Carbon Dioxide Use for Euthanasia of Laboratory Animals

This document reviews the current scientific literature on the use of carbon dioxide (pure and various mixtures) for euthanasia of laboratory animal species. It outlines the various issues that Victorian Animal Ethics Committees should consider when making recommendations to their institutions.

Authors: Dayna Johnson, Kate Blaszak: Bureau of Animal Welfare, DPI Victoria
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Summary:

The literature on the use of carbon dioxide for inhalation euthanasia of laboratory animals can be considered separately for rodents and poultry. It discusses measurement of aversion to carbon dioxide at different concentrations and with different methods of use, the importance of user training and the variation amongst existing international guidelines. Recent literature recommends the use of halothane for rats and enflurane for mice as the most humane methods of inhalation euthanasia. For chickens a mixture of 60 % argon and 30% carbon dioxide mixture in air is considered to be the most humane method of inhalation euthanasia.

The Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits states that euthanasia by inhalation for rats and mice using carbon dioxide is an ‘acceptable’ method. The use of halothane, isoflurane and methoxyflurane is stated as ‘acceptable with reservations’. The reservations are based on occupational health and safety considerations that can be overcome with the use of appropriate scavenging equipment. Based on the literature, the promotion of the use of these inhalation agents as the preferred method of euthanasia is advisable.

A table of recommendations of various euthanasia methods for adult rodents and experimental needs based on existing literature is provided. The literature also indicates that gaseous euthanasia in the home cage amongst familiar conspecifics has a measurable welfare benefit.

Introduction

Carbon dioxide is commonly used to euthanase adult rats, mice and chickens in the laboratory setting, the focus species of this review. Its use is a subject of debate within the animal research community. Human studies report that carbon dioxide inhalation is adversive, with a linear relationship between concentration and distress and/or pain sensation at concentrations ranging from 7-100%(HSUS 2002). This has raised concern as to the humaneness of the use of carbon dioxide for animals, which has been the subject of numerous studies and recent literature reviews.

Scientific Literature On The Use and Effects Of Carbon Dioxide

Reviewing the actual physiological mechanism of excessive carbon dioxide helps to understand the associated clinical signs, which may vary between species and individuals to some degree. The primary mechanism of anaesthesia and death is the direct action of carbon dioxide on vital systems (Pritchett et al, 2005). Simplified, the gas molecule essentially diffuses into the blood, body and brain from the lungs. Faced with excessive levels of the gas, the inherent capacity of the blood to buffer for carbon dioxide is overwhelmed and results in acidosis (the lowering of the pH of the blood and associated fluids). Low to moderate concentrations of carbon dioxide (ranging from 5-35%, Conlee et al, 2005) cause mild respiratory acidosis leading to a compensatory increase in depth and rate of respiration in an effort to 'blow off' the excess carbon dioxide (hyperventilation), changes in heart rate and blood pressure. Higher concentrations then lead to more profound respiratory acidosis, suppressing the respiratory centres of the brain leading to a slow, gasping respiratory pattern. Without the buffering capacity of blood, the pH of the cerebrospinal fluid (CSF) drops precipitously which is directly related to anaesthetic depth and subsequent insensibility to pain, stupor and finally unconsciousness and death. In addition, another mechanism is the acidosis-induced depression of heart muscle, precipitating heart arrhythmias and failure.

Of welfare interest, however, is the duration and effect prior to insensibility and unconsciousness (ie. anaesthesia). A number of articles refer not only to the sensation and signs of respiratory distress and asphyxia (including some histological indications of a state similar to conscious drowning) but also to the acidification of mucous membranes (eg. eyes, mouth, respiratory). The particular sensory capacity of nasal mucosa associated with degrees of discomfort and pain is reported in humans and animals (Conlee et al 2005).

The literature on the use of carbon dioxide in rats and mice differs to that of chickens due to behavioural differences between rodents and chickens and also the methods of killing of poultry for food production. Methodological limitations create the tendency to compare various inhalation agents, rather than to compare other methods of euthanasia or aspects of the euthanasia process. The earlier studies involving rodents vary in the methods used to assess welfare whereas the most recent and vigorous studies include exploratory behaviour in order to gain insights into the least aversive agents.

In comparison the literature on chicken euthanasia is more extensive due to their use as a food producing species. The nature of the research is mostly applicable to the food industry but still useful when considering the laboratory setting. This literature has moved beyond behavioural indicators, which indicated that carbon dioxide is aversive. It currently focuses on the effectiveness of less aversive agents and mixtures using spontaneous electroencephalograms (EEGs) and somatosensory evoked potentials (SEPs) as indicators of levels of consciousness and the establishment of death.

Rodents

Rodent studies have investigated behavioural, physiological and histological changes associated with carbon dioxide use. They have examined the effects of different concentrations, adding oxygen and the use of pre-filled chambers versus gradual induction. Observations that may be consistent with pain and/or distress that have been reported with carbon dioxide use include: increased locomotion, excitation and serious agitation, increased rearing, defecation and urination, irritation of mucosal membranes (including frequent washing), hyperventilation, gasping and 'heads turned upwards and backwards'. These signs generally increase in frequency and intensity with the increase in concentration of carbon dioxide from 25-100%, until anaesthesia is induced. Studies considering histological effects are comprehensively reviewed in Conlee et al, 2005.

Recent literature on rats and mice concludes that carbon dioxide is the least preferred inhalation agent when compared to halothane, isoflurane, enflurane, desflurane, sevoflurane and argon. These species show aversion to carbon dioxide at concentrations high enough to cause a loss of consciousness. Variations in use including humidification, mixture with argon or oxygen, the use of pre-filled chambers versus gradual increase in concentration all failed to remove the aversive effects (Leach et al 2002a; Leach et al 2002b). The degree of averseness of the previously mentioned inhalation agents was established by evaluation of the willingness of rodents to enter and dwell in chambers containing these agents. The only agents the authors concluded were non aversive are halothane and sevoflurane for rats. All of these inhalation agents had some degree of aversion in the case of mice. The authors concluded (Leach & Morton 2004):

> 'Therefore, based on the findings of these studies the recommended anaesthetic agent for rats is halothane and for mice is enflurane as at appropriate concentrations they induced a rapid and effective induction with the minimum of distress. The recommended method of euthanasia using a single agent would be argon. However, inducing unconsciousness with a volatile liquid anaesthetic (eg halothane and enflurane) and subsequently rapidly killing with carbon dioxide after the animals are unconscious can be considered more humane than argon alone. As once an animal is unconscious then exposure to carbon dioxide, which is an effective killing agent, is not a welfare issue.'

Although outside the scope of this report, the impact on animal welfare by the manner in which inhalation agents are used is deserving of attention. There is evidence to suggest that the use of the home cage and inclusion of a familiar companion animal may offer a significant welfare advantage during the euthanasia process (Maguire & Arthur 2003). This is consistent with the broader literature on stress in animals. The use of telemetry and new technologies may see increased research specifically investigating these sorts of parameters during the euthanasia process (Williams 2004).

Chickens

The specialised anatomy and physiology of the avian respiratory system, which is highly adapted for efficient gaseous exchange, makes poultry extremely sensitive to inhaled gases and therefore makes it vital that inhalation methods are demonstrated to be humane. Carbon dioxide in older and adult birds is currently still a commonly used technique for the euthanasia of large numbers of poultry, although not permitted in UK slaughterhouses since 1995 (NCCAW 2005). There is substantial recent international review of controlled atmospheric stunning (CAS) techniques, a commercial killing practice of chickens which provides an alternative to the most common process involving electrical stunning or the use of carbon dioxide alone. There are a number of types of gas mixtures that stun birds by different mechanisms involving various combinations of carbon dioxide, inert gases (Argon or Nitrogen) and oxygen or air, and the mixtures must be carefully controlled. Choosing between these mixtures is arguably one of the most contentious questions about CAS (Wathes 2004).

The use of EEG and SEP technology has provided evidence to suggest that carbon dioxide inhalation is more aversive to chickens than argon and argon-carbon dioxide mixes (Raj et al 1998). The use of 90% argon in air results in greater time to isoelectric EEG and cessation of SEPs consistent with loss of consciousness and death compared to 60% argon and 30% carbon dioxide in air. Residual oxygen levels when euthanasing chickens with 90% argon in air are critical as levels of greater than 2% oxygen can increase the time to death and decrease mortality rates substantially (Raj & Whittington 1995). This critical effect of residual oxygen does not occur in the 60% argon and 30% carbon dioxide in air mixture when euthanasing batches of chickens (Raj et al 1992).

There are however, still some differing opinions on the interpretation of some welfare indicators of inhalation euthanasia of chickens. Most experts and recent studies consider specific behaviours such as gasping, head shaking, wing flapping, defecation as indicators of aversion, but some still consider them autonomous (involuntary) responses. Further research results are pending. It is also hoped that a new statistical technique, validated in human anaesthesia may provide further information of the complex EEG wave and its extent to which it indicates level of consciousness (Wathes 2005).

Guidelines, Codes and Scientific Review Bodies

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes advises that decisions regarding the animal's welfare must be based on the assumption that where pain and distress cannot be easily evaluated in animals, it must be assumed that animals experience pain in a similar manner to humans unless there is evidence to the contrary. This principle is in line with the U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals. In light of these policies and the reviewed body of scientific literature, various international guidelines would appear to be in conflict on the use of carbon dioxide as a sole euthanasia agent as follows:

The 2001 Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) guidelines on the Euthanasia of Animals for Scientific Purposes recommends the use of carbon dioxide with specialised equipment for rodents, preferring gradual induction but acknowledging and accepting the variation in information and thus methodology of carbon dioxide use. Based on these guidelines, the Australian Veterinary Association (AVA) policy also currently 'approves' such carbon dioxide application. The American Veterinary Medical Association (AVMA) 2000 Panel Report on Euthanasia recommends unreferenced) 70% CO_2 in a pre-filled chamber for the euthanasia of rodents, similar to a 1996 European Commission report. The UK Home Office Code of Practice (1997), which is currently being reviewed, indicates that carbon dioxide is appropriate only for rodents, rabbits and birds up to 1.5 kg and recommends exposure to a rising concentration. The UK University Federation of Animal Welfare (UFAW) and Canadian Council for Animal Care (CCAC) acknowledge that neither slow nor swift carbon dioxide induction is stress-free (HSUS 2002). One author comments on behalf of 2 independent scientific review committees (Morton,2005):

> 'the overwhelming consensus of published work in animals including humans shows clear evidence of aversion. This is the view arrived at by both the EU European Food Standards Authority's Scientific Panel on Animal Health and Welfare as well as the UK's Farm Animal Welfare Council'.

McDonald's Animal Welfare Team have also recently considered the scientific literature concerning the existing methods of stunning and slaughter of poultry and is moving towards the use of controlled atmospheres using inert gases (McDonald's 2005). The (Australian) National Consultative Committee for Animal Welfare reviewed methods of poultry euthanasia in 2005. This report acknowledges that there is an increasing discrepancy between the more recent scientific literature and some international policies and recommendations (NCCAW 2005).

Conclusions:

The evidence of distress and aversive responses associated with the use of carbon dioxide as an anaesthetic agent or as a sole agent for the euthanasia of laboratory animals is considered conclusive by a number of independent review bodies and recent scientific studies. Leach et al (2004) specifically conclude that exposing rats and mice to carbon dioxide in any form, either for anaesthesia or for euthanasia, is likely to cause considerable pain and distress and is therefore unacceptable when efficient and more humane alternatives are readily available.

It is recommended that carbon dioxide be coupled with inhalant pre-anaesthetic gases, such as isoflurane, halothane or methoxyflurane if used for euthanasia. The use of other gaseous combinations for euthanasia of rodents or chickens, as discussed, are also preferred over the sole use of carbon dioxide. It should be noted that argon/oxygen/air and various argon/carbon dioxide gas mixtures are commercially available from BOC Gases Ltd.

Proper training of personnel in all techniques involving euthanasia is also fundamental. Importantly the welfare of rodents and chickens undergoing inhalation euthanasia could be further enhanced by drafting institutional recommendations as to what constitutes preferred practical procedure(s).

In summary, it is recommended that the scientific community carefully reconsider the routine use of carbon dioxide as a sole agent for anaesthesia or euthanasia and adopt these more humane practices wherever possible.

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Table comparing recommendations for various euthanasia methods for rodents, based on existing literature (HSUS 2002, Conlee et al 2005).Note this table does not include the use of injectable anaesthetics or barbiturates for euthanasia which are considered acceptable by the Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits.

	CO2 alone	*Pre-anaesthetic &Co2	Argon/O2	**Decapitation	Cervical Dislocation
Large numbers surplus rodents	No	Yes	Yes	No	No
Few-moderate numbers of rodents on experiments	No	Yes	Yes	No	Yes***
Few-moderate numbers of rodents where no contamination is permitted	No	No	Maybe	When Justified	Yes***

*Pre-anaesthetic refers to the gaseous agents, as mentioned in the conclusion, with appropriate scavenging equipment.

** Decapitation produces a quicker loss of consciousness (3-6 seconds) than sole carbon dioxide use. With regard to the adverse impact on the animal, however, the entire process from handling to execution must be considered. This method may be considered acceptable to be carried out by trained personnel for a small number of small rodents and chickens where other resources are currently unavailable (Conlee et al, 2005).

*** Authors' note: The original table does not recommend cervical dislocation for any category. There is limited published information on cervical dislocation, but it is accepted that this method does not induce instantaneous unconsciousness and is very reliant on operator technique and skill (Gregory and Wotton, 1990). In combination with this discussion and the Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits, cervical dislocation may be considered acceptable for small numbers of rodents less than 150 grams conducted by a skilled operator.

References

Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, seventh edition. National Health Medical Research Committee, 2004

Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART). Euthanasia of Animals Used for Scientific Purposes: second edition. ANZCCART 2001

Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits. Victorian Department of Primary Industries. 2004

Conlee. K.M., Stephens M.L., Rowan A.N. and King L.A. 2005. Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. Laboratory Animals 39(2):137-161

Gregory. N.G, and Wotton. S.B, 1990 Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. Veterinary Record 126: 570-572

Humane Society of the United States (HSUS) 2002. Carbon dioxide for euthanasia and anaesthesia: Concerns regarding pain and distress. Cited 4 August 2005. http://www.hsus.org/ace/11427

Leach M.C., and Morton D.B., 2004 How humane is induction with common agents of anaesthesia and euthanasia in laboratory rodents? ANZCCART News 17(3): 1-3

Leach M.C., Bowell V.A., Allan T.F., and Morton D.B, 2004 Measurement of aversion to determine humane methods of euthanasia. Animal Welfare 13: S77-86

Leach M.C., Bowell V.A., Allan T.F., and Morton D.B, 2002a Aversion to gaseous euthanasia agents in rats and mice. Comparative medicine 52(3): 249-257

Leach M.C., Bowell V.A., Allan T.F., and Morton D.B, 2002b Degrees of aversion shown by rats and mice to different concentrations of inhalational anaesthetics. The Veterinary Record 150: 808-815

Maguire S., Arthur S., 2003 Influence of exposure cage, method of carbon dioxide exposure and pairing on cardiovascular parameters, activity, and stress related plasma values to carbon dioxide anaesthesia and euthanasia in rats. Proceedings of American Association of Laboratory Animal Science Conference, Seattle

Morton D.B. 2005 Letters. Comparative Medicine, 55 (1): 11

National Consultative Committee for Animal Welfare (NCCAW) Review: Methods of Poultry Euthanasia. Prepared by Jacobsen N and Hood J. 2005

Pritchett K., Corrow D., Stockwell J., Smith. 2005. Euthanasia of neonatal mice with carbon dioxide. Comparative Medicine. 55 (3): 275-281

Raj A.B., Whittington P.E. 1995 Euthanasia of day-old chicks with carbon dioxide and argon. The Veterinary Record. 136(12): 292-4

Raj A.B., Wotton S.B., McKinstry J.L., Hillebrand S.J., Pieterse C., 1998 Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of broiler chickens during exposure to gas mixtures. British Poultry Science 39(5): 686-95

Raj A.B., Wotton S.B., Whittington P.E., 1992 Changes in the spontaneous and evoked electrical activity in the brain of hens during stunning with 30 per cent carbon dioxide in argon with 5 per cent residual oxygen. Research in Veterinary Science. 3(1): 126-9.

United Kingdom Home Office. Code of Practice for the Humane Killing of Animals under Schedule 1 of the Animals (in Scientific Procedures) Act 1986.1997. Cited 3 March 2004 http://www.homeoffice.gov.uk/docs/hc193.html

Wathes C. 2005 Controlled atmosphere stunning of poultry: an integrated approach (comment on a Report of a workshop held at Silcoe Research Institute in June

2004 on Controlled Atmosphere Stunning of poultry) Animal Welfare 2005: 14 : 83-84

Williams V., 2004 The use of carbon dioxide for euthanasia and anaesthesia. Surveillance 31(4): 28-30