



### Policy on Rodent Euthanasia Using Carbon Dioxide

*Euthanasia* is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. According to the *Guide for the Care and Use of Laboratory Animals* (1), methods of euthanasia should be consistent with the *AVMA Guidelines on Euthanasia* (2), unless a deviation is justified for scientific or medical reasons. Carbon Dioxide (CO<sub>2</sub>) inhalation is the most common method of euthanasia used for rodents. In addition to the Guide and the AVMA Guidelines, the NIH Office of Laboratory Animal Welfare (OLAW) (3) has also provided specific guidance on the use of CO<sub>2</sub> to euthanized rodents. The UGA Institutional Animal Care and Use Committee (IACUC) has adopted the following policy to conform to this guidance and to ensure effective euthanasia of rodents used in research.

Rodents must be euthanized by trained personnel using appropriate technique, equipment and agents. This is necessary to ensure a death without pain or distress that satisfies research requirements. As a general rule, a gentle death that takes longer is preferable to a rapid, but more distressing death. The euthanasia method must be appropriate to the species and approved in the Animal Use Protocol.

Key elements in complying with this policy are as follows:

- CO<sub>2</sub> euthanasia is recognized as a humane form of euthanasia for adult rats and mice. Therefore, we recommend its use in accordance with the *Guide for the Care and Use of Laboratory Animals* and the *AVMA Guidelines on Euthanasia*.
- **Compressed gas cylinders are the only acceptable source of carbon dioxide for euthanasia.** The use of compressed gas and handling gas cylinders can be hazardous. Please see UGA Environmental Safety Division guidance on compressed gas safety (4).
- **The practice of immersion of animals into a container pre-filled or pre-charged with CO<sub>2</sub>, is unacceptable.** Sudden exposure of conscious animals to carbon dioxide concentrations of 70% or greater has been shown to be distressful.
- **Animals must be placed in uncharged chambers and flow rates must be used which displace 30-70% of the chamber volume per minute.** (For a 10-liter volume chamber, use a flow rate of approximately 3-7 liter(s) per minute.) After the animals become unconscious, the flow rate can be increased to minimize the time to death.
  - **Displacement rate is critical to the humane application of CO<sub>2</sub>, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size container being utilized is absolutely necessary.**
- Overcrowding of the euthanasia chamber has been noted to lead to inadequate asphyxiation. The number of animals must be limited to allow free flow of CO<sub>2</sub> to each animal and allow animals to turn around.

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- Only one species is allowed in the chamber at a time. Use of the home cage as the euthanasia chamber reduces stress and is the preferred method to employ. Furthermore, mixing of unfamiliar or incompatible animals is discouraged.
- Neonatal animals (up to 10 days of age) are resistant to the effects of CO<sub>2</sub>, therefore, alternative methods are recommended (5). Carbon dioxide may be used to induce anesthesia/unconsciousness of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades).
- Unintended recovery of animals after apparent death from CO<sub>2</sub> is a documented occurrence. Such incidents constitute serious noncompliance with the PHS policy and serious deviation from the provisions of the *Guide for the Care and Use of Laboratory Animals*.
- Death of the animal must be ensured prior to disposal of the carcass. Therefore, the IACUC is requiring that a secondary method that ensures irreversibility of the procedure is used. Acceptable secondary methods include:
  - exsanguination
  - decapitation
  - cervical dislocation
  - bilateral thoracotomy

### References:

1. *Guide for the Care and Use of Laboratory Animals*, 8<sup>th</sup> Edition
2. *AVMA Guidelines for the Euthanasia of Animals*: 2020 Edition.
3. PHS POLICY ON HUMANE CARE AND USE OF LABORATORY ANIMALS CLARIFICATION REGARDING USE OF CARBON DIOXIDE FOR EUTHANASIA OF SMALL LABORATORY ANIMALS
4. UGA ESD Guidelines for Compressed Gas Safety
5. NIH Guidelines for the Euthanasia of Rodent Fetuses and Neonates