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Evaluation of the acute toxicity of CO_2

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Introduction

Carbon Dioxide Capture and Storage technology is the latest hot development in reducing carbon dioxide (CO_2) exhaust globally. During industrial processes the combustion product CO_2 is captured and transported to another location by using (old) pipelines and stored underground. In this way, an attempt is made to reduce exhaust of CO_2 by industries. Transport and storage of CO_2 is kept under relatively high pressure to deal with the large amounts of CO_2 which are formed during processes. Recently, a pipeline has been taken into service again for the transport of carbon dioxide (CO_2). This underground pipeline passes through building areas were new dwellings are planned to be built. It is expected that high air concentrations of CO_2 may occur in case of a leakage. However, the toxicity and lethality of such high concentrations of CO_2 are unknown.

For the above reasons, an urgent need exists to perform a quantitative risk assessment (QRA) for acute exposures. Probit functions are used within the framework of the External Safety (Establishments) Decree to calculate the mortality rate due to exposure to toxic substances at a given exposure concentration and duration. In principle, only formally established probit functions may be used in a quantitative risk assessment (QRA) performed under the Decree.

Previously, a probit-relation has been proposed by TNO (2006), but upon evaluation this function was considered to be not suitable because it included too many uncertainties and was based on very limited unverifiable data. In the UK, the Health and Safety Executive (HSE, 2007) derived a concentration-timet relationship for CO_2 lethality (50% lethality) based on human data without lethality. Since the point of departure was a non-lethal effect level and the relationship between concentration and time was assumed, it was concluded that the derived relationship contained too many uncertainties for use in a QRA.

In 2007, the Ministry of Housing, Spatial Planning and the Environment (Ministry of VROM) requested the RIVM to evaluate the acute (lethal) toxicity of CO_2 and if possible, to derive a probit function for CO_2. RIVM then concluded that the available data were insufficient for the derivation of a sufficiently reliable probit function. Subsequently, an animal lethality study for CO_2 according to the C x t protocol in the rat was performed by order of the Ministry of VROM. The present document is an update of the2007 evaluation in which the most recent developments are included.

Probit function

A probit function for lethality describes the relationship between concentration, time and response (lethality). The general equation to describe the probit function is as follows:

$$\mathbf{Pr = -a + b \times \ln(C^n \times t)}$$

where C is the concentration in mg/m^3 and t is time in minutes. The probit (Pr) is normalized to 5, which represents the probability of 50% mortality. Ideally, acute toxicity studies should include several concentrations and exposure durations resulting in a response ranging from 0-100% mortality.

CO_2 toxicity

It is generally believed that CO_2 toxicity is caused by displacing oxygen, leading to asphyxiation, similar to the mode of action as inert gases. This is only partly true. The inhalation of high concentrations of CO_2 can lower the pH of the blood and thus trigger effects on the respiratory, cardiovascular and central nervous systems (HSE, 2007).
In summary, CO_2 exposure can give rise to a variety of effects, including an increase in inhalation rate, in heart rate and in blood pressure and it can induce cardiovascular effects. Mortality is most likely due to effects on the respiratory tract and oxygen supply. These effects are caused by two mechanisms: asphyxia and by direct effects of CO_2 on the regulation of respiration. Respiration is a tightly controlled phenomenon in mammals (including humans). It is primary 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. A too high level of CO_2 in blood and tissues will lead to acidosis which is harmful for mammalian tissues, especially those with a high sensitivity (e.g. brain).

The normal concentration of CO_2 in air is low – below 1 Vol% - while oxygen (O_2; 21 Vol%) and nitrogen (N_2; 79 Vol%) are the main constituents. When CO_2 is introduced into an environment, concentrations of O_2 will decrease because of displacement by CO_2. A drop of the oxygen level below a certain level will be life threatening. An O_2 concentration below 15 Vol% will lead to a decrease in awareness in healthy human beings, below 10 Vol% to unconsciousness and below 6 Vol% death will occur rapidly. CO_2 concentrations of approximately 25 Vol%, 52 Vol% and 70 Vol% will give rise to these respective O_2 levels by displacement. In rats, a 30-min LC_{50} of 47 Vol% (leading to an oxygen level of approximately 11 Vol%) has been found in rats indicating that mortality is not only caused by asphyxiation but also by direct effects of CO_2 itself. This is probably similar in humans.

Available acute toxicity data on carbon dioxide

Human data

Human data on acute toxicity after CO_2 exposure is limited to one accidental death and subjects reaching a state of unconsciousness (Table 1). Carbon dioxide has been tested as anaesthetic in humans in the past, but was abandoned as anaesthetic because better alternatives were available. Studies on CO_2 as anaesthetic have not been evaluated to date, but are unlikely to present information on human lethality after CO_2 exposure. For these reasons a quantitative concentration-time-response relationship for lethality cannot be obtained from the human data.

Table 1: summary of available human acute toxicity data

Concentration CO_2 (Vol%)	Exposure duration (min)	Mortality	Remarks	Reference
Human mortality data				
17	20-52 seconds	-	Unconsciousness	COT, 1996
18.6	< 2	-	Unconsciousness	COT, 1996
20-22	Not stated	Mortality reported	Survivors unconscious	COT, 1996
48	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 gasses will have occurred.

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% 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 rapidly occur. Linde Gas Benelux BV indicates that the information is based on medical data and aimed to protect their employees and customers. The data underlying these statements have not been obtained at the moment.

Animal data

Information on the acute toxicity of CO_2 was sought for in the HSDB and IUCLID databases; in addition a literature search in the public literature was performed. Although several experiments addressing CO_2 toxicity were retrieved many studies focus on combined exposure or maintained the oxygen level at 21 Vol% by adding extra oxygen. Since within the present context the focus is on mortality due to CO_2 exposure concurrently leading to decreased oxygen levels the latter studies are not relevant.

Three studies were identified that investigated the lethal effects of high CO_2 exposure. A detailed discussion of individual studies is beyond the scope of the present document. A general overview of the data retrieved from the mentioned sources will be given with emphasis on the practical suitability for a QRA. The results are summarized in Table 2.

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 in Table 2. The concentrations were controlled; temperatures were at least 85 F (about 30˚C).

In 1988, Levin and colleagues conducted a lethality study in Fischer 344 rats (6 animals per group) for 30 minutes to varying concentrations of CO_2, without controlling the O_2 concentration. The results show that exposure concentrations in the range of 40-50 Vol% will induce mortality. The lowest concentration showing mortality was 42 Vol% (1/6 died), whereas the highest concentration showing no mortality was just under 26 Vol%. Five out of 6 animals died at 49.6 Vol% (Levin, personal communication, 2007).
Very recently, TNO (Muijser, 2009 draft), at the request of the Ministry of VROM, performed a lethality study in the rat (male and female Wistar WU (Crl:WI(WU), outbred)) using the Cxt protocol. In total 14 C x t combinations were used in the study including 1 animal/sex/C x t

combination. The highest Cxt combination were both animals survived was 40.6 Vol% for 180 minutes exposure. Exposure to 40.4 Vol% of CO_2 for 240 minutes resulted in the death of the male rat.

Both studies (Levin (2007) and Muijser (2009 draft)) show an apparent cut-off for lethality at about 40 Vol%. Concentrations reaching 50 Vol% are almost 100% lethal indicating that the dose-response relationship is extremely steep. Based on the results found by Muijser it was not possible to derive a realistic probit function that covers the entire concentration range. The relation between the logarithm of concentration and the logarithm of time could best be described as an asymptote at approximately 40% carbon dioxide, below which irrespective of duration no mortality is expected to occur, and rapidly occurring mortality at higher concentrations. Above a concentration of 44% carbon dioxide, the 'n' from C^n x t could not be established because the 95% reliability limits are -8.6 and 12.1. However, in the range 40-43% CO_2 concentration is much more important than exposure time in the probit function, i.e. the 'n' in C^n x t is estimated to be 24.2 with 95% reliability limits of 15.6 and 32.7.

Table 2: summary of available animal lethality data

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)
40.4	240	1/2	Wistar rats	Muijser, 2009 draft
40.6	180	0/2		
41.7	180	1/2		
44.4	30	0/2		
44.6	30, 30, 60	0/2; 1/2; 2/2		
44.7	20	0/2		
44.8	89	2/2		
44.9	15	0/2		
51.5	20	0/2		
51.6	15	0/2		
51.7	20	2/2		

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

Guideline levels for CO_2

No ERPG or AEGL-values have been derived for CO_2; an AGW value of 50,000 mg/m^3 (27,500 ppm) and an LBW value of 100,000 mg/m^3 (55.000 ppm) have been derived. The LBW is based on data obtained from the HSDB database indicating that no mortality occurred in humans exposed to 91,500 mg/m^3 (50,000 ppm) for 30 min or up to 183,000 mg/m^3 (100,000 ppm) for 5-10 min.

Spacecraft Maximum Allowable Concentrations (SMACs) have been derived for CO_2 (COT, 1996). SMACs refer to concentrations of airborne substances that will not compromise the performance of specific tasks by astronauts during emergency conditions or cause serious or permanent toxic effects. The underlying document does not describe any relevant acute toxicity study providing mortality data following CO_2 exposure. Reported fatalities after accidental exposures lack proper exposure information. Nevertheless, these data may serve as a quality check to control outcomes of derived exposure-response relations for CO_2.

Discussion and Conclusion

In the previous note by RIVM (Ter Burg and Bos, 2007), it was concluded that there is a need for an accurate acute animal toxicity study (without controlling oxygen levels) for the derivation of an adequate probit-relation for CO_2. Data gathered up until that moment did not provide input for a sufficiently reliable probit function. Instead, semi-quantitative estimates were suggested as a conservative guideline for human exposures up to one hour of exposure:

- no deaths are expected at CO_2 concentrations of up to 5-10 Vol%;
- serious effects and possible mortality may start to occur at about 10-15 Vol%;
- a high level of mortality may occur at about 20-25 Vol%.

Since the previous note by RIVM, one new study was performed, i.e. by Muijser et al., (2009, draft). The acute toxicity study in rats by Muijser et al., (2009, draft) was set up according to the C x t protocol that provides relevant estimates for the derivation of a probit function. The results of the study revealed that CO_2 acute toxicity in the rat is best described by an asymptotic function (see page 2). Separate probit analyses were needed for the concentration ranges of 40-43 Vol% and of 43-50 Vol% to describe the results. The confidence interval for the estimated n-value for the concentration range of 43-50 Vol% was too broad (1.8; 95% CI: -8.6-12.1) to draw conclusions. The estimated n-value for the concentration range of 41-43 Vol% was very high (24.4; 95% CI: 15.6-32.7). The software in the Dutch QRA model uses linear probit functions only and thus cannot deal with an asymptotic function.

An explanation for the observations by Muijser et al. (2009) - indicating an asymptotic function with a threshold for lethality in rats at approximately 40 Vol% CO_2 in the air - cannot be provided yet. Questions were raised whether the lethality threshold and asymptotic description of lethality exist for humans as well and, if so, at what exposure levels. What toxic mechanisms are the driving factors for animal and human lethality?

To answer these questions insight in the physiology of animals and humans is necessary. First, the relevant physiological/haematological parameters that play an important role in CO_2 lethality need to be determined. Possibilities are the PCO_2, the PO_2 and the blood/tissue pH. An additional literature study should reveal the most important parameters. Next, animal

experiments should be performed to determine the relationship between these parameters and CO_2 exposure concentrations and how they are related to mortality. Finally, these findings need to be extrapolated to humans and related to CO_2 concentrations in air. Historical literature concerning non-lethal effects in humans, such as CO_2 use as an anaesthetic might provide valuable input for understanding CO_2 acute toxicity in humans.

The C x t study performed by Muijser et al., did not provide a basis for a probit function for CO_2 but showed that the relation between CO_2 concentration, exposure duration and lethality is complex for this compound. It showed that CO_2 acute toxicity in the rat is best described by an asymptotic function. Further studies are necessary to provide more insight in this relationship before a relationship in humans can be described.

In conclusion, although more insight is obtained in the complexity of the effects following acute CO_2 exposures, a probit function to be used in a QRA for CO_2 can still not be recommended. Therefore, the conservative guidelines as presented above (and in the previous note) are presently considered to be the best option until scientific research provides a scientific basis for sufficiently reliable input for QRA.

Acknowledgement
A draft of this document has been discussed within the Dutch Expert Panel for probit functions as well as two Dutch university representatives working in the area of inhalation physiology. Based on their comments this document has been adjusted.

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