | *N/A* |
| | |
| Assessment of Reliability | ***B2*** *Well-performed study, including multiple concentration-time combinations. However, only the target concentrations were presented.* |

Additional remark:
The authors state that "Death generally occurred within 2 to 8 hours after the time of gassing, and the majority of deaths occurred within 24 hours".

**Results**

| Species | Concentration ($mg/m^3$) | | Exposure duration (min) | Lethality |
|---|---|---|---|---|
| | Target | Adjusted | | Male |
| | | | | Dead/tested |
| Mouse | 9.6 | N/A | No deaths, time increments up to and including 1440 min. However no information on total number of animals per group. In total 52 mice exposed. | |
| Mouse | 19 | N/A | | |
| Mouse | 38 | N/A | | |
| Mouse | 77 | N/A | 60 | 0/13 |
| Mouse | 86 | N/A | 180 | 0/10 |
| Mouse | 96 | N/A | 60 | 0/5 |
| Mouse | 96 | N/A | 120 | 0/5 |
| Mouse | 96 | N/A | 240 | 0/5 |
| Mouse | 96 | N/A | 480 | 0/5 |
| Mouse | 96 | N/A | 1440 | 5/10 |
| Mouse | 144 | N/A | 60 | 1/6 |
| Mouse | 144 | N/A | 120 | 2/6 |
| Mouse | 144 | N/A | 240 | 5/6 |
| Mouse | 144 | N/A | 480 | 6/6 |
| Mouse | 191 | N/A | 30 | 2/10 |
| Mouse | 191 | N/A | 60 | 8/10 |
| Mouse | 191 | N/A | 120 | 13/14 |
| Mouse | 191 | N/A | 240 | 10/10 |
| Mouse | 191 | N/A | 480 | 10/10 |
| Mouse | 239 | N/A | 5 | 0/6 |
| Mouse | 239 | N/A | 30 | 4/6 |
| Mouse | 239 | N/A | 60 | 6/6 |
| Mouse | 239 | N/A | 240 | 6/6 |
| Mouse | 383 | N/A | 5 | 4/6 |
| Mouse | 383 | N/A | 10 | 6/6 |
| Mouse | 383 | N/A | 20 | 6/6 |

**Probit function**

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

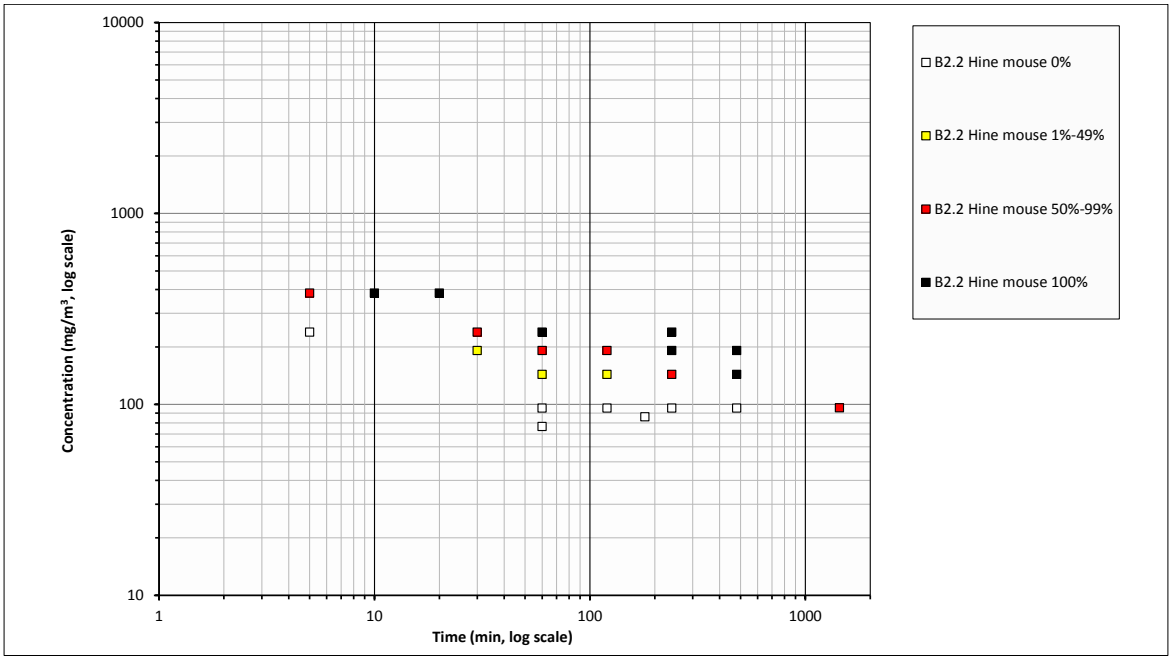
$Pr = a + b \times lnC + c \times lnt$

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

| Probit function | Species | a | b | c | n-value |
|---|---|---|---|---|---|
| | Mouse | -36.5 | 6.76 | 1.53 | 4.414 (3.842-4.986) |

| Duration (min.) | $LC_{50}$ ($mg/m^3$) 95%-C.I. Male |
|---|---|
| 10 | 275 (252-299) |
| 30 | 214 (201-227) |
| 60 | 183 (173-192) |

A graphical overview of the data is presented below. Each concentration-time combination (with 5-14 male animals) represents one point in the plot.



# Study ID: B2.3

***Author, year:*** ***Hine et al, 1970***

Substance: Nitrogen dioxide
Species, strain, sex: guinea pig, strain not specified, male+female
Number/sex/concentration group: variable number/group (range: 2-6)
Age and weight: No information available on age of the animals; 3.0±1.0 kg
Observation period: varying from 30 to 360 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 | *The temperature of the hood (in which the delivery apparatus for the gas mixture was enclosed) was maintained at 30°C in order to prevent $NO_2$ in the reservoir from condensing. No further information provided.* |
| Use of vehicle (other than air) | *N/A* |
| Whole body / nose-only (incl. head/nose-only) exposure | *Whole body* |
| Type of restrainer | *N/A* |
| Pressure distribution | *To protect the experimenters from accidental leaks, the delivery apparatus was enclosed in a separately ventilated hood with a sliding glass front. The temperature of the hood was maintained at approximately 30°C in order to prevent the $NO_2$ in the reservoir from condensing, giving uneven concentrations or causing mechanical difficulties by plugging of the line.* |
| Homogeneity of test atmosphere in breathing zone of animals | *Test atmosphere was generated by mixing $NO_2$ with air drawn from the room. Adequate mixing of chamber air was obtained by baffle plates and circulating fans.* |
| Number of air changes per hour | *5-20 air changes/hour.*<br>*Exposures were carried out in chambers of 200, 1000 and 10,000 liter capacity; not specified which chamber volume was used for which species.*<br>*It is however stated that the animal volume to chamber volume ratio never exceeded 1:50.* |
| Equilibration time (t95) | *Insufficient information to calculate t95* |
| Start of exposure relative to equilibration | *No information* |

| | |
|---|---|
| Actual concentration measurement | *$NO_2$ was determined spectrophotometrically. Once an equilibrium state was reached the concentrations were monitored with a frequency commensurate with the duration of exposure, generally hourly.* |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | *N/A* |
| | |
| Assessment of Reliability | ***B2*** *Well-performed study, including multiple concentration-time combinations. However, only the target concentrations were presented.* |

Additional remark:
The authors state that "Death generally occurred within 2 to 8 hours after the time of gassing, and the majority of deaths occurred within 24 hours".

**Results**

| Species | Concentration (mg/m$^3$) | | Exposure duration (min) | Lethality |
|---|---|---|---|---|
| | Target | Adjusted | | Male+ female |
| | | | | Dead/tested |
| Guinea pig | 9.6 | N/A | No deaths, time increments up to and including 1440 min. However no information on total number of animals per group. In total 18 guinea pigs exposed. | |
| Guinea pig | 19 | N/A | | |
| Guinea pig | 38 | N/A | | |
| Guinea pig | 77 | N/A | 60 | 0/6 |
| Guinea pig | 77 | N/A | 480 | 2/6 |
| Guinea pig | 96 | N/A | 60 | 1/6 |
| Guinea pig | 96 | N/A | 120 | 1/6 |
| Guinea pig | 96 | N/A | 480 | 4/6 |
| Guinea pig | 144 | N/A | 60 | 1/4 |
| Guinea pig | 144 | N/A | 120 | 3/4 |
| Guinea pig | 144 | N/A | 240 | 2/4 |
| Guinea pig | 144 | N/A | 480 | 4/4 |
| Guinea pig | 191 | N/A | 30 | 1/2 |
| Guinea pig | 191 | N/A | 60 | 2/2 |
| Guinea pig | 191 | N/A | 120 | 3/4 |
| Guinea pig | 287 | N/A | 30 | 3/4 |
| Guinea pig | 287 | N/A | 120 | 3/3 |
| Guinea pig | 383 | N/A | 5 | 2/2 |

**Probit function**

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

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

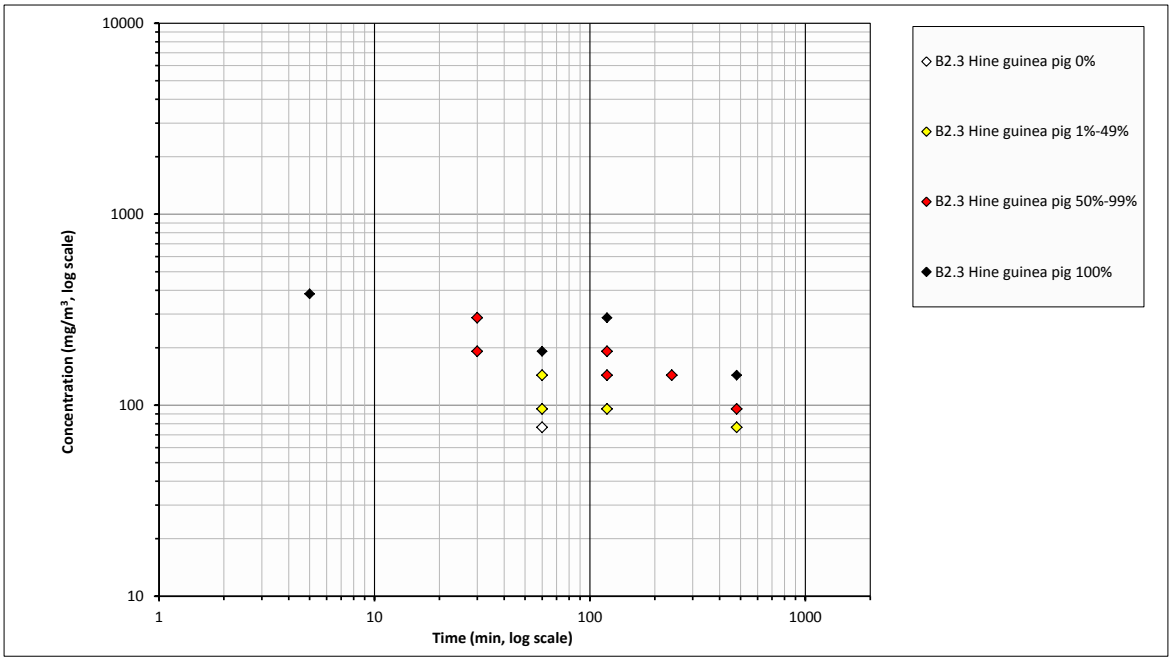
with C for concentration in mg/m$^3$, t for time in minutes.

| Probit function | Species | a | b | c | n-value |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| | Guinea pig | -11.2 | 2.64 | 0.686 | 3.854 (2.051-5.657) |

| Duration (min.) | $LC_{50}$ ($mg/m^3$) 95%-C.I. Male+female |
|---|---|
| 10 | 247 (169-359) |
| 30 | 186 (144-238) |
| 60 | 155 (129-186) |

A graphical overview of the data is presented below. Each concentration-time combination (with 2-6 male and female animals) represents one point in the plot.



# Study ID: B2.4

***Author, year:*** ***Hine et al, 1970***

Substance: Nitrogen dioxide
Species, strain, sex: rabbit, strain not specified, male+female
Number/sex/concentration group: variable number/group (range: 2-8)
Age and weight: No information available on age of the animals; 2.5±0.5 kg
Observation period: varying from 30 to 360 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 | *The temperature of the hood (in which the delivery apparatus for the gas mixture was enclosed) was maintained at 30°C in order to prevent $NO_2$ in the reservoir from condensing. No further information provided.* |
| Use of vehicle (other than air) | *N/A* |
| Whole body / nose-only (incl. head/nose-only) exposure | *Whole body* |
| Type of restrainer | *N/A* |
| Pressure distribution | *To protect the experimenters from accidental leaks, the delivery apparatus was enclosed in a separately ventilated hood with a sliding glass front. The temperature of the hood was maintained at approximately 30°C in order to prevent the $NO_2$ in the reservoir from condensing, giving uneven concentrations or causing mechanical difficulties by plugging of the line.* |
| Homogeneity of test atmosphere in breathing zone of animals | *Test atmosphere was generated by mixing $NO_2$ with air drawn from the room. Adequate mixing of chamber air was obtained by baffle plates and circulating fans.* |
| Number of air changes per hour | *5-20 air changes/hour.*<br>*Exposures were carried out in chambers of 200, 1000 and 10,000 liter capacity; not specified which chamber volume was used for which species.*<br>*It is however stated that the animal volume to chamber volume ratio never exceeded 1:50.* |
| Equilibration time (t95) | *Insufficient information to calculate t95* |
| Start of exposure relative to equilibration | *No information* |

| | |
|---|---|
| Actual concentration measurement | *$NO_2$ was determined spectrophotometrically. Once an equilibrium state was reached the concentrations were monitored with a frequency commensurate with the duration of exposure, generally hourly.* |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | *N/A* |
| | |
| Assessment of Reliability | ***B2*** *Well-performed study, including multiple concentration-time combinations. However, only the target concentrations were presented.* |

Additional remark:
The authors state that "Death generally occurred within 2 to 8 hours after the time of gassing, and the majority of deaths occurred within 24 hours".

**Results**

| Species | Concentration (mg/m$^3$) | | Exposure duration (min) | Lethality |
|---|---|---|---|---|
| | Target | Adjusted | | Male+ female |
| | | | | Dead/tested |
| Rabbit | 9.6 | N/A | No deaths, time increments up to and including 1440 min. However no information on total number of animals per group. In total 12 rabbits exposed. | |
| Rabbit | 19 | N/A | | |
| Rabbit | 38 | N/A | | |
| Rabbit | 96 | N/A | 60 | 0/4 |
| Rabbit | 96 | N/A | 480 | 0/4 |
| Rabbit | 96 | N/A | 1440 | 0/4 |
| Rabbit | 144 | N/A | 60 | 1/8 |
| Rabbit | 144 | N/A | 120 | 0/6 |
| Rabbit | 144 | N/A | 240 | 2/8 |
| Rabbit | 144 | N/A | 480 | 6/8 |
| Rabbit | 191 | N/A | 30 | 1/3 |
| Rabbit | 191 | N/A | 60 | 0/4 |
| Rabbit | 191 | N/A | 120 | 2/4 |
| Rabbit | 191 | N/A | 240 | 3/4 |
| Rabbit | 287 | N/A | 5 | 0/2 |
| Rabbit | 287 | N/A | 60 | 1/6 |
| Rabbit | 287 | N/A | 240 | 3/4 |
| Rabbit | 383 | N/A | 5 | 0/2 |
| Rabbit | 383 | N/A | 10 | 1/2 |
| Rabbit | 383 | N/A | 20 | 2/4 |

**Probit function**

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

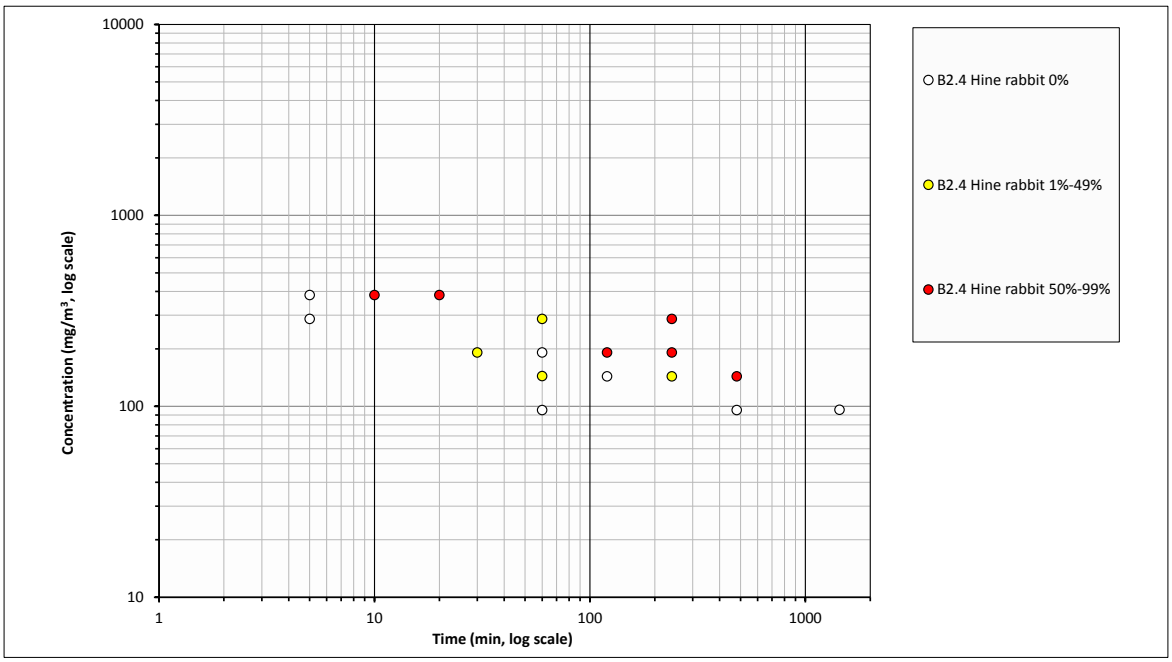
$Pr = a + b \times \ln C + c \times \ln t$

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

| Probit function | Species | a | b | c | n-value |
|---|---|---|---|---|---|
| | Rabbit | -10.3 | 2.23 | 0.658 | 3.390 (2.102-4.678) |

| Duration (min.) | $LC_{50}$ ($mg/m^3$) 95%-C.I. Male+female |
|---|---|
| 10 | 491 (358-940) |
| 30 | 355 (285-587) |
| 60 | 289 (243-442) |

A graphical overview of the data is presented below. Each concentration-time combination (with 2-8 male and female animals) represents one point in the plot.



# Study ID: B2.5

***Author, year:*** ***Hine et al, 1970***

Substance: Nitrogen dioxide
Species, strain, sex: dog, strain not specified, male+female
Number/sex/concentration group: variable number/group (range: 1-4)
Age and weight: No information available on age of the animals; 10±4.0 kg
Observation period: varying from 30 to 360 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 | *The temperature of the hood (in which the delivery apparatus for the gas mixture was enclosed) was maintained at 30°C in order to prevent $NO_2$ in the reservoir from condensing. No further information provided.* |
| Use of vehicle (other than air) | *N/A* |
| Whole body / nose-only (incl. head/nose-only) exposure | *Whole body* |
| Type of restrainer | *N/A* |
| Pressure distribution | *To protect the experimenters from accidental leaks, the delivery apparatus was enclosed in a separately ventilated hood with a sliding glass front. The temperature of the hood was maintained at approximately 30°C in order to prevent the $NO_2$ in the reservoir from condensing, giving uneven concentrations or causing mechanical difficulties by plugging of the line.* |
| Homogeneity of test atmosphere in breathing zone of animals | *Test atmosphere was generated by mixing $NO_2$ with air drawn from the room. Adequate mixing of chamber air was obtained by baffle plates and circulating fans.* |
| Number of air changes per hour | *5-20 air changes/hour.*<br>*Exposures were carried out in chambers of 200, 1000 and 10,000 liter capacity; not specified which chamber volume was used for which species.*<br>*It is however stated that the animal volume to chamber volume ratio never exceeded 1:50.* |
| Equilibration time (t95) | *Insufficient information to calculate t95* |
| Start of exposure relative to equilibration | *No information* |

| | |
|---|---|
| Actual concentration measurement | *$NO_2$ was determined spectrophotometrically. Once an equilibrium state was reached the concentrations were monitored with a frequency commensurate with the duration of exposure, generally hourly.* |
| Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure | *N/A* |
| | |
| Assessment of Reliability | ***B2*** *Well-performed study, including multiple concentration-time combinations. However, only the target concentrations were presented.* |

Additional remark:
The authors state that "Death generally occurred within 2 to 8 hours after the time of gassing, and the majority of deaths occurred within 24 hours".

**Results**

| Species | Concentration ($mg/m^3$) | | Exposure duration (min) | Lethality |
|---|---|---|---|---|
| | Target | Adjusted | | Male+ female |
| | | | | Dead/tested |
| Dog | 9.6 | N/A | No deaths, time increments up to and including 1440 min. However no information on total number of animals per group. In total 4 dogs exposed. | |
| Dog | 19 | N/A | | |
| Dog | 38 | N/A | | |
| Dog | 77 | N/A | 480 | 0/3 |
| Dog | 96 | N/A | 60 | 0/1 |
| Dog | 96 | N/A | 120 | 0/2 |
| Dog | 96 | N/A | 240 | 0/2 |
| Dog | 96 | N/A | 480 | 0/2 |
| Dog | 144 | N/A | 60 | 0/2 |
| Dog | 144 | N/A | 120 | 0/2 |
| Dog | 144 | N/A | 240 | 1/3 |
| Dog | 144 | N/A | 480 | 1/4 |
| Dog | 191 | N/A | 30 | 0/2 |
| Dog | 191 | N/A | 120 | 1/3 |
| Dog | 191 | N/A | 240 | 2/2 |
| Dog | 287 | N/A | 60 | 2/3 |
| Dog | 383 | N/A | 20 | 2/2 |

**Probit function**

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

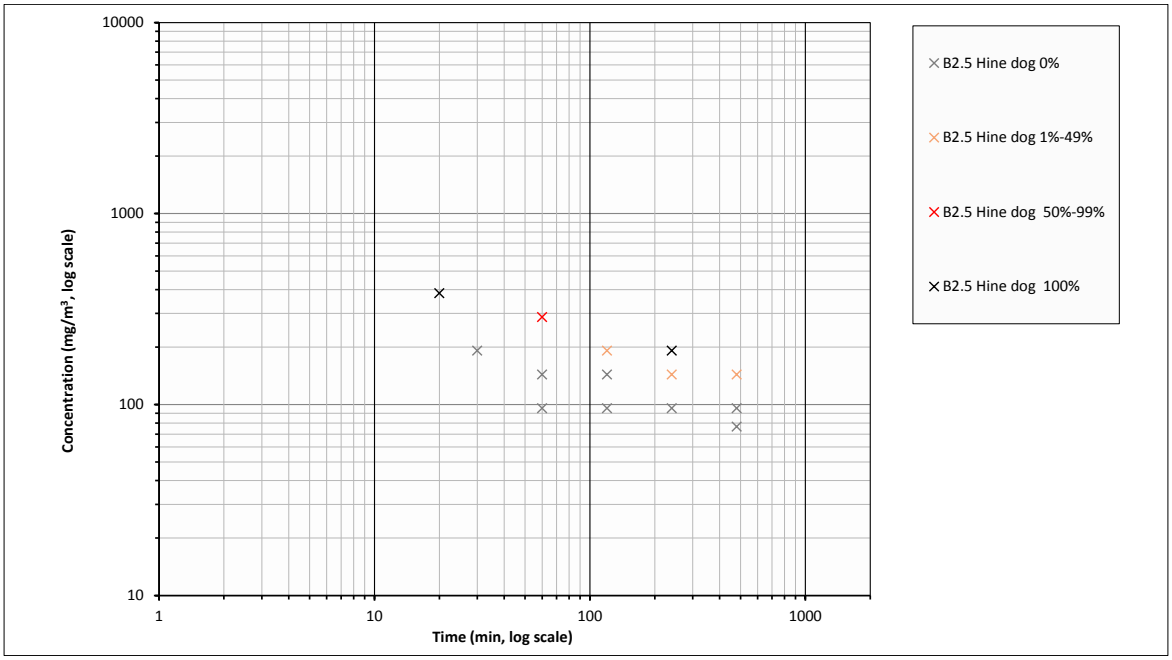
$Pr = a + b \times \ln C + c \times \ln t$

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

| Probit function | Species | a | b | c | n-value |
|---|---|---|---|---|---|
| | Dog | -28.9 | 5.31 | 1.18 | 4.495 (1.826-7.164) |

| Duration (min.) | $LC_{50}$ ($mg/m^3$) 95%-C.I. Male+female |
|---|---|
| 10 | 354 (161-535) |
| 30 | 278 (172-369) |
| 60 | 238 (176-296) |

A graphical overview of the data is presented below. Each concentration-time combination (with 1-4 male and female animals) represents one point in the plot.



# Study ID: C studies

Steadman et al., 1966 exposed rats, guinea pigs, rabbits, monkeys and dogs for 8 hours to 123 mg/$m^3$ nitrogen dioxide (whole-body, simultaneously). Signs of eye and nose irritation were noted during the first hour of exposure, accompanied by anorexia and lethargy. The dogs appeared to be the least affected. Lethality ratios after 20 days were 6/15 for rats, 13/15 for guinea pigs, 1/3 for rabbit, 2/2 for dog, 3/3 for monkey. All deaths occurred within the first 72 hours, where in total 3 animals died in the 25-72hour time slot.

Carson *et al*. (1962) exposed rats, rabbits and dogs (strains not specified) whole-body to nitrogen dioxide for 5, 15, 30 and 60 minutes (concentrations not specified). The authors reported $LC_{50}$ values of 416 ppm (796 mg/$m^3$; rats, 5-min), 201 ppm (385 mg/$m^3$; rats, 15-min), 315 ppm (603 mg/$m^3$; rabbits, 15-min), 162 ppm (310 mg/$m^3$; rats, 30-min), 115 ppm (220 mg/$m^3$; rats, 60 min). As no full information on the lethality (at each applied exposure concentration) was available, the quality of these calculated $LC_{50}$–values could not be evaluated.

Gray *et al*. (1954) exposed albino rats to nitrogen dioxide whole body. The authors reported $LC_{50}$ values of 1445 ppm (2764 mg/$m^3$, 2 min), 833 ppm (1594 mg/$m^3$, 5 min), 420 ppm (803 mg/$m^3$, 15 min), 174 ppm (333 mg/$m^3$, 30 min), 168 ppm (321 mg/$m^3$, 60 min), 88 ppm (168 mg/$m^3$, 240 min). In addition, the effect of ambient temperature on $NO_2$ toxicity was studied at 100 to 105F (38-41°C) for 30 minute exposure duration. An $LC_{50}$ of 139 ppm (266 mg/$m^3$) was reported for this temperature (i.e. 20°C above room temperature). As no full information on the lethality (at each applied exposure concentration) was available, the quality of these calculated $LC_{50}$–values could not be evaluated.

Greenbaum *et al*. (1967) exposed anaesthetized mongrel dogs (n = 1/concentration) to $NO_2$ in two experiment. In experiment 1, dogs were exposed to 0.1% (1,000 ppm; 1913 mg/$m^3$) for 136 min, 0.5% (5,000 ppm; 9565 mg/$m^3$) for 35 min, or 2% (20,000 ppm; 38260 mg/$m^3$) for 15 min. During the latter two exposures, the dogs were ventilated artificially. Those dogs that survived the exposure were sacrificed before recovering from anaesthesia. In experiment 2, dogs were exposed to 0.5% (5,000 ppm; 9565 mg/$m^3$) for 5-45 min. These animals were sacrificed within 1, 24 or 48 hours after exposure. The applied post-exposure observation period is considered not sufficient to cover for possible delayed deaths (see also Steadman et al., 1966). Therefore the true lethality incidence could not be ascertained.

Meulenbelt *et al*. (1992a) exposed female Wistar rats (n=6/group) nose-only to nitrogen dioxide. The first experiment included exposure to 0, 25, 75 or 200 ppm nitrogen dioxide (corresponding to 48, 143 or 383 mg/$m^3$) for 10 minutes. The animals were observed during a post-exposure period of 24 h. The second experiment included exposure to 175 ppm (335 mg/$m^3$) for 10, 20 or 30 minutes, while exposure in the third experiment was 400 ppm (765 mg/$m^3$) for 5, 10 or 20 minutes. A fourth experiment was performed with rats exposed for 10 minutes to 175 ppm (335 mg/$m^3$). Animals were observed for clinical effects during the next 24 hours.
The second experiment resulted in lethality in rats of the 20- and 30-min exposure group (175 ppm, 35 mg/$m^3$), i.e. 5/6 died in each group within 3-4 and 2-3 h of exposure, respectively. The third experiment resulted in lethality in all rats of the 10- and 20 minute groups shortly after exposure.
The applied post-exposure observation period is considered not sufficient to cover for possible delayed deaths (see also Steadman et al., 1966). Therefore the true lethality incidence could not be ascertained.

Meulenbelt *et al*. (1992b) exposed female Wistar rats (n=6/group) nose-only to nitrogen dioxide at concentrations of 0, 75, 125 or 175 ppm (0, 143, 239 or 335 mg/m$^3$) during 10 minutes. Animals were divided in four groups with postexposure observation periods of 6h, 24h, 3d, or 7d. In the exposure group of 335 mg/m$^3$, one rat died within 18 h of exposure; other animals survived.
The applied post-exposure observation period is not considered sufficient to cover for possible delayed deaths for all exposure groups (see also Steadman et al., 1966). Therefore the true lethality incidence could not be ascertained.

Meulenbelt *et al*. (1994) exposed female and male rabbits (sedated with midazolam) nose-only to nitrogen dioxide. A low- and high concentration experiment and a confirmation experiment was performed. For the low exposure, the animals were exposed for 10 min to 0, 125, 175 or 250 ppm nitrogen dioxide (corresponding to 0, 239, 335, 478 mg/m$^3$). The high exposure group was exposed to 0, 250, 400, 600, 800 ppm (corresponding to 0, 478, 765, 1148, 1530 mg/m$^3$) nitrogen dioxide. A post-exposure observation period of 1 day was included for these two experiments. No lethality was observed in the low-exposure group. In the high exposure group, 2/3 animals died after exposure to 800 ppm (1530 mg/m$^3$).
A confirmation experiment was included in which rabbits were exposed to 0 and 600 ppm $NO_2$ (0 and 1148 mg/m$^3$) for 10 min and were autopsied at 1, 3 or 5 days postexposure. Lethality was not observed in this experiment.
The applied post-exposure observation period is considered not sufficient to cover for possible delayed deaths (see also Steadman et al., 1966). Therefore the true lethality incidence could not be ascertained.

Stavert and Lehnert (1990) exposed male Fischer 344 rats to nitrogen dioxide. Groups of rats were exposed to 10, 25, 50 or 100 ppm $NO_2$ (corresponding to 19, 48, 96, 191 mg/m$^3$) for durations of 5, 15, or 30 min. No rats died. A postexposure observation period of 24 hours was included. No significant changes in lung weight occurred in rats exposed at 10 ppm for 30 min or at 25-50 ppm for up to 15 min. Significant increases in lung wet weight and right cranial-lobe dry weight were found after exposure at 50 ppm for 30 min or at 100 ppm for 5 and 15 min. However, histologic evidence of lung injury was seen in animals exposed at 25 ppm for 30 min, 50 ppm for ≥5 min, and 100 ppm for 5 and 15 min. Findings included accumulation of fibrin, increased numbers of polymorphonuclear neutrophils and macrophages, extravasated erythrocytes, and type II pneumocyte hyperplasia, the severity of which increased with concentration and duration of exposure.

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