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| <b>IACUC SOP:</b> | <b>Statement on Humane Euthanasia of Animals Used in Research or Teaching</b> |                                |
| <b>SOP#112.00</b> | <b>IACUC Approval: 11/4/2022</b>  | <b>IO Approval: 12/20/2022</b> |

**Purpose**

To provide guidance regarding humane euthanasia of animals used in research or teaching.

**Policy**

- All euthanasia methods must be described in the applicable Animal Use Protocol (AUP) and approved by the IACUC. Unless a deviation is justified for scientific or medical reasons, euthanasia methods should be consistent with the AVMA Guidelines on Euthanasia (AVMA 2020 or later editions). The 2020 AVMA Guidelines on Euthanasia can be found at (<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>).
  - o In cases where specific guidelines are absent for a species or situation, methods must be described and justified. Justification may be supported by citations including published guidelines from professional societies.
  - o Protocols that do not include euthanasia of animals for the study should still describe a method of euthanasia in case of emergency.
- The method of euthanasia of animals intended for human consumption must be compatible with current state and federal food safety regulations, as well as the [2016 AVMA Guidelines for the Humane Slaughter of Animals](#).
- As part of the TAMUSA disaster plan, emergency euthanasia of large numbers of animals will be managed as per the AVMA Guidelines for Depopulation of Animals in the event that the euthanasia guidelines cannot be met.
- All euthanasia procedures require confirmation of death. Many species and methods of euthanasia require a secondary physical method. Unintended recovery of animals after apparent death constitutes serious noncompliance.

**Training**

All personnel performing animal euthanasia must be trained, knowledgeable, and proficient in the chosen techniques and verification of death. Training must be documented. Personnel using physical methods of euthanasia without anesthesia must be certified to verify proficiency for each type of physical method used.

Principal Investigators (PI) are responsible for ensuring that their research staff and students are trained in the relevant euthanasia method and its associated conditions. All training should be documented in writing and training documents should be made available for the IACUC and other regulatory agencies upon request. For supplementary information, assistance, or training in any euthanasia method, please contact the IACUC office.

**Minimizing Pain and Distress**

Pain and distress prior to and during euthanasia should be avoided.

1. Regardless of the technique used, the animal should be carefully handled and/or gently restrained in an appropriate and safe manner prior to euthanasia.
2. “Distress vocalizations, fearful behavior, and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals. Therefore, for sensitive species, it is

desirable that other animals not be present when individual animal euthanasia is performed.” (pg 14, AVMA 2020). In general, animals should not be euthanized in the animal housing room/area to minimize stress on the remaining animals. Exceptions may occur given scientific justification (e.g. to prevent spread of infectious disease), for emergency euthanasia when an animal cannot be readily moved, or situations that do not induce stress on the remaining animals (e.g., Aquatic animals euthanized by chemical methods or ice bath submersion).

3. Chambers used to expose multiple animals to inhaled euthanasia agents must not be overcrowded.
4. Animals with a propensity for fighting should not be mixed together for transport to and during euthanasia (i.e. unfamiliar adult male mice, hamsters, and other incompatible animals).

**Procedure Guidelines**

1. Listed below are some commonly used and accepted euthanasia and secondary confirmation methods for different species. This list is not inclusive. Please see the [AVMA Guidelines on Euthanasia](#)<sup>1</sup> for further information.
2. Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia. Methods acceptable with conditions are those techniques that may require certain conditions to be met to consistently produce humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure death. Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method can be met.

*For preservation as scientific specimens, reptiles may be euthanized using buffered tricaine methanesulfonate (MS222) injected intracoelomically into a cooled animal followed by freezing to ensure mortality.*

**Rodent Species<sup>1</sup>:**

| <u>Acceptable Methods</u>  | <u>Acceptable with Conditions</u>   |
|--|---|
| <ul style="list-style-type: none"> <li>• Injected barbiturates and barbiturate Combinations</li> <li>• Injected dissociative agent combinations</li> </ul> | <ul style="list-style-type: none"> <li>• Inhaled anesthetic overdose</li> <li>• CO<sub>2</sub><sup>2</sup></li> <li>• Cervical dislocation</li> <li>• Decapitation</li> </ul> |

<sup>1</sup>Confirmation of death must have secondary physical method: cervical dislocation, decapitation, cardiac perfusion, removal of vital organs, pneumothorax, or exsanguination.

<sup>2</sup>Conditions required for CO<sub>2</sub> euthanasia:

- Source is from a compressed gas CO<sub>2</sub> cylinder (dry ice and other sources not acceptable)
- Flow rate displaces 30-70% of the chamber volume per minute (prefilled chambers are not acceptable).
- CO<sub>2</sub> should be maintained for one minute after respiratory arrest
- If necessary, a physical method of euthanasia may be applied once the animal is completely unresponsive to external stimuli (unconscious) but prior to complete respiratory and cardiovascular arrest. (See below)
- For inhalant methods, animals should be euthanized in their home cages whenever possible
- Chamber should be cleaned after each use

**Rodent Neonates**



- o Neonates up to 10 days of age:
  - o Decapitation using scissors or a sharp blade is acceptable for altricial neonates up to 10 days of age.
- o Neonatal rodents are resistant to the effects of CO<sub>2</sub>, thus adjunctive physical methods must be performed after the neonate is unresponsive to stimuli.

**Fish<sup>1</sup>:**

| <u>Acceptable</u>   | <u>Acceptable with Conditions</u>   |
|---|---|
| <ul style="list-style-type: none"> <li>• Injected barbiturates</li> <li>• Immersion in:<sup>2</sup> <ul style="list-style-type: none"> <li>o Buffered benzocaine or benzocaine HCl</li> <li>o Ethanol</li> <li>o Buffered tricaine Methanesulfonate (MS222) (250-500 mg/L)</li> <li>o 2- phenoxyethanol</li> </ul> </li> <li>• Rapid chilling (2° to 4°C)<sup>3</sup> <ul style="list-style-type: none"> <li>o tropical fish - up to 3.8cm in length</li> </ul> </li> </ul> | <ul style="list-style-type: none"> <li>• Eugenol, isoeugenol, Clove oil<sup>2</sup></li> <li>• CO<sub>2</sub>-saturated water (aquarium-fish facilities/fisheries)</li> <li>• Decapitation</li> <li>• Cervical transection</li> <li>• Manually applied blunt force trauma followed by pithing</li> <li>• Rapid chilling followed by adjunctive method (aquarium-fish facilities)</li> </ul> |

<sup>1</sup>Confirmation of death must include secondary method such as decapitation, pithing, exsanguination, or chemical methods for destroying brain function.

<sup>2</sup>Fish should be left in anesthetic solution for a minimum of 30 minutes after cessation of opercular movement.

<sup>3</sup>Exposure time for adult zebrafish is a minimum of 10 minutes following the loss of opercular movement. Fish must not come in direct contact with the ice in the water.

**Amphibians<sup>1</sup>:**

| <u>Acceptable Methods (as appropriate by species)</u>   | <u>Acceptable with Conditions</u>   |
|---|---|
| <ul style="list-style-type: none"> <li>• Injected barbiturates</li> <li>• Injected dissociative agents or anesthetics</li> <li>• Topical buffered MS-222</li> <li>• Benzocaine hydrochloride</li> </ul> | <ul style="list-style-type: none"> <li>• Inhaled anesthetics</li> <li>• CO<sub>2</sub></li> <li>• Penetrating Captive Bolt</li> <li>• Blunt force trauma to the head</li> </ul> |

<sup>1</sup>Confirmation of death must include secondary method such as decapitation followed by pithing, double pithing, exsanguination, or chemical methods for destroying brain function.

**Reptiles:**



| <u>Acceptable</u>   | <u>Acceptable With Conditions</u>  |
|---|--|
| <ul style="list-style-type: none"> <li>As appropriate by species—injecte d barbiturates, dissociative agents and anesthetics as specified.</li> <li>In the field, species may be euthanized by decapitation and pithing.</li> </ul> | <ul style="list-style-type: none"> <li>As appropriate by species—Inhaled anesthetics as specified, CO<sub>2</sub>, penetrating captive bolt or firearm, manually applied blunt force trauma to the head, rapid freezing for animals &lt; 4 g.</li> </ul> |

Because it is often difficult to confirm that a reptile is dead, the application of two or more euthanasia procedures is usually recommended. Consulting multiple references on reptile euthanasia is advised as a means of identifying methods that are most appropriate for a given species and set of circumstances.

**Avian species<sup>1</sup>:**

| <u>Acceptable Methods</u>   | <u>Acceptable with Conditions</u>   |
|---|---|
| <ul style="list-style-type: none"> <li>Injected barbiturates</li> </ul> | <ul style="list-style-type: none"> <li>CO<sub>2</sub><sup>2</sup></li> <li>Inhaled anesthetics</li> <li>Cervical dislocation</li> <li>Decapitation</li> </ul> |

<sup>1</sup>Confirmation of death must have a secondary method such as decapitation, exsanguination or cervical dislocation.

<sup>2</sup>Pre-fill the euthanasia chamber with CO<sub>2</sub>, place the bird in the chamber, and wait for spontaneous movement to cease.

**Eggs**

Avian Eggs at <80% incubation may be destroyed by prolonged exposure (>20 minutes) to CO<sub>2</sub>, cooling (<4°C for 4 hours) or freezing. If egg is over 80% incubation, must use similar method as listed above.

Reptile/Amphibian Eggs may be euthanized using buffered MS222 and rapid chilling.

**Tissue Collection Following CO<sub>2</sub> or Inhalant Anesthesia Exposure**

If necessary, the animal can be removed from the CO<sub>2</sub> or inhaled anesthetic before complete cessation of breathing and euthanized by an appropriate physical method 8 (decapitation of neonatal to adult rodents, cervical dislocation of adults, or other means). The physical method must be applied only when the animal is completely unresponsive to external stimuli (unconscious). This technique may apply if the collection of animal tissue or fluid is required at or just before the time of death.

## References

1. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition  
<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
2. AVMA Guidelines for the Depopulation of Animals: 2019 Edition  
<https://www.avma.org/sites/default/files/resources/AVMA-Guidelines-for-the-Depopulation-of-Animals.pdf>
3. Guide for the Care and Use of Laboratory Animals 8th edition, 2011 (the Guide)  
<https://olaw.nih.gov/sites/default/files/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf>
4. Guide for the Care and Use of Agricultural Animals in Research and Teaching 3rd edition, 2010 (the Ag Guide).  
[https://www.adsa.org/Portals/default/SiteContent/docs/AgGuide3rd/Ag\\_Guide\\_3rd\\_ed.pdf](https://www.adsa.org/Portals/default/SiteContent/docs/AgGuide3rd/Ag_Guide_3rd_ed.pdf)
5. AVMA Guideline for the Humane Slaughter of Animals: 2016 Edition  
<https://www.avma.org/sites/default/files/resources/Humane-Slaughter-Guidelines.pdf>
6. NIH Guidelines for the Euthanasia of Rodent Fetuses and Neonates:  
[https://oacu.oir.nih.gov/sites/default/files/uploads/aracguidelines/b4\\_rodent\\_euthanasia\\_pup.pdf](https://oacu.oir.nih.gov/sites/default/files/uploads/aracguidelines/b4_rodent_euthanasia_pup.pdf)