20220608-carbon dioxide
-INHOUDELIJK VASTGESTELD

National Institute for Public Health
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Probit function technical support document

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substance name	CAS number
Carbon dioxide	**124-38-9**

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 "inhoudelijk vastgesteld" (approved content).

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 carbon dioxide

1. Substance identification

CAS-number:	124-38-9
IUPAC name:	dioxomethane
Synonyms:	dioxomethane, methanedione
Molecular formula:	CO_2
Molecular weight:	44 g/mol
Physical state:	gas (at 20°C and 101.3 kPa)
Boiling point:	-78.5°C (at 101.3 kPa)
Vapour pressure:	N/A
Saturated vapor conc:	N/A
Conversion factor:	1 mg/m^3 = 0.546 ppm (at 20°C and 101.3 kPa)
	1 ppm = 1.83 mg/m^3 (at 20°C and 101.3 kPa)
	1 Vol% = 10.000 ppm[1]
Labelling:	no labelling for human hazards

2. Mechanism of action and toxicological effects following acute exposure

Acute effects: Carbon dioxide at high concentrations can lower the pH of the blood and trigger effects on the respiratory, cardiovascular and central nervous systems as well as an asphyxiating effect by replacing oxygen in air. Respiration is a tightly controlled phenomenon in mammals (including humans). It is primarily regulated by the CO_2 tension in arterial blood ($PaCO_2$) and the concentration of hydrogen ions (pH). Arterial PO_2 is not the major driving force for ventilation under normal circumstances. So, any condition that increases $PaCO_2$ will result in a stimulation of ventilation in order to eliminate the surplus of CO_2. The main target for inhalation exposure to carbon dioxide is the respiratory regulation system and consequently the oxygen supply (asphyxiation). Increased levels of CO_2 in air will result in hypercapnia and hypoxemia, disruption in buffer capacity and decreasing pH levels, causing respiratory acidosis, which in case exposure continues will result in hypoxia and metabolic acidosis.

The health endpoints are increased inhalation rates (hyperventilation) and cardiovascular effects including increased heart rate and blood pressure. A too high level of CO_2 in blood and tissues is harmful for mammalian tissues, especially those with a high sensitivity (e.g. brain) causing CNS effects.

Symptoms of high exposure are hyperventilation, headache, shortness of breath, unconsciousness and respiratory arrest. According to Pauluhn (2016) incapacitation smoothly progresses to fatality and thus incapacitation can be considered as state of impendent death if exposure continues. Lethality results from respiratory arrest.

Long-term effects: Long-term effects from acute exposure may result from the lack of oxygen supply to highly sensitive tissues, such as the brain. This is however based on a theoretical view of carbon dioxide toxicity. In case the high exposure is survived, other acute effects will disappear. It is however unclear if the effects of hypercapnia leading to long-term effects on the lungs itself and/or the muscle tissues and diaphragma surrounding the lungs are caused by the deleterious effects of CO_2 or caused by fatigue from the increased ventilation.

Chronic exposure produces similar effects as acute exposure.

[1] Throughout the TSD the dose metric volume percentage (Vol%) will be used along with mg/m^3, as Vol% is the most commonly used metric for carbon dioxide levels in air.

3. Human toxicity data

Human data on incapability and lethal responses following acute CO_2 exposure

Documented human data on acute toxicity after CO_2 exposure is limited to one accidental death, a notion of mortality in the Spacecraft Maximum Allowable Concentration (SMAC) document (COT, 1996), and subjects reaching a state of unconsciousness (Table 1).

Table 1: summary of available human acute toxicity data – case reports

Concentration CO_2 (Vol%)	Concentration CO_2 (mg/m³)	Exposure duration (min)	Mortality	Remarks	Reference
			Human data		
17	311000	20-52 seconds	-	Unconsciousness	COT, 1996
18.6	340380	< 2	-	Unconsciousness	COT, 1996
20-22	366000-402600	Not stated	Mortality reported	Survivors unconscious	COT, 1996
48	878400	120	1 accidental death[a]	6 Vol% O_2	Anonymous, 1987

a: Considering that 48 Vol% CO_2 would lead to a concurrent O_2 concentration of approximately 11 Vol% and since the O_2 concentration was only 6 Vol% it is likely that co-exposure to other gases will have occurred.

The following data sources provide information on levels where CO_2 may cause unconsciousness or death, which were used by other organisations to set lethal levels, safety levels or probit functions.

The Immediately Dangerous to Life or Health (IDLH value) by CDC/NIOSH (1994) of 40000 ppm (4 Vol%; 73,200 mg/m³) is based on statements by ACGIH (1971) that a 30-minute exposure to 50000 ppm (5 Vol%; 91500 mg/m³) produces signs of intoxication (Aero 1953), and a few minutes of exposure at 70000 ppm (128100 mg/m³) and 100000 ppm (183000 mg/m³) produces unconsciousness (Flury and Zernik 1931; Hunter 1975). In addition according to CDC/NIOSH, the IDLH is supported by reports on submarine personnel who were exposed continuously at 30000 ppm (54900 mg/m³) and being only slightly affected, provided the oxygen content of the air was maintained at normal concentrations (Schaefer 1951). It is noted by the author of this TSD, that under conditions of accidental release of carbon dioxide, oxygen levels will drop and therefore such results by Schaefer (1951) are not relevant for acute toxic effects related to emergencies.

The DNV TECHNICA/SCANDPOWER A/S (2001) reports concentration-time combinations for carbon dioxide with corresponding effects on humans (see Table 2). DNV TECHNICA/SCANDPOWER A/S cites two references in which the values were previously reported (German handbook for dangerous substances (1980) and Bryan (1986) on effects on personnel from fire toxicants. The data in Table 2 seem to originate from Pryor (1968; as cited in Bryan, 1986) on fire hazards. Based on those reviews, DNV TECHNICA/SCANDPOWER A/S recommends 100% fatal limits at 15 Vol% (274500 mg/m³) for durations up to 5 minutes, 12 Vol% (219600 mg/m³) for 5 to 30 minutes and 10 Vol% (183000 mg/m³) for >30 minutes.

Table 2 Human acute toxicity data (DNV TECHNICA/SCANDPOWER A/S, 2001).

Concentration CO_2 (ppm)	Concentration CO_2 (Vol%)	Concentration CO_2 (mg/m^3)	Remarks
45000	4.5	823500	Reduced concentration capability after more than 8 hours exposure, adaptation possible
55000	5.5	100650	Breathing difficulty, headache and increased heart rate after 1 hour
65000	6.5	118950	Dizziness, and confusion after 15 minutes exposure
70000	7.0	128100	Anxiety caused by breathing difficulty effects becoming severe after 6 minutes exposure
100 000	10	183000	Approaches threshold of unconsciousness in 30 minutes
120 000	12	219600	Threshold of unconsciousness reached in 5 minutes
150 000	15	274500	Exposure limit 1 minutes
200 000	20	366000	Unconsciousness occurs in less than 1 minute

Linde Gas Benelux BV (2007) (formerly known as Hoek Loos) provides some qualitative information in a document on 'How to work safely with gases: lack of oxygen' (in Dutch). It is stated that at CO_2 concentrations of 7-10 Vol% (127100-183000 mg/m^3) a surplus of CO_2 in the blood may result in death after 4 hours of exposure. At air concentrations above 20 Vol% death will occur rapidly. Linde Gas Benelux BV indicates that the information is based on medical data and aimed to protect their employees and customers, thus intrinsically conservatively set.

In a note by RIVM (Ter Burg and Bos, 2007 and revised in 2009 based on additional experimental data) it was concluded that semi-quantitative estimates can be used as a conservative guideline for human exposures up to 60 minutes of exposure, rather than deriving a human probit function:

- no deaths are expected at CO_2 concentrations of up to 5-10 Vol% (91500-183000 mg/m^3);
- serious effects and possible mortality may start to occur at about 10-15 Vol% CO_2 (183000-274500 mg/m^3);
- a high level of mortality may occur at about 20-25 Vol% CO_2.

The UK HSE (2008; 2009) used the data that were collected by IDLH (and references therein) and DNV TECHNICA/SCANDPOWER A/S to derive a human probit function for CO_2. The probit function is given as:
$Pr = -90.8 + 1.01\ln(C^8 \times t)$, where C is in ppm and t in minutes.
Using this probit function the UK HSE derived the following estimates for lethality (Table 3).

Table 3: lethality estimates derived by the UK HSE (2008; 2009).

Concentration / approximate exposure time for % lethality					
1-5% lethality			**50% lethality**		
ppm	mg/m^3	min	ppm	mg/m^3	min
63000	115290	60	84000	153720	60
69000	126270	30	92000	168360	30
72000	131760	20	96000	175680	20

79000	144570	10	105000	192150	10
86000	157380	5	115000	210450	5
105000	192150	1	140000	256200	1

Tebodin (2008) used two data points to derive a human probit function. At 30 minutes no lethality was assumed to occur at 5 Vol% (91500 mg/m^3), whereas at 10 Vol% (183000 mg/m^3) 100% lethality was assumed, providing:
Pr = -98.8 + ln(C^9 x t), where C is in Vol% and t in minutes.
The probit function was based on levels causing unconsciousness and estimates for lethality as previously stated by UK HSE, Ter Burg and Bos, and DNV TECHNICA/SCANDPOWER A/S.

Human volunteer study

In a recent human volunteer study (Leiden University Medical Centre/Shell (manuscript in preparation)), 6 participants per exposure group were exposed to levels of CO_2 ranging from 6 Vol% to 9 Vol% (109800-164700 mg/m^3) for durations up to 60 minutes or until subjects were pulled out for safety reasons. The volunteers were healthy males aged 18-35 years with a BMI between 18-25 kg/m^2 and bodyweight between 50 and 100 kg. The order of experiments was escalating, if none of the stopping criteria (pH <7.20, heart rate > 180 bpm, systolic blood pressure > 200 mmHg, diastolic blood pressure > 120 mmHg or subjectively experienced side effects warranting discontinuation) were observed in the six participants, a subsequent group of 6 participants was exposed to a higher CO_2 concentration. At 6 Vol% and 7.5 Vol% (109800 and 137250 mg/m^3) during the first 10 minutes 18 participants were included, after which 6 participants were pulled out at random, leaving 12 to fulfil the 30 minute exposure after which an additional 6 participants were pulled out, so that 6 participants completed 60 minutes exposure. In addition, 10 subjects were exposed to 10 Vol% (183000 mg/m^3) and 2 subjects were exposed to 12 Vol% (219600 mg/m^3) for 10 minutes. Carbon dioxide was administered through a mask that was fitted around the head of the participant. A controlled mixture of CO_2, O_2 [2] and N_2 was administered via the mask with an air flow of 45 L/min. Care was taken to ensure that there was no pressure built-up within the mask due to increased respiratory rate by the volunteer. While being exposed, certain physiological parameters such as the arterial pressure of O_2 and CO_2 were monitored alongside the blood pH level, heart rate, and blood pressure, all to ensure the safety of the participant and to generate the data concerning the response to the exposure.

Table 4. Tolerability of the exposure (copied from LUMC/Shell; manuscript in preparation).

	CO_2 exposure level										
	6 %			***7.5 %***			***9 %***			***10 %***	***12 %***
Intended duration, minutes	***10***	***30***	***60***	***10***	***30***	***60***	***10***	***30***	***60***	***10***	***10***
Number participants	***6***	***6***	***6***	***6***	***6***	***6***	***6***	***6***	***6***	***10***	***2***
Completed regardless intended duration, No. (%)	18 (100)	12 (100)	6 (100)	18 (100)	12 (100)	6 (100)	12 (66.7)	4 (33.3)	1 (16.7)	3 (30)	0 (0)
Actual exposure duration, mean (SD)	10 (0)	30 (0)	60 (0)	10 (0)	30 (0)	60 (0)	10 (0)	15.7 (12.2)	22.2 (21.5)	7.1 (2.6)	6.5 (2.1)

[2] The oxygen levels administered corresponded to levels that would be acquired by oxygen displacement by the increased levels of carbon dioxide.

All subjects could tolerate levels up to 7.5 Vol% (137250 mg/m^3) during the assigned exposure period up to 60 minutes. At 9 Vol%, 10 Vol% and 12 Vol% (164700, 183000 and 219600 mg/m^3) the exposure was unbearable for some (9 Vol% 10 min), most (9 Vol% (164700 mg/m^3) for 30 and 60 min, and 10 Vol% (183000 mg/m^3) for 10 min) or all (12 Vol%; (219600 mg/m^3)) (Table 4). Symptoms reported were mild headache already at 7.5 Vol% (137250 mg/m^3) at 30 minutes, while panic and hyperventilation were reasons for discontinuing exposure at 9 Vol% (164700 mg/m^3) after 10 minutes or longer. During 10 minutes 10 Vol% (183000 mg/m^3) CO_2 exposure, 3 out of 10 participants were able to complete the full 10 minutes of exposure. At 10 Vol% (183000 mg/m^3), one participant was discontinued due to a stopping rule (pH < 7.2). Four participants discontinued due to the occurrence of dissociative phenomena (i.e. altered sensation extremities, disinhibition of emotions, blurry vision). The self-reported panic was the reason for discontinuation by two participants and the inability to maintain increased respiratory efforts was reported by three participants as reason for discontinuation. Two participants were exposed to 12 Vol% (219600 mg/m^3) CO_2, exposure lasted 6.5 ± 2.1 minutes. Panic and dissociation were reasons for discontinuation.

Physiological results showed similar profiles for changes in arterial concentration of HCO_3^- and PaO_2 across the exposure range up and including 10 Vol% (183000 mg/m^3). The exposure results in a relatively rapid increase (or decrease) of the arterial pressure which stabilizes before the cessation of exposure. However for the 12 Vol% (219600 mg/m^3) the stabilization is not (yet) reached, but it seems to stabilize at the same magnitude of change as seen for the lower concentrations though seemingly requiring more time to reach that state. A similar profile is seen for the increase in in arterial blood CO_2 and the decrease in pH levels, however, the increase or decrease is much more prominent from a visual inspection of the data and is not stabilized yet at 9 Vol% (164700 mg/m^3), with data for 10 Vol% (183000 mg/m^3) and 12 Vol% (219600 mg/m^3) showing steeper increase or decrease of the parameter where the deviation from normal is greater as well. The pH levels at the end of the exposure duration for 6, 7.5, 9, 10 and 12 Vol% (after 60, 60, 30, 10, and 4 mins) were approximately 7.34, 7.26, 7.20, 7.18 and 7.21 respectively.

Based on these observations and those in the accompanying rat study, a predictive model was derived to assess the drop in pH levels at higher concentrations (Figure 1).

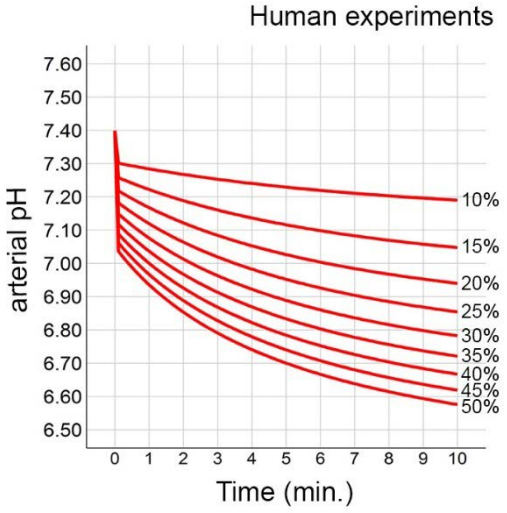


Figure 1. Modelled arterial pH levels in humans by CO_2 exposure concentration in Vol% and exposure duration in minutes (copied from LUMC/Shell; manuscript in preparation)

The authors of the study cautioned that the predictive model for pH changes in humans is not, by itself, a reliable predictor for lethality in humans. The rat modelled data for which the rat experiments did provide lethal data for concentrations up to 50 Vol%, indicated that the pH levels in rats that succumbed the exposure to carbon dioxide was around a pH level of 6.7 or lower (experimental data are available from the LUMC/Shell study; see Appendix 1, C-studies for more details on the experiment in rats on the lethal data; see section 6 for comparisons between rats and humans based on the LUMC/Shell study).

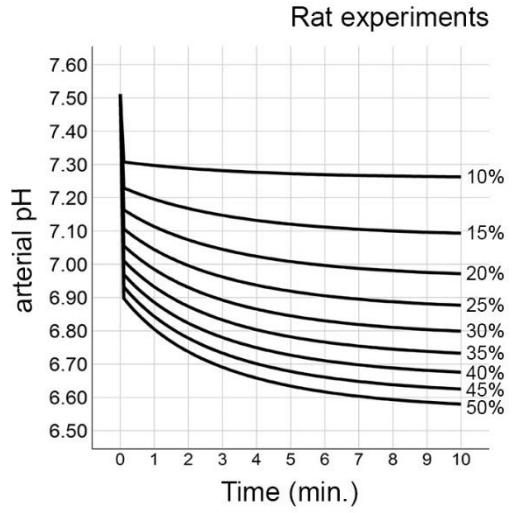


Figure 2. Modelled data on arterial pH in rats by CO_2 exposure concentration in Vol% and exposure duration in minutes (copied from LUMC/Shell; manuscript in preparation).

4. Animal acute toxicity data

During the literature search the following technical support documents and databases were consulted:

1. No AEGL TSD, ERPG document nor EU RAR has been drafted for carbon dioxide. Carbon dioxide is not registered under REACH. Documents by the UK Health and Safety Executive (HSE) and by the Spacecraft Maximum Allowable Concentration (SMAC) document by the US National Research Council were consulted.
2. An additional search 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 five studies were identified -with five datasets for two species- with data on lethality following acute inhalation exposure. One dataset was assigned status A for deriving the human probit function, four 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 carbon dioxide, without any type of co-exposure or oxygen suppletion (see appendix 1 on C-studies), are displayed in Figure 3.

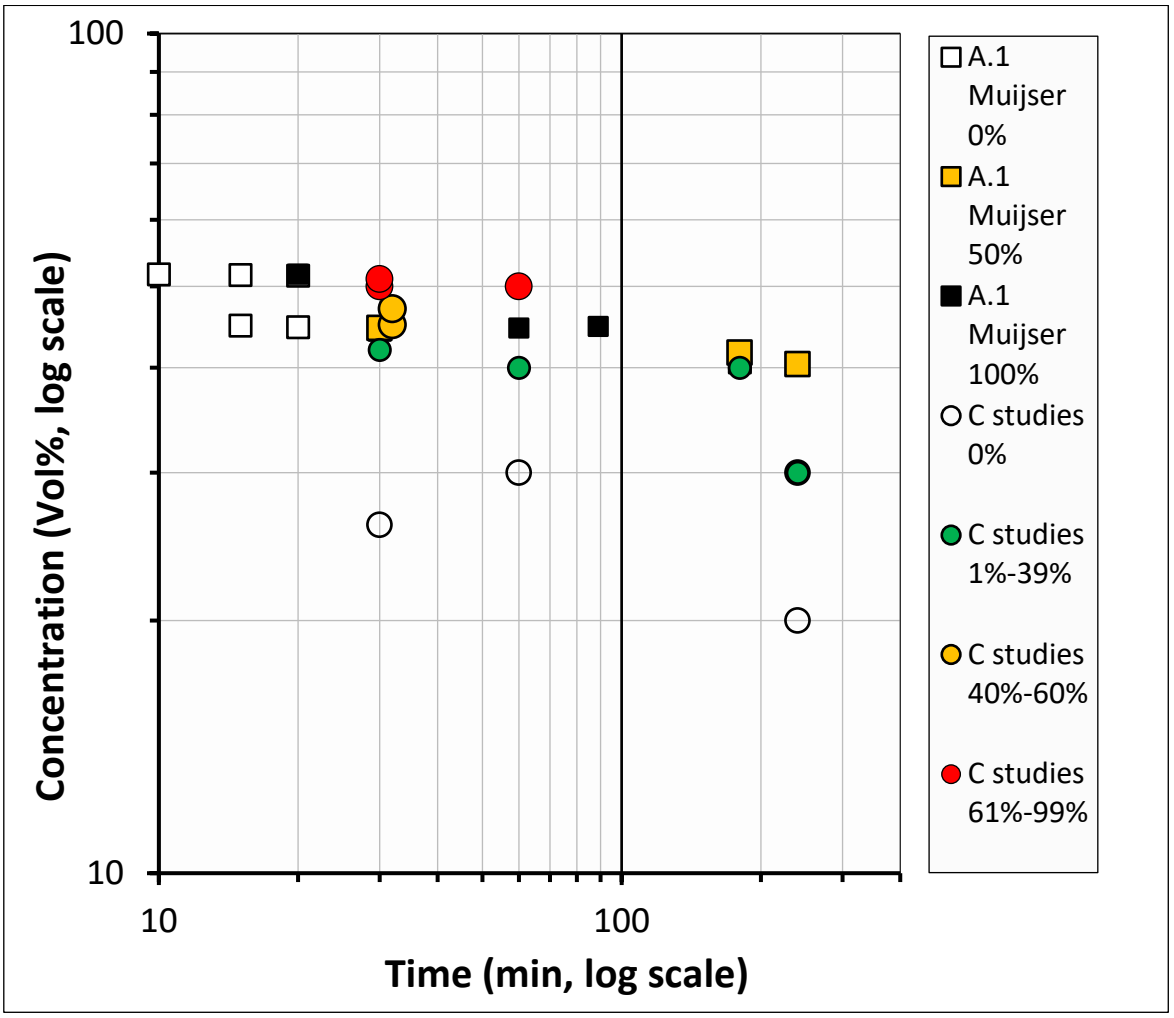


Figure 3 *All available acute animal lethality data for carbon dioxide.*

The data that were selected for initial analysis of the animal probit function are presented in Table 5 and Figure 4.

The only available A-study (A.1; Muijser and Van Triel, 2010) was selected for derivation of the animal probit function for carbon dioxide.

The probit function has been calculated and reported in Appendix 1, which deviates from the original calculations by the study authors. Because of the data Muijser and Van Triel derived separate probit functions for the concentration ranges 40 to 43 Vol% and 43 to 50 Vol%. The probit function for the lower concentration range and longer exposure durations did provide realistic estimates with a very high n-value of approximately 24 (see appendix 1) and the LC_{50} seems to reach an asymptotic value at 40 Vol% for long exposure durations. The results of the calculations according to

the RIVM methodology are presented in Table 5 for the probit function without sex as covariate.

Table 5 *Data selected for initial analysis of the animal probit function of carbon dioxide.*

Study ID	Species	Probit (C in Vol% or mg/m^3, t in min)	LC_{50}, 30 minutes (Vol% or mg/m^3) 95% C.I.	n-value 95% C.I.
A.1 (Vol %)	Rat	-42.0 + 11.0×lnC + 1.23×lnt	49.6 (47.3 – 1.1 x 10^{4*})	8.94 (4.31-13.57)
A.1 (mg/m^3)	Rat	-149 + 11.0×lnC + 1.23×lnt	908400 (86900 – 2.1 x 10^{8*})	8.94 (4.31-13.57)

*unrealistic value.

The data of the A study with rats are presented graphically below.

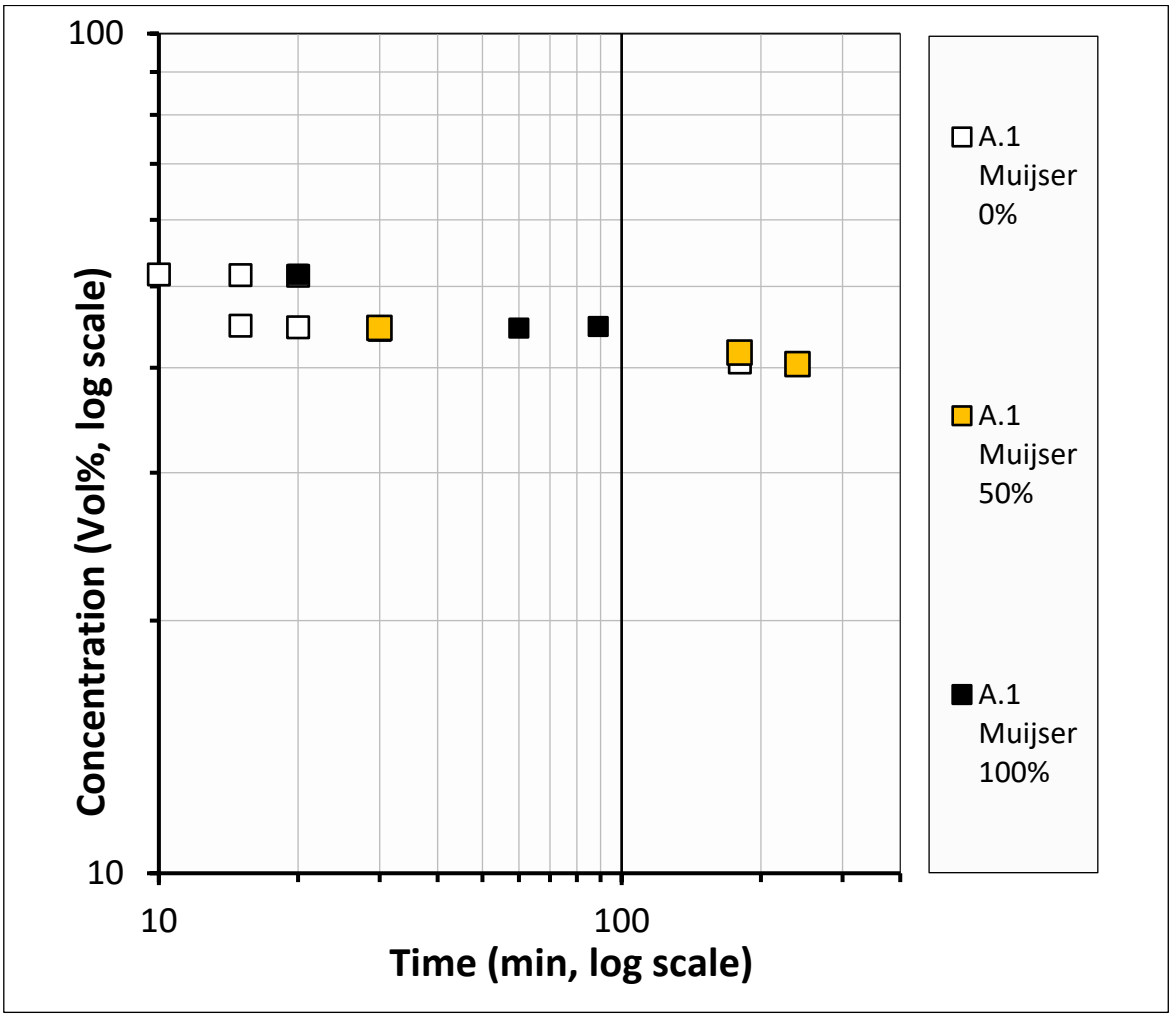


Figure 4 *Data selected for the initial analysis for the derivation of the animal probit function of carbon dioxide.*

Based on criteria outlined in the guideline the data from Muijser and Van Triel (2010; study A.1) should be selected for the final dataset for the derivation of the animal probit function. However, the animal probit function, regardless of the probit functions

by Muijser and Van Triel or according to the RIVM methodology, shows an ill fit on the higher mortality ranges and does not provide an accurate fit for the entire exposure duration or concentration range. Since the previous notes in 2007 and 2009 by Ter Burg and Bos, the view on deriving a human probit function based on the animal probit function has not been changed, despite having more information on the physiological effects coming from the LUMC/Shell study. The results in appendix 1 and in section 5 underline this view. Therefore, the animal probit function is not used for human probit function derivation nor for setting lethal values (see section 6 for further explanation and derivation of lethal estimates).

6. Derivation of the human probit function

In the RIVM notes of 2007 and 2009 (Ter Burg and Bos 2007; 2009) it was concluded that the acute animal lethality data, including the Muijser and Van Triel study (2010; A.1), cannot provide a human probit function that would generate proper predictions of lethal effects after short-term accidental release.

Since then, one new study became available. This study was conducted by LUMC/Shell to investigate the physiological parameters in humans (see section 3 above) and rats (see appendix 1: C-studies) following the recommendations previously made in Ter Burg and Bos (2009). In the rat, physiological parameters could be studied up to lethal concentrations. This study has generated insight in physiological changes in both humans and rats as well as insight in gross pathology in the rat following acute CO_2 exposure. The results concerning the rat study of LUMC/Shell supported the lethal findings observed earlier by Muijser and Van Triel (2010; A.1). The new study did not change the view regarding the predictive value of the animal probit function for human lethality since it showed similar results compared to the Muijser and Van Triel study and thus the previous conclusion in the RIVM note (ter Burg and Bos 2007; 2009) is not invalidated by the new observations. The Expert panel on probit functions concluded that the acute animal lethality data cannot be fitted to a scientifically justified (human) probit function.

A clear explanation cannot be presented as to why a human probit function cannot be derived that would fit the entire exposure and lethal response range. The LUMC/Shell study does suggest that many factors may play a role simultaneously in the cause of death, such as the decrease of pH in blood to critical levels, physiological stress in the lungs (hyperventilation) and cardiovascular system, as well as effects following oxygen deprivation. Apparently, the many processes involved cannot be captured by a ‘simple’ human probit function. As an alternative, human probit functions have been derived for CO_2 based on conservative/protective estimates of human lethal response relying on reports of unconsciousness due to CO_2 exposure (see section 3 referring to the UK HSE and Tebodin probit functions). These human probit functions seem to be set from a conservative point of view side and are based on human data, but lack data on lethality. It is noted however by UK HSE (2008), DNV TECHNICA/SCANDPOWER A/S (2001), and Pauluhn (2016) that incapacitation due to CO_2 exposure is a predictor for levels where mortality starts to occur. The use of these probit functions can be justified from a pragmatic (conservative and protective) point of view.

The Expert Panel on probit functions therefore decided to provide estimations of CO_2 air concentrations at various time points where lethal effects may start to occur, instead of proposing a human probit function. To this end, human data from the volunteer study of LUMC/Shell and cases of unconsciousness (DNV TECHNICA/SCANDPOWER A/S 2001; UK HSE 2008; COT 1996) are considered together with findings on sub-lethal and lethal responses in the rat (LUMC/Shell; Muijser and Van Triel 2010; Levin et al. 1995; 2007) to support those estimations. To evaluate the applicability of rat data, the observations by Pauluhn (2016) and

LUMC/Shell are considered to provide information about the predictive value of the rat data for human toxicity.

Pauluhn (2016) states that rodents, especially burrow dwellers, respond to increased CO_2 exposures by reducing their body temperature and thus reducing the oxygen demand and CO_2 formation in the tissue. Although the basic physiology between rodents and humans is regarded similar, it is the hypothermic reaction in rodents that may result in lower physiological disruption caused by excessive CO_2 exposure compared to humans. The data by LUMC/Shell do provide more information related to differences between human and rat responses to CO_2 exposure and seem to support the hypothesis by Pauluhn, where the decrease in pH levels and increase in CO_2 levels in rats appear to be less steep compared to humans. Taking the pH levels in arterial blood a comparison was made between humans and rats, by taking results from the LUMC/Shell experiment and parameterising that into a model prediction (see Figures 1 and 2 in section 3 of this TSD). Using the model approach, the pH changes at levels above 10 Vol% can be modelled in humans. Based on the model predictions up to 10-mins it appears that the drop in pH level is slightly steeper in humans compared to rats. It is noted that almost identical pH levels are found at 10 minutes especially at the higher exposure range >25 Vol% (457500 mg/m^3) between humans and rats (modelled data for both species). Overall, the results in $PaCO_2$, PaO_2, HCO_3^- show similar responses to CO_2 exposure in humans and rats, with minor differences in favour of the rat from a toxic effect perspective (LUMC/Shell). It seems that humans are more susceptible to CO_2 exposure compared to the rat, but respond in a similar way.

With respect to intraspecies (or inter individual) data it is noted that practically no information is available that describes differences between humans. Some participants in the volunteer study by LUMC/Shell stopped the experiment as it was perceived as unbearable while others could complete the study. However these observations cannot be interpreted as interindividual differences as the decision was not based on toxicological or physiological effects but rather on perceived (dis)comfort. Based on the observations in the healthy male volunteers and pathological effects observed in the rats, the authors of the LUMC/Shell study hypothesize that individuals, with for example respiratory disorders, might succumb sooner from the respiratory stress to the lungs and muscle tissue surrounding the lungs. The effects observed in the rats are caused by extreme increases in cardiac output, pulmonary vasoconstriction and extreme hyperventilation following high CO_2 exposures, that cannot be endured if the respiratory or cardiovascular systems are already compromised. Such differences and their magnitude are not covered by the available data and thus cannot be quantified.

Taking all the information from the rats and humans together a weight of evidence approach is pursued in setting the human lethal estimates. As humans seem to be more susceptible to CO_2 exposure than rats and unconsciousness seems to be a conservative predictor for lethal outcomes, more weight has been given to human data, including the human volunteer study. Table 6 presents estimations of CO_2 air concentrations at various time points where lethal effects may start to occur in humans, arbitrarily set at 1% lethality. The lethal values are given for the 5, 10, 30 and 60 minutes together with the rationale for that value. As the data set is fairly limited for durations > 60 minutes, no estimates will be provided for durations exceeding 60 minutes.

Table 6. Estimations of CO_2 air concentrations at various time points where lethality may start to occur in humans, assumed to equal 1% lethality.

Duration (min)	**Air concentration (Vol%; (mg/m^3))**	**Rationale**
5	20 (366000)	The human data on 5 minute exposure to CO_2 are limited. The LUMC/Shell study with human volunteers showed that two participants could tolerate an exposure to 12 Vol% for an average duration of 6.5 minutes without any overt signs of toxicity, where one participant stepped out before 5 minutes was reached due to panic and dissociation; exposure could not be tolerated. The LUMC/Shell study showed that 10 minute exposure to 10 Vol% was without toxicological and physiological effects that deemed cessation of exposure necessary. Information gathered by DNV TECHNICA/SCANDPOWER A/S indicate that 5 minute exposure to 12 Vol% is the threshold for unconsciousness. At 20 Vol% unconsciousness was observed within one minute. DNV TECHNICA/SCANDPOWER A/S derives an exposure limit indicating a 100% fatal outcome for 15 vol% for durations up to 5 minutes. Contextual information is not available from DNV/Scandpower supporting these statements but considering the LUMC/Shell data, they seem to be set from a conservative point of view. As the effects observed in the LUMC/Shell study are still at the level of tolerability up to 12 Vol% and unconsciousness is seen rapidly at 20 Vol% (DNV TECHNICA/SCANDPOWER A/S), though at a shorter duration, it seems appropriate to set the estimate for 1% lethality for five minutes at 20 Vol%.
10	15 (274500)	The LUMC/Shell study showed that 10-minute exposure to CO_2 at 10 Vol% could be completed without producing signs of toxicological or physiological effects that would prompt cessation of the exposure, according to the safety standards, except for 1/10 participant with too low pH. At 12 Vol% both participants stepped out as the exposure became unbearable (average about 6.5 min exposure), but without signs of toxicological or physiological effects. Taking into consideration that the effects did not include signs of unconsciousness (though panic and dissociation were noted) on the one hand and the study involved healthy males on the other hand led to the estimate of 15 Vol% at 10-minutes as the 1% lethality value. The margin between lethal effects observed in

Duration (min)	**Air concentration (Vol%; (mg/m^3))**	**Rationale**
		the rat at 50 Vol% and the lethal value estimated for humans seems sufficiently large.
30	10 (183000)	DNV TECHNICA/SCANDPOWER A/S indicated that a level of 10 Vol% for 30 minutes approaches the threshold for unconsciousness. Further they assumed 100% fatal outcomes for this concentrations > 30 minutes. The LUMC/Shell study showed that 10 Vol% could be tolerated for up to 10 minutes (3 out of 10 participants), but 12 Vol% became rapidly intolerable within approximately 6.5 minutes (for 2 out of 2 subjects). In one subject (10 Vol% group) the pH level became too low (pH < 7.2) according to safety standards in the study. Physiological data showed that the decrease in pH and increase in blood pressure did not yet reach equilibrium in the participants, indicating that effects were still worsening. On the other side, 7.5 Vol% did not appear to produce serious toxicity, indicating that lower estimates for lethality than 10 Vol% are not justified. Therefore, extrapolating these findings to 30 minute exposure provides some limited support to the statement by DNV TECHNICA/SCANDPOWER A/S. The rat studies indicate that the exposure concentration appears to be the predominant driver for the toxic effect rather than duration (high n-values were derived). Physiological results in rats support this where equilibrium is found at 10 Vol% after 30 minutes. Lethal responses in the rat start to occur at 30-min to 42 Vol% (data by Levin et al.). No lethal response was seen at 30 Vol% up to 60 minutes exposure in rat studies. Lethal response was seen at 40 Vol% after almost 60 minute exposure, but the mortality rate is relatively low. Taking the above into consideration, 10 Vol% was considered appropriate as lethal value.
60	10 (183000)	There is no information available on levels of unconsciousness in humans at 60 minutes. The estimate for 60 minutes of 10 Vol% is based on the same rationale as for the 30-min lethal value. Adverse physiological changes have not been observed up to 60 minutes at 7.5 Vol% in

Duration (min)	Air concentration (Vol%; (mg/m^3))	Rationale
		the LUMC/Shell study supporting the choice for 10 Vol%. Based on the rat lethality data, it is considered that the duration plays a minor role in CO_2 acute toxicity (from 30 minute and longer durations). This provides some confidence that the 10 Vol% for 60 minutes is appropriate. The rat data further show a sufficient large margin between levels with lethal response and the estimated lethal values for humans of 10 Vol%.

The above estimates of the human lethal air concentrations should be considered with some reserve. Due to absence of appropriate data and the complexity of the physiological processes following CO_2 exposure, they are set from a conservative point of view. Most supporting data comes from the human volunteer study by LUMC/Shell and can be considered describing effects at the level of tolerability rather than at the level of unconsciousness. Especially, the impact of exposure duration is difficult to assess. The available data from the rat experiments suggests a threshold concentration for mortality, especially for exposures longer than 30 minutes. This supports the decision of setting the 30 and 60 minute at the same lethal value. The data with human volunteers exposed to 7.5, 9, 10 and 12 Vol% indicate that the number of individuals dropping out significantly increases with a small increase in concentration.

Appendix 1 Animal experimental research

Study ID: A.1

Author, year: Muijser and Van Triel, 2010 (also reported in Muijser et al., 2014)

Substance: carbon dioxide
Species, strain, sex: Rats, Wistar WU (Crl:WI(WU), outbred), male and female

Number/sex/conc. group: 16 males and 16 females, 1 animal/sex per C x t
Age and weight: 6 weeks, weight 226 – 300 g (m); 160-187 g (f)
Observation period: 14 days

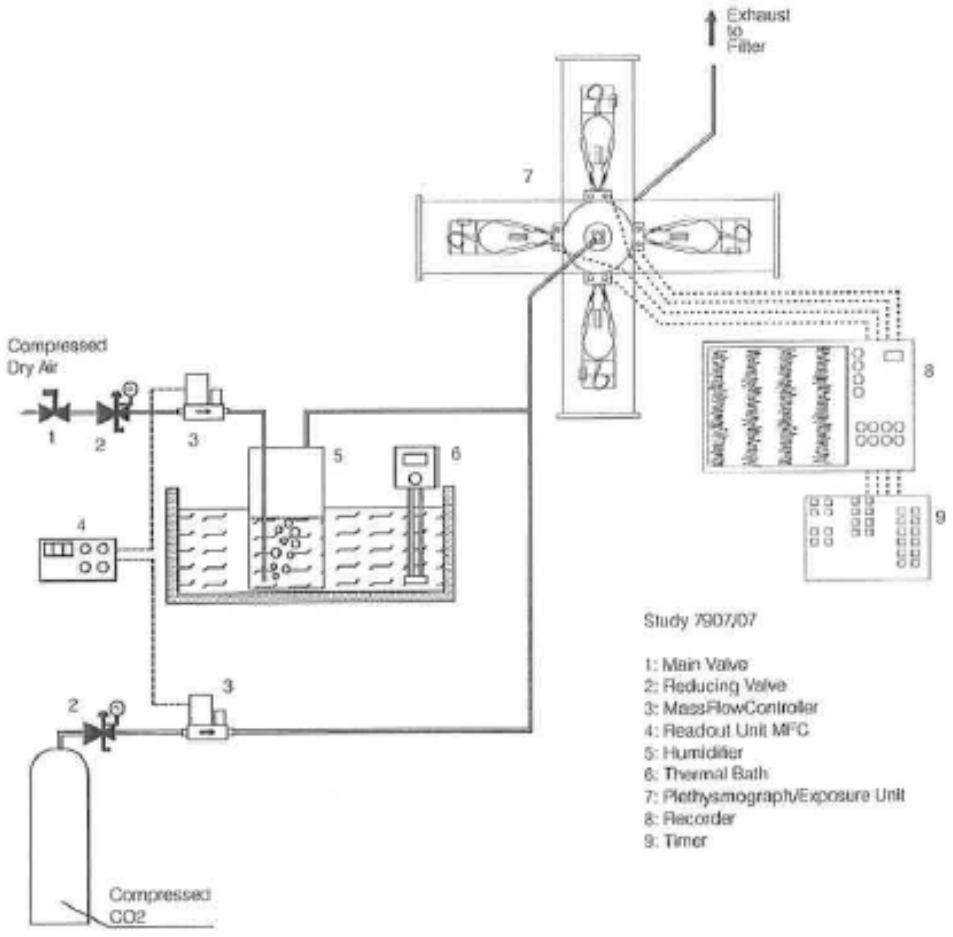
Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*yes*
Study carried out according to OECD 403 guideline(s)	*yes*
Stability of test compound in test atmosphere	*stable*
Use of vehicle (other than air)	*Humidified air*
Whole body / nose-only (incl. head/nose-only) exposure	*head only*
Type of restrainer	*Battelle fitted with collar*
Pressure distribution	*Positive pressure was secured at the central column*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere was generated as a gas from the cylinder and controlled by a mass flow controller. This flow was mixed with controlled mass flow with humidified air. System was calibrated by a volumetric flow meter.*
Number of air changes per hour	*10 l/min/animal, central column has a volume of 0.8 l.*
Equilibration time (t95)	*N/A*
Start of exposure relative to equilibration	*Generation was started with animals placed in the plethysmograph/exposure system as the volume in the plethysmograph is low compared to test atmosphere flow. In 2 of the 4 'legs' exposuse was administered. In the third leg the analytical concentration was measured and at the fourth leg was used to monitor temperature and relative humidity of the test atmosphere.*
Actual concentration measurement	*At one of the 4 'legs' of the plethysmograph/exposure system. The oxygen level was measured and used to calculate the CO_2 volume percentage.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*

Assessment of Reliability	***A*** *Well-performed study, including multiple exposure duration - concentration combinations.*

Note: in contrast to the common approach in acute inhalation toxicity studies the oxygen displacement by carbon dioxide was not compensated by adding oxygen to the generated test atmosphere. The exposure to carbon dioxide in high concentrations would, therefore, mimic real-life exposure situations and is more appropriate for the purpose of the study.

Figure 1 - Schematic diagram of the generation and exposure system



Results

Species	Concentration (Vol%)		Exposure duration (min)	Lethality	
	Target	Calculated		Male	Female
				Dead/tested	

Rat	40	40.4	240	1/1	0/1
	40	40.6	180	0/1	0/1
	50	51.7	20	1/1 (15)*	1/1 (14)*
	43	44.8	89	1/1 (14)*	1/1 (31)*
	43	44.9	15	0/1	0/1
	50	51.7	10	0/1	0/1
	43	44.7	20	0/1	0/1
	43	44.6	30	0/1	0/1
	43	44.6	60	1/1 (46)*	1/1 (33)*
	43	44.4	30	0/1	0/1
	43	44.6	30	0/1	1/1
	50	51.6	15	0/1	0/1
	50	51.5	20	0/1	0/1
	40	41.7	180	1/1	0/1

*Numbers in brackets are time of death (in minutes) if that occurred before the end of exposure.

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 Vol% and mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male). Please note that the time of death, if occurred before the end of exposure as reported in the Muijser and Van Triel study, have been taken as input for duration.

Probit function	Species	a	b	c	d	n-value
Sex as variable	Rat (vol%)	-7.75	-0.91	0.045	0.38	-19.984 (-572 - 532)*
Sexes combined	Rat (Vol%)	-42.0	11.0	1.23		8.94 (4.31 - 13.57)
	Rat (mg/m^3)	-149	11.0	1.23		8.94 (4.31 - 13.57)

*The results for the probit function with sex as variable resulted in an unrealistic estimate for lethality over time (data not shown) as estimates for lethal concentrations have a negative correlation with time. The probit function cannot be used to indicate differences between the sexes. Based on the results provided in the table above and by visual inspection of the results as shown in the figures below it does not seem that sex differences in regard to CO_2 toxicity exist.

Using the 'sexes combined' probit function provides the following estimates for lethality:

Duration (min.)	LC_{50} (Vol%) 95%-C.I. Combined	LC_{50} (mg/m^3) 95%-C.I. Combined
10	56.1 (51.8 – 7.9 x 10^5*)	1027000 (947100 – 1.43 x 10^{10})
30	49.6 (47.3 – 1.1 x 10^4*)	908400 (86900 – 2.1 x 10^8)
60	45.9 (43.5 – 812*)	840600 (795800 – 1.47 x 10^7)

*the upper ranges of the confidence intervals for 10-min, 30-min and 60-min are above 100 Vol% and unrealistic.

The study authors (Muijser and Van Triel) noticed that the probit function derivation resulted in unrealistic values, inline what is presented here. Therefore they decided to perform probit function analyses for the concentration ranges 40-43 Vol% and 43-50 Vol% separately as it seems that there is an asymptotic relation for C x t lethality of CO_2. A reliable probit function could only be derived for the lower concentration range with a rather high n-value of 24.2 (95% C.I. 15.6 – 32.7). They derived the following lethal estimates, noting that confidence intervals could not be derived:

Duration of exposure	Estimated LC50 (vol % CO_2)	
(min)	Males (sex=0)	Females (sex=1)
15	45.8	46.3
30	44.5	45.0
60	43.3	43.7
120	42.0	42.5
240	40.8	41.3

A graphical overview of the data is presented below, copied from Muijser and Van Triel (2010), for male and female rats separately.

Figure 2A – Concentration – time –mortality plot of male animals

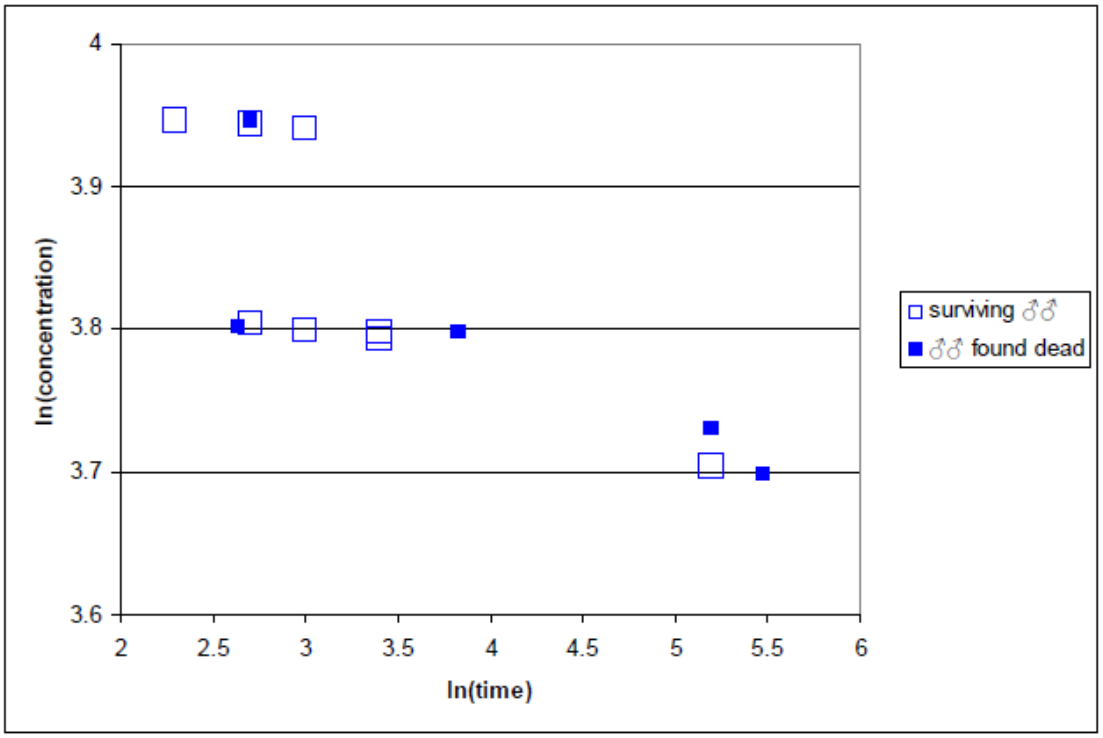
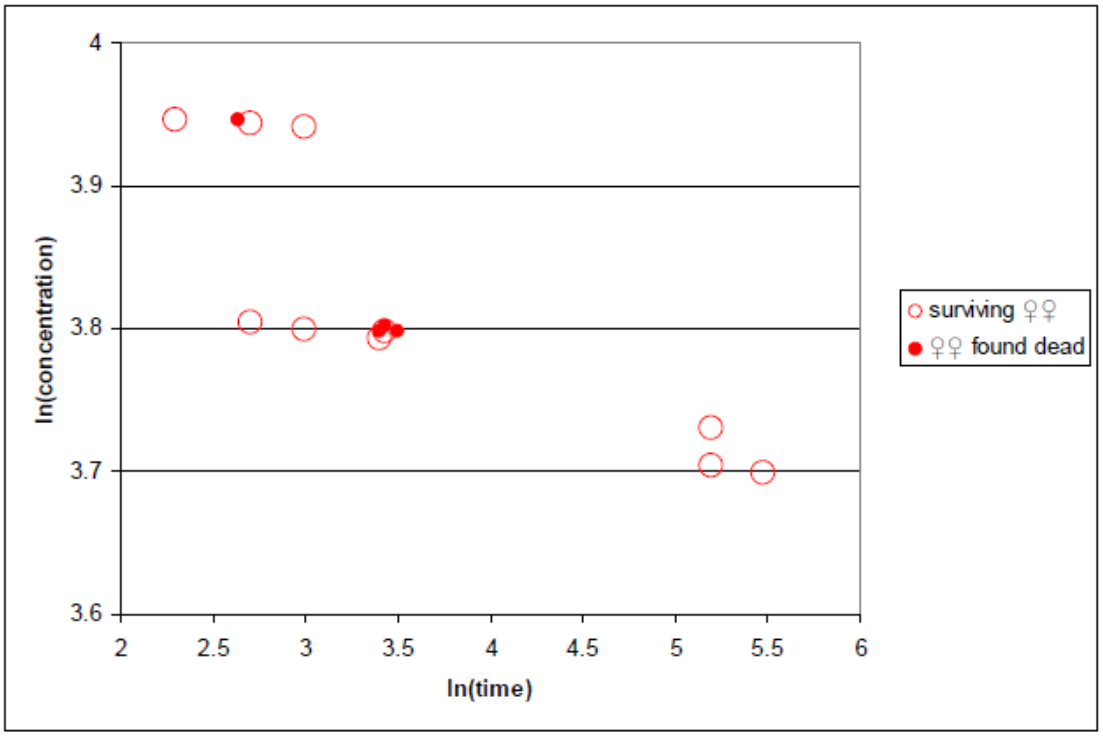


Figure 2B - Concentration – time –mortality plot of female animals



1
2

Study ID: C studies

Animal studies with acute lethality of CO_2 alone are limited in number. There are a number of studies where co-exposure with either oxygen, temperature, or fire related substances occurred. These studies show that the lethal response is influenced by the co-exposures either reducing lethal response by administering oxygen (Pryor et al. 1974, see table below) or increasing lethal response by co-exposure to heat combined with other substances such as CO (Schaefer et al. 1963). Besides the Muijser and Van Triel study there are two other studies that concerned exposure to lethal levels of CO_2 without controlling oxygen levels, i.e. a study by Levin et al. 1995 and a study conducted at the Leiden University Medical Centre (to be published).

Pryor et al. (1974) performed a series of experiments in male mice. Male mice were exposed to different CO_2 concentrations for 4 hours. Although most of the experiments were aimed at combined exposures and effects of other parameters like temperature on mortality, a few results are relevant within the present context. These are summarized below. The concentrations were controlled; temperatures were at least 85 F (about 30°C).

The study by Levin et al. (1995) only reports a 30-min LC_{50} value of 47 vol% CO_2 in F344 rats. Via personal communication unpublished data were obtained regarding the concentrations used in their study, provided in the table below, but no further detailed information was obtained.

The SMAC further reports a 21% mortality rate in rats at 40 vol% for 180 minutes (COT, 1996), but its origin is unknown.

Concentration CO_2 (Vol%)	Exposure duration (min)	Mortality	Remarks	Reference
Animal mortality data				
20	240	No mortality	mice, 16 Vol% O_2 *	Pryor et al. (1974)
30	240	No mortality	mice, 16 Vol% O_2 **	Pryor et al. (1974)
30	240	20% mortality	mice, 13.5 Vol% O_2 **	Pryor et al. (1974)
40	180	21% mortality	rats	COT, 1996
1.3 – 26	30	0/6	F344 rats	Levin et al., (2007 unpublished data)
42	30	1/6		
44.5	30	3/6		
47	30	3/6		
49.6	30	5/6		
50.2	30	2/6		
51	30	5/6		
47	30	LC_{50}	F344 rats	Levin et al. (1995)

*: concurrent O_2 concentration would be approximately 16 Vol% under normal circumstances
**: concurrent O_2 concentration would be approximately 14 Vol% under normal circumstances

The Leiden University Medical Centre/Shell (unpublished) studied the effects of CO_2 exposure in a human volunteer study as well as in a rat acute toxicity study. The human volunteer study is briefly described under section 3 of this TSD. The rat acute toxicity study was designed to evaluate physiological parameters such as pH, CO_2 and O_2 pressure, and gross pathology at high CO_2 concentrations up to 60 minutes.
Five cohorts consisting of 8 male CD IGS rats each (weight: ± 300 g) were exposed to one of five concentrations 10%, 20%, 30%, 40% or 50% of CO_2, with adjusted O_2 concentrations of 18.9%, 16.7%, 14.6%, 12.5% and 10.4%, respectively (the O_2 adjustment mimics the air replacement by CO_2; in standard toxicity testing the oxygen level is kept constant at normal levels). Two separate sample regimens were implemented. The first four animals (group 1) were submitted to a more frequent sample regimen which means that the baseline sample immediately before exposure was followed by samples obtained at two-minute intervals (0, 2, 4, 6, 8, 10 min.) followed by two samples at 15 and 20 minutes after exposure. This sampling regimen provided valuable information on the acute changes in blood physiological parameters shortly after CO_2 exposure. The second sampling regimen (group 2) was applied to four other animals. Following the baseline sample, a less frequent sampling interval was implemented up until the end of exposure (0, 5, 10, 20, 30, 40, 50, 60 min). Information obtained from group 2 animals was valuable for the more chronic effects of CO_2 exposure on blood physiological parameters. All animals were monitored in terms of behaviour, and moribund animals reaching mentioned endpoints were euthanized immediately by means of pentobarbital injection.
No animals died up to 30 Vol%. At 40 Vol% one animal died (at 52 minutes), whereas at 50 Vol% one animal survived the 60 minute exposure duration (animals died at 14 (2 animals), 22, 25 , 50, 58 minutes).

Appendix 2 Reference list

Anonymous (1987). Fatal Accident Circumstances and Epidemiology (FACE) report: Worker dies in fermentation tank in Montana. Govt Reports Announcements & Index (GRA & I), Issue 23.

Bryan, J.L: "Damageability of Buildings, Content and Personnel from Exposure to Fire", Fire Safety Journal, 11, 1986

Burg W ter, Bos PMJ. (2007). Evaluation of the acute toxicity of CO_2. RIVM

Burg W ter, Bos PMJ. (2009). Evaluation of the acute toxicity of CO_2: update. RIVM

CDC/NIOSH (1994). https://www.cdc.gov/niosh/idlh/124389.html

COT (1996). Carbon Dioxide. In: Spacecraft Maximum Allowable Concentrations for selected airborne contaminants – Volume 2. National Research Council. National Academy Press, Washington D.C. ISBN: 0-309-05478-8.

➔ NASA statement on SMACs: https://www.nasa.gov/sites/default/files/atoms/files/jsc_20584_signed.pdf.

DNV Technica/Scandpower A/S (2001) Human resistance against thermal effects, explosion effects, toxic effects and obscuration of vision.

UK HSE (2007). Carbon dioxide Dangerous Toxic Load assessment, received by e-mail.

UK HSE (2009). Comparison of risks from carbon dioxide and natural gas pipelines. Report RR 749

Leiden University Medical Centre/Shell (2020, manuscript in preparation).

Levin, B.C., Braun, E., Navarro, M., and Paabo, M. (1995). Further development of the N-Gas mathematical model – An approach for predicting the toxic potency of complex combustion mixtures. NIST, Gaithersburg, MD 20899. In: Fire and Polymers II: Materials and tests for hazard prevention. Chapter 20.

➔ Levin, B.C. (2007; unpublished data) by personal communication.

Linde Gas Benelux BV (2007). Veilig werken met gassen – zuurstofgebrek. http://www.lindegasbenelux.com/international/web/lg/nl/likelghlnl30.nsf/repositorybyalias/pdf_zuurstofgebrek/$file/Zuurstofgebrek.pdf

LUMC/Shell (unpublished data). Homeostasis and acute exposure to CO_2.

Muijser and Van Triel, 2010. Acute (concentration - time) inhalation toxicity study with carbon dioxide in rats. TNO report V7901-07

Muijser, H., van Triel, J.J., Duistermaat, E., Bos, P.M., 2014. Acute toxicity of high concentrations of carbon dioxide in rats. Regul. Toxicol. Pharmacol. 69, 201–206.

Pauluhn J. 2016. Risk assessment in combustion toxicology: Should carbon dioxide be recognized as a modifier of toxicity or separate toxicological entity? Toxicology Letters 262 (2016) 142–152

Pryor A.J., Fear., F.A., and Wheeler R.J. (1974). Mass life hazard: Experimental study of the life hazard of combustion products in structural fires. JFF/Combustion Toxicology 1: 191-235

Tebodin 2008. Veiligheidsanalyse Ondergrondse Opslag van CO_2 in Barendrecht. Documentnummer: 3800784

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

Schaefer KE (1963). The effects of CO_2 and electrolyte shifts on the central nervous system, in Schade JP, McMehemy WH (eds): Selective vulnerability of the brain in hypoxemia. Oxford, Blackwell Scientific Publications, pages 101-23