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.

Carbon dioxide inhalation is the preferred method of rodent euthanasia. Intraperitoneal barbiturate overdose and isoflurane overdose (with secondary procedure) may be used in certain circumstances. Any other method of euthanasia requires justification in the Animal Protocol.

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

The following guidelines provide important criteria for the successful implementation of inhalant anesthesia.

- 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 room.
- The euthanasia method must be appropriate to the species, approved in the animal study proposal, and conform to the most recent Report of the AVMA Panel on Euthanasia. The use of inhalant agents for euthanasia 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>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0-14 days gestation</td>
<td>Euthanize dam</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>15 days gestation to birth</td>
<td>Barbiturates, Cervical dislocation, Decapitation, Overdose of injectable anesthesia</td>
<td>Euthanize dam with carbon dioxide. Fetuses are resistant to inhalant euthanasia at this stage should be euthanized with one of the methods listed on the left. If age of gestation is unknown, use procedure for 15 days to birth.</td>
</tr>
<tr>
<td>Hamster</td>
<td>0-10 days old</td>
<td>Anesthetic overdose (Ketamine/Xylazine, Barbiturates), Cervical dislocation/Decapitation under anesthesia, Isoflurane/sevoflurane</td>
<td>At this state, neonates are resistant to carbon dioxide euthanasia.</td>
</tr>
<tr>
<td>Gerbil</td>
<td>Adult (&gt;10 days old)</td>
<td>Barbiturates</td>
<td>-Cervical dislocation is appropriate for rats and mice less than 200g.</td>
</tr>
</tbody>
</table>
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

**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 100% carbon dioxide.
- Animals should be left in the container until clinical death has been ensured. Unintended recovery must be prevented by the use of appropriate CO₂ concentrations and exposure times or by other means.
- 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.

**References**
1. AVMA Guidelines for the Euthanasia of Animals, 2013
2. NIH Office of Animal Care and Use. Guidelines for Euthanasia of Rodent Fetuses and Neonates, rev 06/22/16
   http://oacu.od.nih.gov/ARAC/index.htm

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<table>
<thead>
<tr>
<th>Guinea pig</th>
<th>Inhalant anesthesia: (CO₂, isoflurane, sevoflurane) Cervical Dislocation/Decapitation</th>
<th>Decapitation is acceptable when justified by experimental conditions.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-34 days gestation</td>
<td>Euthanize dam</td>
<td>-</td>
</tr>
<tr>
<td>35 days gestation to birth</td>
<td>Anesthetic overdose (Ketamine/Xylazine, Barbiturates) Decapitation under anesthesia</td>
<td>At this stage, neonates are resistant to inhalant euthanasia.</td>
</tr>
<tr>
<td>Birth → adult</td>
<td>Anesthetic overdose (Ketamine/Xylazine, Barbiturates) Carbon dioxide</td>
<td>-</td>
</tr>
</tbody>
</table>

Zebrafish - - See IACUC Policy 23

Other USDA-covered - - Consult the Institutional Veterinarian
4. NIH Notice NOT-OD-02-062 released 7/27/2002