

Guidelines for the Humane Euthanasia of **NEONATAL Rodents** Utilizing CO₂

General considerations:

- Animals must be euthanized in their home cage in a familiar group when possible.
- Cage density must not exceed housing space requirements.
- Transport animals to procedure room in a quiet and stress free manner.
- Keep other animals away from those being euthanized to avoid stress pheromones.
- Euthanasia **MUST** be performed via the method described in the IACUC-approved *Proposal*.

Euthanasia of NEONATAL Mice and Rat (less than 21 days)

1. Remove the cage lid and replace it with the Plexiglass plate attached to the CO₂ flow meter.
2. Set the CO₂ flow meter as follows:

Cage Type	CO ₂ flow meter setting
All Mouse Cages	3 liters per minute
All Rat Cages	10 liters per minute

3. Maintain CO₂ level for at least 1 minute after cessation of breathing or until they are nonresponsive to painful stimuli
4. ***Death must be confirmed.*** A secondary physical method of euthanasia must be performed. Acceptable methods include:
 - Cervical dislocation performed by personnel with demonstrated proficiency
 - Decapitation (animals < 7 days only) with sharp scissors or blades
5. Place animal carcass in bags, and place in the freezer
6. Turn off CO₂ meter and wipe off the Plexiglass lid
7. Return lid to top of cage and place it on the rack for dirty cages

Alternatives to CO₂ :

- **Fetuses and Neonates:** Cervical dislocation without anesthesia performed by personnel with demonstrated proficiency.
- Decapitation without anesthesia (**animals < 7 days only**) with sharp scissors or blades.
- Gradual cooling (**animals < 7 days only**) using measures to prevent direct contact with ice or pre-cooled surfaces.