IACUC GUIDELINE
CARBON DIOXIDE EUTHANASIA OF RODENTS

To the greatest extent possible, animals being euthanized should not experience pain, fear, or other significant stress prior to their death. Carbon dioxide (CO₂) is a frequently used euthanasia agent for small laboratory animals due to its rapid onset of action, safety, low cost, and ready availability (ACLAM). The University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) has adopted the following guideline to: (1) assist the research community by clarifying the specific procedures relating to the use of CO₂ and (2) promote best practices and ensure that pain and distress are minimized in euthanized laboratory rodents.

Guidance is derived from the Public Health Service (PHS), Office of Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH), the American Association of Veterinary Medicine (AVMA), peer institutions, and laboratory animal medicine experts; this guideline concentrates on the following topics regarding the use of CO₂ euthanasia:

- Species
- Administering CO₂
- Confirming death
- Exemptions

SPECIES

Any rodent used for research may be euthanized by CO₂ by following the guidance described below. Examples include mice of the genus Mus, rats of the genus Rattus, in addition to hamsters, gerbils, and other laboratory rodents. Although CO₂ is considered an “acceptable” form of euthanasia for other species (AVMA), the IACUC will require specific justification for CO₂ use in non-rodents.

ADMINISTERING CO₂

1. All personnel administering CO₂ to rodents must be properly trained (Guide). All Principal Investigators (PI) must assure that their research staff is duly trained and adhere to animal care and use protocols, policies, and guidelines. Training on the use of the equipment and appropriate methods of euthanasia is available from the ULAR Training Division.  
   “Institutions must ensure that all individuals responsible for administering CO₂ euthanasia are appropriately qualified and monitored, and that they adhere to IACUC-approved protocols and institutional policies.” (PHS)

2. Compressed gas is the only acceptable source of CO₂ for euthanizing rodents. Dry ice, fire extinguishers and other sources of CO₂ may not be used.  
   “[Compressed CO₂ in cylinders is the only...recommended source of CO₂ for euthanasia purposes.]” (PHS) (AVMA) (OLAW)

3. Euthanasia chambers should be constructed of clear material (e.g. Plexiglas) and must be kept clean to minimize odors that might distress animals subsequently euthanized. Gas should be delivered in a predictable and controllable fashion, at a low-flow rate.
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“\textit{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.}” (NIH)

4. Euthanasia should occur in a procedure room or laboratory, away from other rodent housing. “\textit{Euthanasia should not be performed in the animal room.}” (NIH)

5. When possible, rodents should be euthanized in their home cages. **Mixing or combining of animals from multiple cages, into a single cage, is prohibited.**

   “\textit{Researchers and animal care staff should... minimize the stress experienced by rodents that undergo CO\textsubscript{2} euthanasia...[by] performing euthanasia in the home cages... and minimizing regrouping to prevent social aggression are simple approaches to lessening potentially stressful conditions.}” (ACLAM)

6. Do not “pre-fill” the euthanasia chamber with CO\textsubscript{2}. Start with room air then slowly fill the chamber with CO\textsubscript{2} over several minutes (OLAW). CO\textsubscript{2} is heavier than room air, thus the chamber may need to be “purged” between groups of cages.

   “\textit{Because inspiration of high concentrations of CO\textsubscript{2} is both aversive and painful, a recommended procedure is to place animals into a chamber that contains room air and then to gradually introduce CO\textsubscript{2}.}” (ACLAM)

7. CO\textsubscript{2} first renders the animal anesthetized and then, with adequate exposure time, will result in death by CO\textsubscript{2} narcosis. Animals should be left for additional time within the euthanasia chamber, after spontaneous movements have ceased, with CO\textsubscript{2} continuing to flow.

   “\textit{Animals should be left in the container until clinical death has been ensured.}” (NIH)

Resistance to hypoxia results in a prolonged time to unconsciousness when CO\textsubscript{2} inhalation is used as a euthanasia agent in neonatal rodents. The duration of exposure to carbon dioxide varies with the age of the neonate compared with adult rodents.

<table>
<thead>
<tr>
<th>AGE</th>
<th>Minimum time in 100% CO\textsubscript{2}</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><strong>MICE</strong> (Pritchett et al. 2005)</td>
</tr>
<tr>
<td>Non-haired pups</td>
<td>60 minutes</td>
</tr>
<tr>
<td>0–6 days</td>
<td></td>
</tr>
<tr>
<td>Haired pups, eyes closed</td>
<td>20 minutes</td>
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<tr>
<td>7–13 days</td>
<td></td>
</tr>
<tr>
<td>Haired pups, eyes open, preweaning</td>
<td>10 minutes</td>
</tr>
<tr>
<td>14–20 days</td>
<td></td>
</tr>
<tr>
<td>Weanlings and adults</td>
<td>5 minutes</td>
</tr>
<tr>
<td>21+ days</td>
<td></td>
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</tbody>
</table>

Fetuses also require extended exposure to inhalant anesthetics, including CO\textsubscript{2}. When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother should ensure cerebral anoxia to the fetus and minimally disturb the uterine milieu to minimize fetal arousal. A recommended method for euthanasia of the mother is CO\textsubscript{2} exposure followed by cervical dislocation (ACLAM).

**CONFIRMING DEATH**

Since the anesthetic effects of CO\textsubscript{2} are reversible, animals that are prematurely removed from the chamber prior to death can recover (OLAW). Furthermore, death should be confirmed by personnel
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who have been specifically trained to recognize cessation of vital signs in rodents (Guide). Therefore, all animals being euthanized with CO₂ overdose must also receive a confirmatory method of euthanasia to ensure death. These confirmatory methods, to be performed after carbon dioxide overdose, include exsanguination, decapitation, cervical dislocation, bilateral thoracotomy, or at least 50% additional time in the euthanasia chamber filled with 100% CO₂.

Death must be verified after euthanasia and prior to disposal. Unintended recovery must be obviated by the use of appropriate CO₂ concentrations and exposure times or by other means.” (PHS)

Death of the animal must be ensured prior to disposal of the rodent carcass. Failure to confirm death of a euthanized rodent is a significant non-compliance, reportable to the appropriate regulatory and accrediting agencies.

"Unintended recovery of animals after apparent death from CO₂...constitute[s] serious noncompliance with the PHS Policy and... the IACUC, through the Institutional Official, must promptly provide OLAW with a full explanation of the circumstances and actions taken.” (PHS)

EXEMPTIONS

Methods of euthanasia used will be consistent with the recommendations of the AVMA Guidelines on Euthanasia, unless a deviation is justified for scientific reasons in writing by the investigator. Any departures from these guidelines will require the PI to provide strong scientific and clinical justifications for the exemption. These justifications must be submitted via an “Exemption Form” and will be reviewed by the IACUC in consultation with ULAR veterinarians. As designees of the Attending Veterinarian, ULAR veterinarians are given the authority to decide how a rodent should best be euthanized.

REFERENCES

American College of Laboratory Animal Medicine Report of the ACLAM Task Force on Rodent Euthanasia (ACLM)
American Veterinary Medical Association Guidelines for Euthanasia (2007) (AVMA)
Guide for the Care and Use of Laboratory Animals (Guide)
National Institutes of Health Guidelines for Euthanasia of Rodents Using Carbon Dioxide (NIH)
Office of Laboratory Animal Welfare IACUC Guidebook (OLAW)
Public Health Service Policy: Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small Laboratory Animals (PHS)