



A. PURPOSE

To ensure that researchers follow acceptable practices of euthanasia of mouse and rat fetuses and neonates when necessary in the course of animal research.

B. GUIDELINES

In general, the IACUC will not approve CO₂ as the sole method of euthanasia for mouse and rat fetuses and neonates.

Mouse and rat fetuses and neonates (up to 10 days of age) show extraordinary tolerance for CO₂ exposure, with up to 50 minutes of exposure required to cause death. Therefore, CO₂ is not recommended as a sole method of euthanasia in neonatal mice and rats. The primary concerns are the potential for distress in the animal undergoing CO₂ exposure, distress associated with animals reawakening after presumed euthanasia due to insufficient CO₂ exposure, and the prolonged exposure times needed when used as a sole method to ensure death.

Acceptable methods:

- CO₂ may be used as per I-IC-GU-214 “Guidelines for CO₂ Euthanasia in Rodents” (see link below) to induce anesthesia in neonatal rodents, **provided it is followed by a secondary physical method** of euthanasia, such as cervical dislocation or decapitation, which is applied to the unconscious animals.
- Decapitation without pre-sedation is acceptable for fetal and neonatal mice and rats < 7 days of age.

Decapitation, whether as a primary or secondary means of euthanasia, requires sharp, well-maintained scissors. Scissors should be assessed prior to use, must be clean, sharp, and their action should be smooth. After use, scissors must be washed and dried. Scissors must be sharpened or replaced periodically depending on frequency of unit use, but at a minimum either sharpened/disposed of yearly.

C. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

- AVMA Guidelines on for the Euthanasia of Animals: 2020 Edition:
www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf
- [Guidelines for Euthanasia of Rodents Using Carbon Dioxide, NIH Office of Animal Care and Use, 2024:](https://oacu.oir.nih.gov/system/files/media/file/2024-01/b5_euthanasia_of_rodents_using_carbon_dioxide.pdf)
oacu.oir.nih.gov/system/files/media/file/2024-01/b5_euthanasia_of_rodents_using_carbon_dioxide.pdf
- [I-IC-GU-214 Guidelines for CO₂ Euthanasia in Rodents](#) (link to AWP Guidance page)