Introduction

Carbon dioxide (CO\textsubscript{2}) is widely used in Australia for the euthanasia of wildlife (both native and introduced species) and laboratory animals. Carbon dioxide is relatively safe and easy to use, is cheap and readily available and can be used to kill large numbers of animals within a short period of time. However, there is much scientific debate about the humaneness of using CO\textsubscript{2}. While recent research suggests that it is highly aversive and causes distress in many animal species (e.g. Conlee et al. 2005), in many cases, such as in the laboratory environment or with wild animals, killing animals using a chamber filled with CO\textsubscript{2} is considered by some to be less stressful than other manipulations (e.g. injections) which require individual handling (Hawkins et al. 2006). Thus, there is conflicting evidence in the literature about the degree of pain and distress that animals suffer when exposed to this gas.

Current recommendations

The 2001 ANZCCART (Australian and New Zealand Council for the Care of Animals in Research and Teaching) Guidelines for the Euthanasia of Animals used for Scientific Purposes recommend (with reservations in some cases) CO\textsubscript{2} for killing a number of animal species including rats, mice, birds, cats and dogs. The ANZCCART Guidelines do not consider the use of CO\textsubscript{2} an acceptable method for rabbits, pigs and fish killed for scientific purposes because there is some concern over the welfare implications in these species.

The 2000 Report of the American Veterinary Medical Association Panel on Euthanasia (AVMA 2000) states that CO\textsubscript{2} is acceptable for killing appropriate species which include small laboratory animals, birds, cats, small dogs, rabbits, mink, zoo animals, amphibians, fish, some reptiles and pigs. This report also states that compressed CO\textsubscript{2} gas in cylinders is the only recommended source of CO\textsubscript{2} because the inflow to the killing chamber can be regulated.

Mode of action

Initially, an animal that is exposed to high CO\textsubscript{2} concentrations (60% or greater) experiences dyspnoea (respiratory distress) exhibited by an acceleration in breathing and heart rate as the body tries to increase the amount of oxygen it is receiving. After a short while, excessive amounts of acid build up in the bodily fluids and, finally, the animal goes into cardiac arrest and dies. According to Conlee et al. (2005), a CO\textsubscript{2} concentration of 60% has been observed to have aversive effects in mice including increased locomotion, rearing, defecation and urination – behaviours that often indicate stress. Inhalation of high concentrations of CO\textsubscript{2} is distressing to animals because it results in asphyxia (suffocation) and because the gas combines with moisture in the nasal cavities to form carbonic acid which causes pain and irritation.
Humaneness

Studies of the use of CO$_2$ for killing animals have shown that mice and chickens experience high levels of distress before succumbing to unconsciousness (e.g. Conlee et al., 2005; Niel and Weary, 2006). Death by CO$_2$, at least in these species, cannot therefore be considered entirely painless or stress-free. However, information from the literature suggests that mice and chickens experience unconsciousness in less than one minute of exposure to 100% CO$_2$ and die within two minutes, suggesting that the period of distress due to CO$_2$ exposure at high concentrations is relatively brief (e.g. Fenwick 2001 and Raj 1995).

There are two principle methods of administering CO$_2$: animals are either placed in a chamber that is pre-filled with a high concentration of the gas or they are placed in a chamber in which the concentration of CO$_2$ is gradually increased. Research into the comparative humaneness of these two methods is predominantly limited to the effects on rodent species and the results are not conclusive (see below). The effects of CO$_2$ exposure can vary significantly between species, which means that the most humane method of CO$_2$ introduction cannot be generalised across species.

Anaesthetic agents that cause anoxia (lack of oxygen supply to the body), such as nitrogen, carbon monoxide and argon, can be added to the gas in order to minimise the negative behavioural responses that result from CO$_2$ exposure before the onset of unconsciousness. However, the use of anaesthetic agents is often not possible or practical because it requires sophisticated equipment to closely monitor and regulate the concentrations of the gases in the chamber.

Summary of the pros and cons of using CO$_2$

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>• CO$_2$ has rapid depressant, analgesic and anaesthetic effects</td>
<td>• Concentrations necessary to induce anaesthesia and death vary amongst species, some species such as fish and burrowing animals have a high CO$_2$ tolerance</td>
</tr>
<tr>
<td>• Readily available and can be purchased as a compressed gas</td>
<td>• High concentrations cause distress and pain in many species</td>
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<tr>
<td>• Inexpensive</td>
<td></td>
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<tr>
<td>• Safe to store and poses minimal hazard to personnel</td>
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Findings of the Newcastle meeting on the use of CO$_2$ for laboratory animals

In February 2006, a scientific meeting was organised in Newcastle upon Tyne in the UK with the aim of forming an international consensus on the use of CO$_2$ for the killing of laboratory animals. The following are the major points of consensus that were reached at this meeting (Hawkins et al. 2006):

• There is no ideal method for killing of animals with CO$_2$ as there are welfare concerns associated with both pre-fill and progressively filled chambers.

• Exposing an animal to CO$_2$ can cause distress because sensitive CO$_2$ chemoreceptors and pH receptors have evolved in vertebrates so that elevated CO$_2$ levels are a potent stimulus for respiratory effort.

• CO$_2$ may also cause discomfort or pain as a result of its conversion to carbonic acid on mucosal surfaces.

• Animals will generally try to avoid or escape from an atmosphere containing CO$_2$ levels above a certain threshold limit.

• Any method that involves exposing animals to elevated or increasing levels of CO$_2$ will have welfare implications because exposure to levels of CO$_2$ that will induce anaesthesia or cause death will result in a degree of aversion.
Key points to consider are 1) time to loss of consciousness and 2) what adverse effects do animals experience before loss of consciousness.

Animals placed into a chamber containing concentrations of CO₂ above 50% will experience at least 10-15 seconds of pain prior to loss of consciousness.

If animals are placed into a chamber of rising concentration of CO₂ they will find it aversive once it reaches a certain concentration and may experience dyspnoea, “air hunger” which is known to be very distressing in humans.

It is not yet possible to recommend the use of any suitable alternative gases or gas mixtures that would cause death by hypoxia such as argon, nitrogen, carbon monoxide, helium or xenon for killing rats or mice.

Hypoxia may be a method preferred by some for killing other non-rodent species, but there are insufficient data available in terms of the impact of these gases on rodents.

The use of volatile anaesthetic agents may be an appropriate alternative to CO₂ but the aversive nature of these gases can vary. They can either be used as the sole euthanasia agent or they may be used to anaesthetise animals prior to completing the process by switching to CO₂.

More research is needed into the physiological and affective responses to a range of gaseous agents in order to identify best practice and/or possible alternatives to CO₂.

Overall, the Newcastle meeting served to highlight two very significant areas of concern relating to the use of CO₂. Firstly, while there is some information on the effect of CO₂ on rodent species, there is a dearth of information about its effects in other animal species and secondly, there is almost no research being conducted into alternative and potentially more humane methods of killing animals.

Conclusion

Research into the humaneness of CO₂ as a method for killing animals has shown that its effects prior to death are aversive. However, it is important to consider the humaneness of CO₂ in the context of the other methods that are currently available for killing animals, especially in large numbers, which is the principle reason for using CO₂. Although information on the time to death by CO₂ is not available for many species, studies on animals such as chickens and rodents have shown that unconsciousness occurs within one minute in the presence of 100% CO₂ with death under these conditions occurring within one to two minutes.

It is clear that, when exposed to high concentrations of CO₂, most animals experience high levels of distress and discomfort prior to unconsciousness. However, most alternative methods for killing require animals to be handled individually which can also lead to suffering and distress before death. In considering the use of CO₂ as a method for killing it is therefore a question of relative humaneness. What has become clear is that, whilst there are welfare concerns over the use of CO₂ for killing, there is little evidence available to clearly recommend an alternative at the present time. The use of other gases or gas mixtures, or the addition of anaesthetic agents, such as halothane, to CO₂ may provide a more humane solution, but more research on the effects of these alternatives on different species is needed to confirm this.

This issue highlights the importance of encouraging research into developing alternative and more humane methods of euthanasia, particularly into the effects of alternative gases or gas mixtures as well as practical delivery methods which incorporate anaesthetic agents.
References


Hawkins, P. et al. (2006). *Newcastle meeting on carbon dioxide euthanasia of laboratory animals. 27th and 28th February Newcastle upon Tyne, UK.*


Other useful references


http://www.hsus.org/animals_in_research/pain_distress/concerns_about_carbon_dioxide_use_in_euthanasia_and_anesthesia/