INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
Policy #9
Euthanasia

In concurrence with
TTUHSC El Paso Assurance #D19-01056
and Federal Regulations and Guidelines

Purpose
To define the regulations for animal euthanasia at TTIHSC El Paso.

Overview
Euthanasia (from the Greek, meaning "good death") is a critical component of humane animal care. In general, the recommendations of the AVMA Guidelines on Euthanasia (2013) serve as the standard for acceptable methods on euthanasia.

The AVMA guidelines describe appropriate methods of rodent euthanasia (described in the table below). Any other method of euthanasia requires justification in the Animal Protocol.

Non-rodent species under USDA jurisdiction must be euthanized by barbiturate overdose. Principal Investigators should consult with the Institutional Veterinarian in these cases.

The following guidelines provide important criteria for the successful implementation of euthanasia.
- Animals must be euthanized only by trained personnel using appropriate technique, equipment and agents. This is necessary to ensure a painless death that satisfies research requirements.
- Death must be induced as painlessly and quickly as possible.
- Upon completion of the euthanasia procedure, death must be confirmed by a secondary method as noted below.
- Euthanasia may not be performed in the animal housing room.
- The euthanasia method must be appropriate to the species, approved in the animal study proposal, and conform to the most recent AVMA guidelines for the euthanasia of animals. The euthanasia method must observe the conditions and precautions in the pertinent sections of that report, and to NIH guidelines in their latest revisions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Methods</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Pregnant dam</td>
<td>Barbiturates, CO₂ inhalation</td>
<td>-Cervical dislocation is appropriate for rats and mice less than 200g when performed under anesthesia. Decapitation is acceptable when justified by experimental conditions in the IACUC protocol.</td>
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<tr>
<td>Rat</td>
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<td>CO₂ inhalation</td>
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<td>Isoflurane/Sevoflurane inhalation</td>
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<td>Hamster</td>
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<td>Cervical dislocation</td>
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<td>Gerbil</td>
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<td>Decapitation</td>
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<tr>
<td>pups in utero</td>
<td>Euthanize dam</td>
<td>According to the AVMA guidelines, rodent fetuses are unconscious in utero and hypoxia does not evoke a response. Therefore it is unnecessary to remove fetuses for euthanasia after the dam is euthanized.</td>
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<tr>
<td>Age</td>
<td>Method</td>
<td>Euthanasia Comment</td>
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</table>
| 0-10 days   | Anesthetic overdose (Ketamine/Xylazine, Barbiturates)  
Cervical dislocation/Decapitation under anesthesia  
Isoflurane/sevoflurane     | At this state, neonates are resistant to carbon dioxide euthanasia. |
| Adult (>10 days old) | Barbiturates  
CO₂ inhalation  
Isoflurane/sevoflurane inhalation  
Cervical Dislocation  
Decapitation | -Cervical dislocation is appropriate for rats and mice less than 200g when performed under anesthesia.  
Decapitation is acceptable when justified by experimental conditions in the IACUC protocol. |
| Guinea pig  | Pregnant dam  
Anesthetic overdose (Ketamine/Xylazine, Barbiturates)  
Carbon dioxide | -  
According to the AVMA guidelines, rodent fetuses are unconscious in utero and hypoxia does not evoke a response.  
Therefore it is unnecessary to remove fetuses for euthanasia after the dam is euthanized. |
|             | pups in utero  
Euthanize dam | -  
According to the AVMA guidelines, rodent fetuses are unconscious in utero and hypoxia does not evoke a response.  
Therefore it is unnecessary to remove fetuses for euthanasia after the dam is euthanized. |
|             | Birth → adult  
Anesthetic overdose (Ketamine/Xylazine, Barbiturates)  
Carbon dioxide | -  
According to the AVMA guidelines, rodent fetuses are unconscious in utero and hypoxia does not evoke a response.  
Therefore it is unnecessary to remove fetuses for euthanasia after the dam is euthanized. |
| Zebrafish   | -  
- | -  
See IACUC Policy 23 |
| Other USDA- covered | -  
- | -  
Consult the Institutional Veterinarian |

As a means of euthanasia, administration of inhalant overdose results in deep depression of all life signs prior to death. It is possible that animals could revive from this state, which can be mistaken for death during a cursory examination. To prevent such an occurrence, the TTUHSC Animal Care and Use Committee has instituted the following policy.

**Secondary Method**
Administration of an inhalant overdose must be followed by one of the following secondary procedures:
- Cervical dislocation
- Decapitation
- Exsanguination
- Bilateral thoracotomy
- Major organ removal

**Guidelines for Euthanasia of Rodents Using Carbon Dioxide**
CO₂ inhalation is the most common method of euthanasia used for mice, rats, guinea pigs, gerbils, and hamsters and must be used as follows:
• 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.
• Compressed CO₂ gas in cylinders is the only recommended source of carbon dioxide, as it allows the inflow of gas to the induction chamber to be controlled without precharging the chamber. Place the animal(s) in the chamber and introduce carbon dioxide at a rate of 30-70% (liters per minute). Appropriate flow rate for each species and cage type will be posted in the LARC.
• Animals should be left in the container until breathing has ceased. Observe for one additional minute and use a secondary method to ensure clinical death
• The use of dry ice for CO₂ euthanasia is not permitted.

Exceptions to these guidelines will be considered by the IACUC on a case-by-case basis.

Related policies
Investigators must comply with all other institutional policies at TTUHSC El Paso and Federal Guidelines. This list includes, but is not limited to, the following:

IACUC policy 5: Cervical Dislocation or Decapitation of Animals
IACUC policy 23: Use of Zebrafish for Research and Teaching

References
1. AVMA Guidelines for the Euthanasia of Animals, 2020
4. NIH Notice NOT-OD-02-062 released 7/27/2002