

TITLE: Euthanasia of Mouse and Rat Fetuses and Neonates

PURPOSE: It was brought to the attention of the IACUC that there has been a great deal of discussion and controversy surrounding the use of CO₂ to euthanize mouse and rat fetuses and neonates because they seem to show extraordinary tolerance for CO₂ and thus it is generally considered a less effective means to quick death. The primary concern is with regard to the potential for mental distress for the animals undergoing this method of euthanasia.

The National Institutes of Health developed Intramural Guidelines for the Euthanasia of Mouse and Rat Fetuses and Neonates which does not allow for euthanizing animals younger than 14 days with CO₂.

A summary of CompMed discussions indicated that many researchers consider the fetal and neonatal rats and mice insufficiently developed to properly be euthanized by CO₂, and thus have instituted policy utilizing other forms of euthanasia. Methods most used were decapitation – after chilling or other pre-sedation, or barbiturate overdose.

University Animal Care Method for Euthanizing Rodent Neonates:

University Animal Care has a lab animal technician assigned to euthanize between 100 and 1000+ rodents daily. A change from the utilization of the CO₂ as the primary method of euthanasia would comprise difficulties with regard to man hours needed to individually handle each animal for barbiturate injection or physical euthanasia. Additionally, alternative methods of euthanasia would require significant special handling training, but would require the hiring of an additional vet tech.

Dr. Sanders personally witnessed a number of rodent neonates being euthanized by CO₂ and noted that the animals showed no symptoms of pain or suffering.

University Animal Care has filed a Waiver request to be authorized to continue using CO₂ euthanasia for fetal and neonate mice and rats.

REVIEW/REVISIONS: Permanent amendment/revisions to this policy must be presented to the IACUC for review before implementation and should be developed by the IACUC Office and/or IACUC membership.

EFFECTIVE DATE: 6/8/00; **Revised/Re-Approved:** 12/7/00, 11/2/06, November 1, 2007

POLICY/PROCEDURES:

IACUC DECISION:

For research personnel: No changes or additions will be made to the recommendation from the 2007 AVMA Panel on Euthanasia noted (**The Recommendation of the 2007 AVMA Panel on CO₂ Euthanasia is attached**)

For University Animal Care: Waiver request to allow for Co2 euthanasia of mouse and rat fetuses and neonates granted.

The AVMA Panel does note that CO₂ concentration for neonates should be especially high. A CO₂ concentration of 60% to 70% with a 5-minute exposure time appears to be optimal.

2007 AVMA Panel Guidelines for Euthanasia of Rodents Using Carbon Dioxide

Rodents must be euthanized by trained personnel using appropriate technique, equipment and agents. This is necessary to ensure a painless death that satisfies research requirements. Death should be induced as painlessly and quickly as possible. Upon completion of the procedure, death must be confirmed by an appropriate method, such as ascertaining cardiac and respiratory arrest or noting an animal's fixed and dilated pupils (1). Euthanasia should not be performed in the animal room. The euthanasia method must be appropriate to the species, approved in the animal study proposal and conform to the most recent Report of the AVMA Panel on Euthanasia (2). CO₂ inhalation is the most common method of euthanasia used at NIH for mice, rats, guinea pigs and hamsters. A few important aspects of this procedure are:

1. The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber: all animals in the chamber must be able to make normal postural adjustments.
2. Compressed CO₂ gas in cylinders is the only recommended source of carbon dioxide as it allows the inflow of gas to the induction chamber to be controlled. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% carbon dioxide at the rate of 10-20% of the chamber volume per minute so as to optimize reduction in distress. (For a 10-liter volume chamber, use a flow rate of approximately 1-2 liter(s) per minute.) After the animals become unconscious, the flow rate can be increased to minimize the time to death. Sudden exposure of conscious animals to carbon dioxide concentrations of 70% or greater has been shown to be distressful (3).
3. Animals should be left in the container until clinical death has been ensured. Unintended recovery must be prevented by the use of appropriate CO₂ concentrations and exposure times or by other means.¹
4. Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂ therefore, alternative methods are recommended (4). Carbon dioxide may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades). Keeping neonates warm during CO₂ exposure may decrease the time to death (5).
5. If an animal is not dead following CO₂ exposure, another approved method of euthanasia (e.g. decapitation) must be added while the animal is under CO₂ narcosis to assure death. Please refer to Appendixes 1 and 2 of the Report of the AVMA Panel on Euthanasia (2) for additional recommended methods.

References

1. NIH Guide for Grants and Contracts. 7/17/2002, notice: OD-02-062. <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html>
2. AVMA Guidelines on Euthanasia. 2007 Report of the AVMA Panel on Euthanasia. [<http://www.avma.org/resources/euthanasia.pdf>]
3. Danneman PJ, Stein S, Walshaw SO. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. *Lab Anim Sci* 1997, 47:376-385.
4. Guidelines for the Euthanasia of Rodent Feti and Neonates. NIH Animal Research Advisory Committee, 2004 [<http://oacu.od.nih.gov/ARAC/euthmous.pdf>]
5. Klaunberg BA, O'Malley J, Clark T, Davis JA. Euthanasia of Mouse Fetuses and Neonates. *Contemp Top Lab Anim Sc* 2004, 43:(5) 29-34.

Approved - 9/12/01

Revised - 10/9/02

Revised - 10/13/04