Introduction to Euthanasia of Mice

Division of Comparative Medicine
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Objectives

Upon completion of this training, you will be able to:

- Comprehend acceptable euthanasia procedures as performed in the rodent.
  - Injectable Barbiturate, Injectable Anesthesia
- Understand adjunctive methods to ensure death.
  - Decapitation, cervical dislocation, bilateral thoracotomy
- Comprehend methods that are deemed “Acceptable with Conditions.”
  - Decapitation or cervical dislocation w/o anesthesia, inhaled anesthesia, carbon dioxide
- Understand special considerations of neonate rodents.
Euthanasia – CO$_2$

- The AVMA Panel on Euthanasia has stated that the use of CO$_2$ for euthanasia of small laboratory animals is “Acceptable with Conditions.”

  - Provides rapid depression with analgesic and anesthetic effects (CO$_2$ narcosis)
  - Does not introduce chemical residues in tissues
  - Typically, initial CO$_2$ delivery to the micro-isolator is accomplished by opening the CO$_2$ cylinder valve so that animal(s) are slowly exposed to increasing levels of CO$_2$ (e.g., displacing approximately 10-30% of the chamber volume per minute)
Euthanasia – CO₂

- Compressed CO₂ in gas cylinders is the only acceptable source of CO₂ for euthanasia.
- Only animals of the same species should be placed into a chamber at any time.
- Euthanasia of more than one animal at a time should always be performed in cohorts of live animals (i.e., live animals must not be placed in the chamber with dead animals).
- To reduce stress, rodents should be euthanatized in their home cage whenever possible.
Euthanasia – CO$_2$

• Sudden exposure to high concentrations of CO$_2$ may be distressful to some species.

• Pre-filling the CO$_2$ chamber is no longer recommended.
Euthanasia – CO$_2$

CO$_2$ delivery to the microisolator is accomplished by opening the CO$_2$ cylinder valve and flow meter so that rodents are slowly exposed to increasing levels of CO$_2$ at the illustrated flow rates:

<table>
<thead>
<tr>
<th>Species</th>
<th>Cage Type</th>
<th>Flow Rate L/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>Static (small)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Static (large)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>IVC (w/o lid)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>IVC (w/ lid)</td>
<td>4</td>
</tr>
<tr>
<td>Rat</td>
<td>Static</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>IVC (w/o lid)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>IVC (w/ lid)</td>
<td>13</td>
</tr>
</tbody>
</table>
Euthanasia - CO$_2$

- A higher flow rate may be required in some experimental settings (e.g., cardiac puncture for the collection of whole blood, or for pulmonary histopathology). However, any exception must be outlined in an approved protocol.

- Gas flow may be increased as loss of consciousness is observed. Flow should be maintained for at least one minute after apparent clinical death (e.g., cessation of cardiovascular and respiratory movements).
Death must be verified by the assurance of:

- cessation of respiratory and cardiovascular movements by observation at room air for at least 10 minutes

  OR

- by employing a secondary method of euthanasia, such as cervical dislocation, decapitation, or bilateral thoracotomy, prior to carcass disposal.
Euthanasia – CO$_2$

- **Euthanasia chambers and lids must be cleaned & dried** between animals or groups of animals to minimize odors/pheromones that might distress animals.

- **Animals must not be euthanatized in animal housing rooms** except during special circumstances, i.e., during quarantine; exposure to infectious agents.
Euthanasia – CO\textsubscript{2} & Neonates

- Neonates (up until approximately 10 days) are resistant to euthanasia by CO\textsubscript{2} due to their inherent resistance to hypoxia. They may require a prolonged exposure time to any type of inhalant.

- Consequently, CO\textsubscript{2} should not be used as the sole means of euthanatizing neonates.

- CO\textsubscript{2} may be used to induce narcosis but must be followed with another acceptable secondary method of euthanasia (e.g., decapitation, cervical dislocation, or bilateral thoracotomy) to ensure death.
When administered at higher/lethal doses, injectable anesthetics can be used for euthanasia.

Barbiturates produce rapid and humane euthanasia. These may be administered intravenously or intraperitoneally.

Barbiturates are controlled substances and must be procured through proper channels. Exact recordkeeping is required.

Commercially prepared “Euthanasia Solution” products are available.
Euthanasia – Inhalant Anesthetics

- With inhalant anesthetics, such as isoflurane, the animal is placed in a closed container.
- Anesthetic agent is introduced via a vaporizer (5% isoflurane).
- Vapors are inhaled until respiration ceases and death ensues.
- This method may be associated with a long induction time.
- Because the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors.
Acceptable (Methods) w/Conditions

• Decapitation or cervical dislocation of deeply sedated or anesthetized rodents and other small animals is an acceptable method of euthanasia.

• A request to perform decapitation or cervical dislocation without sedation or anesthesia requires a scientific justification explaining the experimental design that necessitates this method of euthanasia.

• Staff who intend to perform decapitation or cervical dislocation without sedation or anesthesia must have demonstrated competence under the observation of the Comparative Medicine Training Coordinator.
Acceptable (Methods) w/Conditions


• "[decapitation] is conditionally acceptable…if performed correctly, and it may be used in research settings when its use is required by the experimental design and approved by the IACUC…"

• The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.

• The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal…

• Those responsible for the use of this technique must ensure that personnel who perform decapitation have been properly trained to do so and are monitored for competence."
Acceptable (Methods) w/Conditions


- "Manual cervical dislocation is acceptable with conditions for euthanasia of…mice…when performed by individuals with a demonstrated high degree of technical proficiency.

- In lieu of demonstrated technical competency, animals must be unconscious or anesthetized prior to cervical dislocation.”

- “Those responsible for the use of this method must ensure that personnel performing cervical dislocation have been properly trained and consistently apply it humanely and effectively."
Other Considerations

• Exsanguination is not used as a sole means of euthanasia.

• Animals may be exsanguinated when surgically anesthetized, or immediately following CO\(_2\) narcosis and affirmation of a plane of anesthesia.

• Following all methods of euthanasia, animal death is assured by the determination of the cessation of cardiovascular and respiratory movements.
  - Observe for 10 minutes prior to disposal.
Other Considerations

“Methods of euthanasia likely to elicit distress vocalizations or pheromones that other animals in the room could hear or smell may be best performed in another location…”
Additional Resources

- Lessons 21 - A.L.L. “Working with the Laboratory Mouse”
- USF SOP 401 “Carbon Dioxide Euthanasia in Rodent Species”
- USF IACUC Principles and Procedures of Animal Care and Use
Quiz Questions

1. Mouse pups are considered neonates up until approximately ___ days of age.
   a. 7 days
   b. 10 days
   c. 21 days

2. Animals must be observed at room air for ___ after cessation of breathing, or have a secondary method employed.
   a. 3 minutes
   b. 5 minutes
   c. 10 minutes

3. Injectable euthanasia agents can only be given intravenously.
   a. true
   b. false
3. Decapitation doesn’t need to be scientifically justified as the sole means of euthanasia, as long as it is listed on the protocol.
   a) True
   b) False

4. Having documentation of observation regarding performing cervical dislocation in an unanesthetized mouse is mandatory.
   a) True
   b) False