



The ACC Connection

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WELCOME to the seventh edition of the **ACC Connection**, a bi-monthly newsletter designed to help researchers with questions regarding animal research at the University of Connecticut Health Center. Our seventh issue is going to discuss the issues and concerns surrounding the use of carbon dioxide (CO₂) as a method of euthanasia.

Background

The humane use of laboratory animals is an issue of great importance to the scientific community. This includes the humane euthanasia (derived from Greek terms meaning “good death”) of the laboratory animals used in biomedical research. *The 2000 Report of the American Veterinary Medical Association Panel on Euthanasia* lists numerous euthanasia methods currently approved for use with various laboratory animals. In general, the AVMA finds euthanasia methods to be acceptable if they result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function and if they minimize distress and anxiety experienced by the animal prior to the loss of consciousness recognizing the fact that the absence of pain and distress cannot always be achieved. Federal and institutional regulations stipulate that methods of euthanasia must be approved by the *AVMA Panel on Euthanasia* report unless a deviation is scientifically and/or medically justified and approved by the institution’s IACUC.

Also to be considered when determining the method of euthanasia are the human psychological responses to euthanasia of the animals. Researchers, technicians, and students may become attached to the animals that must be euthanized. This may be particularly hard if any individuals feel responsible for allowing an animal’s medical problem to go unattended which results in the euthanasia of a sick animal. There are ethical implications to be considered when euthanasia involves healthy animals that are not needed during the course of the experiment being performed. Distress experienced by individuals involved with the euthanasia in this type of situation may be minimized by humane euthanasia of the animals.

Euthanizing agents cause death by three basic mechanisms: (1) hypoxia; (2) direct depression of neurons necessary for life; and (3) physical disruption of brain activity and destruction of neurons necessary for life.

In February 2006, a meeting was held at Newcastle Upon Tyne University, in the United Kingdom, which was comprised of a series of presentations that summarized the progress made to date by key researchers in the field of inhalant euthanasia. This was followed by discussion session that addressed how CO₂ should best be administered, possible alternative euthanasia agents, and future research directions. This will address some of the concerns detailed by that meeting. A report of this meeting can be found on the ACC website at <http://clacc.uhc.edu/ACC/DrugDosage.htm>.

Carbon Dioxide (CO₂) Euthanasia

Room air contains 0.04% CO₂ which is heavier than air and nearly odorless. Inhalation of CO₂ at a concentration of 7.5% increases the pain threshold and higher concentrations have an anesthetic effect. Signs of effective CO₂ anesthesia are those associated with deep surgical anesthesia, such as loss of withdrawal reflexes. CO₂ has been used to euthanize laboratory animals including mice, rats, guinea pigs, and rabbits. The use of CO₂ is usually performed in specially designed chambers. Currently, there is no definitive guidance on whether and how CO₂ can be administered humanely. There is also uncertainty about the feasibility of using alternative gaseous euthanasia agents (e.g., nitrogen, argon, carbon monoxide) and inhalant anesthetics with respect to both animal welfare and human health and safety.

Advantages of CO₂

There are advantages of using CO₂ as a method of euthanasia for appropriate species. It is a rapid depressant and its analgesic and anesthetic effects are well established. It is readily available and can be purchased in compressed gas cylinders. It is inexpensive, nonflammable, non-explosive, and poses minimal hazard to personnel when used with properly designed equipment. It does not distort urine cholinergic markers or corticosterone concentrations.

Disadvantages of CO₂

There are disadvantages of using CO₂ as well. Because CO₂ is heavier than air, incomplete filling of a euthanasia chamber may permit animals to climb or raise their heads above the higher concentrations and avoid exposure. Some species may have extraordinary tolerance for it. It may take longer than euthanasia by other means. One of the biggest concerns is that high concentrations of CO₂ may be distressful to animals prior to their death.

Use of CO₂ Euthanasia Recommendations

The acceptability of using CO₂ as a method of euthanasia is dependant upon a number of factors:

Verification of death- Death must be verified after euthanasia and prior to disposal (e.g., by cervical dislocation, decapitation, thoracic trans-section, etc.). Unintended recovery constitutes serious non-compliance with the PHS Policy and is reportable to the Office of Laboratory Animal Welfare (OLAW).

Use of Compressed CO₂ gas in cylinders- Compressed CO₂ gas in cylinders is the only allowable source of CO₂ because inflow to the chamber can be regulated. CO₂ generated by other methods (e.g., dry ice, fire extinguishers, chemical means, etc.) is unacceptable.

No overcrowding of the euthanasia chamber- Species should be separated and chambers in which the euthanasia is performed must not be overcrowded.

Debate Regarding the Use of Carbon Dioxide (CO₂) Euthanasia

Pre-filling of euthanasia chamber versus rising concentrations of CO₂

There is no "ideal way" of killing animals with CO₂- both pre-fill and rising concentrations may cause animal welfare problems. Exposing animals to CO₂ can cause distress because sensitive CO₂ chemoreceptors in pH receptors have evolved in vertebrates with the result that CO₂ is a respiratory stimulant that rapidly induces dyspnea. It can also cause pain or discomfort because it is converted to carbonic acid in the mucosa of the eyes, nose, and mouth. Research has shown that animals placed into a chamber containing >50% concentration of CO₂ will experience at least 10 to 15 seconds of pain in the mucosa of the upper airways before loss of consciousness. If animals are placed into a chamber with a rising concentration of CO₂, they will find it aversive at a certain level and may experience dyspnea which is unpleasant. A general consensus, and the recommendation of the AVMA, is to have rising levels of concentration of CO₂ rather than a pre-filled chamber feeling that it may be better to avoid or minimize pain and distress than it is to ensure a rapid loss of consciousness. High concentrations of CO₂ may be distressful to some species; accordingly, pre-filling of the chamber is not recommended by the AVMA. As conclusive data is not available for all species, it is federally mandated that IACUCs and veterinary staff keep abreast of current peer-reviewed scientific literature on this issue.

Optimum filling rate of euthanasia chamber

The optimum filling rate is uncertain. The use of 100% CO₂ at a flow rate of 20% of the chamber volume per minute has been shown to produce loss of consciousness without evidence of pain but not without evidence of dyspnea. Reduced flow rates can be increased once animals have lost consciousness. The rising concentration technique should not cause pain, but may cause distress. It was recognized that there may be some practical limitations to using slow flow rates; namely, when large numbers of animals need to be euthanized.

Concomitant use of oxygen (O₂)

It is possible that the addition of O₂ to CO₂ may reduce, but not overcome, animal welfare problems caused by pain or dyspnea. It is also possible that high O₂ would prolong consciousness, which may not be desirable. There is currently insufficient information in the literature to reach a clear conclusion on the appropriate level of O₂. Some studies have suggested that the addition of O₂ to CO₂ has made CO₂ euthanasia less aversive to birds, but can this be extrapolated to other vertebrate animals? The addition of O₂ to CO₂ has been shown to reduce agitation and gasping in the rat, but it is not known if the addition of O₂ results in a more humane euthanasia.

Alternative Gaseous Euthanasia Agents

Possible alternative inhalation agents to CO₂ can be divided into two groups: (1) gases that cause hypoxia, and (2) volatile anesthetic agents. Each alternative has its own animal welfare, practical, economic, and human safety issues.

Use of gases such as argon, nitrogen, and carbon monoxide (CO)

In the case of gases that can cause hypoxia, the literature is not sufficiently comprehensive to enable a judgment on the suitability of these gases for rats and mice. It has been shown that nitrogen and argon can be distressful to rats. This method of euthanasia using nitrogen and argon is currently deemed to be conditionally acceptable by the AVMA if O₂ concentrations of <2% are achieved rapidly and animals are heavily sedated or anesthetized. This requirement may result in a delayed death. The use of CO as a method of euthanasia is considered to be acceptable by the AVMA provided that commercially compressed CO is used and appropriate safety precautions are taken.

Use of volatile anesthetic agents

There can be issues with respect to using volatile anesthetics for euthanasia. Euthanasia with these agents takes some time and the suitability of a particular agent depends on whether an animal experiences distress between the time it begins to inhale the agent and the time it loses consciousness. Inhalant anesthetics may be valuable for the euthanasia of smaller animals and many (such as isoflurane and sevoflurane) are nonflammable and non-explosive under ordinary environmental conditions. The AVMA considers the use of these agents to be acceptable for small animals as long as they are used with appropriate safety precautions.

Future Research

It is generally felt that the use of CO₂ for the euthanasia of laboratory animals will be considered to be acceptable for the foreseeable future. However, experts feel that more research is needed into the physiological and affective responses to a range of gaseous agents to identify possible alternatives to CO₂ and define good practice for euthanizing with CO₂. This will require a multidisciplinary approach and communication between researchers. There is an inherent ethical dilemma in conducting studies that may cause pain and distress in animals in order to improve the well-being of other animals. In addition, in the studies that have been done, the studies have focused on rats and other laboratory animals, not mice. This needs to be addressed because extrapolation of data from rats or other animals to mice may not always be appropriate.

Frequently Asked Questions

How should I euthanize my animals by CO₂?

At this point, animals should be euthanized by performing a slow-fill of the euthanasia chamber using compressed gas from a cylinder. Death needs to be confirmed by cervical dislocation, decapitation, or thoracic trans-section.

I have noticed animals being alive after CO₂ exposure and prior to disposal. What should I do?

This is a difficult situation- you believe the animals are dead and are ready to place them in the freezer when you notice a wriggling in the disposal bag. OLAW considers this to be a serious non-compliance issue and this must be reported to the ACC which, in turn, must report this to OLAW. This only happens when no confirmation of death has been performed by the individual performing the euthanasia.

If you have questions you'd like to see answered in future issues, please send them to pohl@uchc.edu and we will do our best to answer as many questions as possible.

Upcoming Training, March 2007 – April 2007

New Animal Users Initial Basic Core Training

Monday, March 19

9:00 am – 12:00 pm

Building 20 conference room

Monday, April 23

9:00 am – 12:00 pm

Building 20 conference room

New Institutional, State, or Federal Regulations

Institutional

ACC Policy 02/22/07 Revised policy on Cage Density. To view policy, go to: <http://clacc.uchc.edu/ACC/Policies/CageDensity.htm>

ACC Policy 02/22/07 Policy on Post Approval Monitoring. To view policy, go to: <http://clacc.uchc.edu/ACC/Policies/PAM.htm>

State

None

Federal

Animal Enterprise Terrorism Act 11/13/06 Signed into law by President Bush



Next Issue: Occupational Health Program

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