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The effects of and tolerances for Carbon Dioxide in relation to recent developments in laboratory animal housing

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Introduction

During the last decades little focus has been on the deleterious effects of CO_2. The majority of studies within the field were performed before 1970. However, as individually ventilated cage systems (IVS), un-ventilated filter-topped cages and other tightly sealed containment systems are increasing being used and are getting tighter allowing little or no air exchange between the cage and the surrounding environment, it is reasonable also to focus on the impact of CO_2. The totally sealed IVS seems to be the ultimate solution; a system which might not be so far from being available on the market. Especially during failure of the individual cage ventilation this is problematic. At present no official limits for acceptable exposure in laboratory animals have been set, although some papers have discussed the item (*Lipman, 1992; Serrano, 1971*) In certain countries human exposures up to 5000 ppm (0.5%) during an eight-hour working day as well as short term exposures up to 10000 ppm (1.0%) are accepted, and several papers recommend these levels for humans to be applied (*Serrano, 1971*; *Lipman, 1992*; *Reeb et al., 1997*). On the other hand, the effects of CO_2 on animals, as investigated during the fifties and sixties, seem to be quite harmless and reversible. In contrast to e.g. ammonia no studies have so far been able to show any toxicological effects caused by CO_2 (*King et al., 1955*; *Schaefer et al., 1968*).
The present paper summarizes the effects of CO_2 revealed in studies during the fifties and sixties in order to put these into a new perspective in relation to housing animals in new containment systems, such as the individually ventilated cage system, and discusses the physiological impact on the animal and human physiology caused by CO_2 and how the effects of CO_2 may be regarded as a common stressor. Finally, possibilities of dealing with the CO_2 as a parameter and of setting limits for CO_2 exposure in relation to animals are discussed.

Stress and how to monitor it

A stress reaction is rather complex and difficult to analyze and interpret. Stress parameters are such as elevated serum corticosterone (*Barnett & Hemsworth, 1990*) and reduced numbers of eosinophils and lymphocytes (*Bohus et al., 1991*). The normal fight-or-flight-reaction accompanied by the release of adrenaline may also be regarded as a stress reaction (*Dantzer & Mormède, 1983*). All reactions are linked in a chain initiated by the release of adrenaline as a direct reaction to the stressor (*Friend, 1980*).

CO_2 production in animals

Atmospheric CO_2 concentration is approximately 0.03%, which has increased from 280 p.p.m. (0.028%) in AD 1800 to 355 p.p.m. (0.036%) at present (*Oeschger, 1993*). In an un-ventilated cage, e.g. as when removed from the rack for experimental manipulation in the laboratory, the CO_2 concentration inside the cage rises as a result

of the respiration of the animals. A 25 g resting mouse consumes 1.65 ml O_2 per g bodyweight per hour converting 1 ml O_2 to 1 ml CO_2. In a filter-topped Type II cage, i.e. a cage with approximately 350 cm^2 floor area and a total height of 19 cm, in which five 30 g mice are allowed (*Council of Europe, 1986*), the total volume is approximately 6.7 liters. During one hour these five mice generate 250 ml CO_2, which, provided the sealing is complete, leads to a final CO_2 concentration in the cage of 3.7%.

The impact of CO_2 on the animal and human physiology

In the 1950´s and 1960´s studies exposed laboratory animals and humans to different levels of CO_2 ranging from 0.5 up to 15%, during which various physiological effects were monitored.

No studies exposing animals to CO_2 levels below 1.0% seem to be available. In humans exposed to CO_2 levels below 1.0% only minor effects were discovered, such as a 24% increase in respiratory minute volume, which indeed is a rather limited effect. No other effects were observed and during long-term exposure levels normalized after 10 - 15 days (*Glatte & Welch, 1967*).

Animal exposure to CO_2 concentrations in the range between 1.0 and 3.0% caused some physiological deviations. Rats and guinea pigs exposed to 1.5% CO_2 responded by increased adrenal secretion and a drop in adrenal cholesterol and ascorbic acid levels (*King et al., 1955*). The numbers of eosinophils and lymphocytes also significantly decreased in animals exposed to 1.5% CO_2 for 42 days. At return into atmospheric air, i.e. 0.03% CO_2, the physiological parameters normalized within ten days. A recent study was not able to register any change in the respiration parameters in mice exposed to CO_2 concentrations up to 2.7% (*Nielsen et al., 1993*). Exposure of humans to 1.5% CO_2 for 42 days caused some effects on the respiratory system (*Schaefer et al., 1963*). The exposure period could be split into two phases. During the first phase of 23 days the respiratory system did not compensate for the exposure of CO_2, but during the second phase lasting from 24 - 42 days, respiratory acidosis, which had occurred during the uncompensated phase, disappeared after day 24. During all 42 days respiratory minute volume as well as tidal volume were increased but returned to normal after four weeks of recovery in atmospheric air. No changes in performance and control of the central nerve system (e.g. co-ordination and power of concentration) at 1.5% CO_2 exposure could be observed (*Glatte & Welch, 1967*).

The effects of CO_2 become more dramatic in animals exposed to a higher level of CO_2. In animals exposed to 3.0% CO_2 respiratory minute volume and tidal volume increased, while the pH of the blood decreased. Slightly elevated serum corticosteroid levels (*Schaefer et al., 1964*), and reduced adrenal gland adrenaline concentration were also observed (*Schaefer et al., 1968*). Minor hyaline membranes observed in the lung tissue disappeared after only one day of recovery (*Schaefer et al., 1964*). In general, all these effects were reversible and values normalized within few days after returning to atmospheric air.

In humans exposed to 3.0% CO_2 acidosis occurred, but this was compensated after 3 - 4 days of continuous exposure (*Schaefer et al., 1963*). Furthermore, a drop in the number of eosinophils in the blood was observed. Some people noticed deeper breathing, which was accompanied by an increase in both respiratory minute volume and tidal volume (*Glatte & Welch, 1967*). Also serum levels of catecholamines, cortisone and steroids increased. All effects normalized after 10 days of recovery in atmospheric air.

The effect on animals of exposure to high levels, i.e. 15% CO_2, seems in some ways to be similar to exposure to 3.0%, i.e. decreased pH value in the blood, increased respiratory minute volume and tidal volume, drop in the number of white blood cells and an increased corticosterone release. However, changes seem to be more distinct. Some changes were also observed in the lungs, which, however, were all reversible and disappeared after a few days in atmospheric air. Exposure lasting several days was compensated and after four days most of the changes normalized (*Schaefer et al., 1964*).

Studies exposing humans to CO_2 levels higher than 5.0% are uncommon so only few data are

available. Exposure to 7.0% CO_2 caused acidosis, increased respiration, increased stress hormone levels and clinical symptoms such as headache and burning eyes (*Glatte & Welch, 1967*).

Only acute studies are available for animals exposed to a CO_2 level higher than 15%. When animals were exposed to 30% CO_2 for 60 minutes an acidosis during the first minutes was seen as well as an increase in all respiration parameters, adrenaline release, and the level of eosinophils, and a drop in the number of lymphocytes (*Schaefer et al., 1955*). The majority of these effects disappeared after one hour in atmospheric air.

The effects of acute and long-term exposure to CO_2 for both animals and humans are summarized in Tables 1-4.

Use of CO_2 for anesthesia and euthanasia

High concentrations of CO_2 are used as short acting, effective and painless anesthetic for mice, rats and guinea pigs (*Kohler et al., 1999*). A mixture of 80% CO_2 and 20% O_2 induces unconsciousness after 60-120 seconds and results in approximately 60 seconds of anesthesia (*Kohler et al., 1999*). Longer lasting exposure to this mixture or pure CO_2 induces respiratory arrest and death within a few minutes (*Iwarsson & Rehbinder, 1993*). CO_2 has also been used successfully as an anesthetic for women in labor (*Meier, 1994*).

Discussion

The reaction of animals exposed to CO_2 seems to mimic a stress reaction. Both conditions induce a rise in serum corticosterone and adrenaline and reduce the number of circulating eosinophils and lymphocytes. It is, therefore, reasonable to consider exposure to CO_2 as a common stressor comparable to handling or exposure to novel environments. When rodents are handled for a few seconds increased corticosterone and adrenaline levels and a drop in the number of white blood cells are also the observed effects (*Brown & Martin, 1974*; *Seggie & Brown, 1975*; *Landi et al., 1988*), and the same patterns are seen when the animals are exposed to novel environments (*Landi et al., 1982*; *Drozdowicz et al., 1990*; *Tuli et al., 1995*). A wise recommendation after such stress would be a few days of recovery, which might also be the appropriate for animals exposed to CO_2. If animals have been exposed to significant levels of CO_2, e.g. when a cage from an IVS accidentally has been left without ventilation for 45 minutes or more, a few days of recovery prior to experimental use should be allowed to ensure recovery from the impact of the CO_2 exposure.

The question is, however, how to set a limit for acceptable CO_2 exposure. At present it is probably not possible to recommend any strict limits, as no studies are able to show how animals react when exposed to different levels of CO_2 for different periods of time or whether they are able to register low levels of CO_2 (1-5%) at all. It is questionable levels below 1% CO_2 are irreversibly harmful to the animals, or whether it only induces a temporary stress reaction in the animal. As no dramatic effects on the physiology can be observed, little concern for CO_2 levels up to 1.0% seems reasonable. Evolutionary differences between humans and animals exist in relation to CO_2. Humans have evolved and have adapted to a life high above the ground in fresh air, whereas many rodent species have evolved and adapted to a life at least partly underground in tight burrows and surroundings with a limited air exchange. Therefore, human limits may be inappropriate as a recommendation for laboratory animals. Also some differences in the reaction to CO_2 between different species of laboratory animals would be expected, and should be considered when some limits are given. More research is needed to investigate the animals' reactions when exposed to different levels of CO_2 before any solid recommendations can be made.

Until such studies are conducted, animals exposed to a CO_2 level significantly higher than the atmosphere, e.g. above 1.5%, should be used for experimental purpose with caution and allowed a few days of recovery after exposure.

Table 1: The effects of acute exposure to CO_2 on animals (< 24 hours)

CO_2-concentration	Blood pH	Blood-cells	Respiratory function	Humoral hormones	Morphological effects	References
< 1.0%	-	-	-	-	-	
1.0 – 3.0%	-	Eosinophils ↓ Lymphocytes ↓	-	Cholesterol ↓ Ascorbic Acid ↓	None	(*King et al., 1955*; *Nielsen et al., 1993*)
3.0 – 5.0%	pH ↓	Eosinophils ↓ Lymphocytes ↓	Resp.Min.Vol. ↑ Tidal Volume ↑	Adrenaline ↑ Corticosteroids ↗	Reversible formation of hyaline membranes in the lungs	(*Schaefer et al., 1964*)
5.0 – 15.0%	pH ↓	Eosinophils ↓ Lymphocytes ↓	Resp.Min.Vol. ↑ Tidal Volume ↑	Corticosteroids ↑	Reversible changes in lung tissues in the lungs	(*Schaefer et al., 1955*)
> 15.0%	pH ↓	Eosinophils ↑ Lymphocytes ↓	Resp.Min.Vol. ↑ Tidal Volume ↑	Adrenaline ↑	-	(*Schaefer et al., 1955*)

↑ : A raise
↓ : A drop
↗ : A slight raise
↘ : A slight drop
→ : No changes
\- : No data available

Table 2: The effects of long-term exposure to CO_2 on animals (> 24 hours)

CO_2-concentration	Blood pH	Blood-cells	Respiratory function	Humoral hormones	Morphological effects	References
< 1.0%	-	-	-	-	-	
1.0 – 3.0%	pH →	Lymphocytes ↓ Eosinophils ↓	-	Cholesterol ↘	-	(*King et al., 1955*)
3.0 – 5.0%	-	-	Tidal Volume ↑ Resp.Min.Vol. ↑	Corticosterone ↗ Adrenaline ↑	Reversible formation of hyaline membranes in the lungs	(*Schaefer et al., 1964*; *Schaefer et al., 1968*)
5.0 – 15.0%	pH ↓ Comp. after 4 days	Lymphocytes ↓ and followed by ↑ after 4 days	-	Corticosterone ↑ Adrenaline ↑ Compensation after 4 days for both.	Reversible formation of hyaline membranes in the lungs	(*Schaefer et al., 1964*)
> 15.0%	-	-	-	-	-	

↑ : A raise ↘ : A slight drop
↓ : A drop → : No changes
↗ : A slight raise - : No data available

Table 3: The effects of acute exposure to CO_2 on humans (< 24 hours)

CO_2-concentration	Blood pH	Blood-cells	Respiratory function	Humoral hormones	Symptoms	References
< 1.0%	pH →	→	Resp.Min.Vol. ↗	→	None	(*Glatte & Welch, 1967*)
1.0 – 3.0%	pH ↘	→	Resp.Min.Vol. ↗	→	None	(*Glatte & Welch, 1967*)
3.0 – 5.0%	pH ↓	Eosinophils ↓	Resp.Min.Vol. ↑ Tidal Volume ↑	-	Deeper breaths	(*Glatte & Welch, 1967*)
> 5.0%	pH ↓	-	Resp.Min.Vol. ↑ Tidal Volume ↑	Catecholamines ↑ Cortisone ↑	Headache, burning eyes	(*Glatte & Welch, 1967*)

↑ : A raise ↘ : A slight drop
↓ : A drop → : No changes
↗ : A slight raise - : No data available

Table 4: The effects of long-term exposure to CO_2 on humans (> 24 hours)

CO_2-concentration	Blood pH	Blood-cells	Respiratory function	Humoral hormones	Symptoms	References
< 1.0%	pH →	-	Resp.Min.Vol. ↗ Compensation after 10-15 days	→	None	(*Glatte & Welch, 1967*)
1.0 – 3.0%	pH ↓ Comp. after 24 days	-	Resp.Min.Vol. ↗ Tidal Volume ↑	→	None	(*Schaefer et al., 1963*) (*Glatte & Welch, 1967*)
3.0 – 5.0%	pH ↓ Comp. after 3-4 days	Eosinophils ↓	Resp.Min.Vol. ↑ Tidal Volume ↑	Catecholamines ↑ Cortisone ↑ Steroids ↑	Deeper breaths	(*Schaefer et al., 1963*) (*Glatte & Welch, 1967*)
> 5.0%	pH ↘	-	Resp.Min.Vol. ↑ Tidal Volume ↑	-	-	(*Glatte & Welch, 1967*)

↑ : A raise ↘ : A slight drop
↓ : A drop → : No changes
↗ : A slight raise - : No data available

Summary

The present paper summarizes the effects of CO_2 in order to put these into a new perspective in relation to housing animals in new containment systems. During the last decades not much focus has been on the deleterious effects of CO_2, but as tightly sealed containment systems are increasingly being used it is reasonable also to focus on the impact of CO_2 on the animals. At present no official limits for acceptable exposure in laboratory animals have been set, but some papers recommend levels for humans to be applied, although the effects of CO_2 on animals seem to be quite harmless and reversible. In an unventilated filter-topped Type II cage with five mice the CO_2 concentration inside the cage during one hour rises to 3.7%. The reaction of animals exposed to CO_2 seems to mimic a stress reaction. In humans exposed to different levels below 1.0% only minor effects, which normalized after 10 - 15 days, were discovered. Animals and humans exposed to higher concentrations respond by increased adrenal secretion and decreased numbers of eosinophils and lymphocytes. Also respiratory parameters may change. In general, these effects are reversible. Further research is needed to investigate the animals' reactions when exposed to different levels of CO_2 before any solid recommendations can be made. Until such studies have been conducted, animals exposed to a CO_2 level significantly higher than the atmosphere, e.g. above 1.5%, should be used for experimental purpose with caution and allowed a few days of recovery after exposure.

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