ANIMAL EYE CONSULTANTS OF IOWA

Animal Eye Consultants of Iowa	
Institutional Animal Care and Use Committee	
Policy #28	**"Euthanasia of Research and Teaching Animals"**
Approval Date: 7/25/2023 (Replacing Version 6/1/2020)	

A. Purpose

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

B. Policy

All euthanasia methods must be described in the applicable Animal Subjects Approval Form (ASAF) and approved by the IACUC prior to being performed. 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).

- 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.
- Protocols involving housing of animals or field studies that do not require euthanasia of animals for the study should still describe a method of euthanasia in case of emergency.
- Projects proposing to utilize a physical method of euthanasia without anesthesia should provide justification within the ASAF (i.e., cervical dislocation without anesthesia, etc.). Please view Section E for special considerations regarding neonatal rodents under 10 days of age.

Animals Used for Human Consumption

If animals will be used for human consumption, the method of slaughter must be compatible with current state and federal food safety regulations, as well as the 2016 AVMA Guidelines for the Humane Slaughter of Animals[5].

Emergency Depopulation

As part of the AEC disaster plan, in the event that the euthanasia guidelines cannot be met, emergency euthanasia of large numbers of animals will be managed as per the AVMA Guidelines for Depopulation of Animals (see section F below).

Confirmation of Death

All euthanasia procedures require confirmation of death and/or a secondary physical method. Unintended recovery of animals after apparent death constitutes serious noncompliance. Please view Section E in this document for a summary of acceptable secondary physical methods and methods to confirm death by species.

C. Training

All personnel performing animal euthanasia must be trained, knowledgeable, and proficient in the chosen techniques and verification of death. Principal Investigators (PIs) are responsible for ensuring that all personnel performing euthanasia under their ASAFs are trained in the relevant euthanasia method and its associated conditions. All training must be kept on record and training documents must be made available for the IACUC and other regulatory agencies upon request.

Physical Methods of Euthanasia without Anesthesia

Personnel using physical methods of euthanasia without anesthesia must have their proficiency evaluated and verified for each type of physical method used by the Office of the AEC Veterinary Staff (AEC) or an AEC-approved Designated Trainer. A designated trainer is someone that has demonstrated high proficiency with use of the physical method previously and has been approved by AEC to verify the proficiency of others. Once the person has been verified, they will be certified to perform these techniques as described on an approved ASAF. The Animal Welfare Program (AWP) staff will review training history for staff on new protocols and three-year renewals to ensure proficiency has been verified and documented by AEC or a Designated Trainer.

Individuals may be deemed exempt from the in-person Verification of Proficiency requirement by AEC on a case-by-case basis. Exemptions are given based on personnel

experience/credentials and may only be issued by AEC veterinary staff (approved designated trainers may not give exemptions).

Methods of Physical Euthanasia that may require Verification of Proficiency:

- Cervical Dislocation without Anesthesia
- Decapitation without Anesthesia (please view Section E for neonatal rodent considerations)
- Penetrating Captive Bolt without Sedation/Anesthesia
- Firearms (may only be performed by highly trained personnel in jurisdictions that allow for legal firearm use)
- Pithing without Anesthesia
- Manually Applied Blunt Force Trauma to the Head

Training and Information Contact

For supplementary information, verification of proficiency, assistance, or training in any euthanasia method, please contact AEC Veterinary Staff (AEC) at 509-335-6246 or animaleyeiowa@gmail.com .

D. Minimizing Pain and Distress

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

- Regardless of the technique used, the animal should be carefully handled and/or gently restrained in an appropriate and safe manner prior to euthanasia.

- 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). Per the 2020 AVMA Guidelines (Pg 14), *"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."*

- Chambers used to expose multiple animals to inhaled euthanasia agents must not be overcrowded.

- 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). Please refer to IACUC Policy 30 for a list of incompatible animals.

E. Procedure Guidelines

Listed below are some commonly used and accepted euthanasia and secondary confirmation methods for different species. This list is not all inclusive. Please see the AVMA Guidelines on Euthanasia[1] for further information.

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 (see below), 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.

Rodent Species*:

Acceptable Methods	Acceptable with Conditions
• Injected barbiturates • injected anesthetic overdose	• Inhaled anesthetic overdose • CO_2** • Cervical dislocation • Decapitation

*Confirmation of death must have secondary physical method: cervical dislocation, decapitation, cardiac perfusion, removal of vital organs, pneumothorax, or exsanguination.

**Conditions required for CO_2 euthanasia:

- Source must be from a compressed gas CO_2 cylinder (dry ice and other sources not acceptable)
- Flow rate must displace 30-70% of the chamber volume per minute (prefilled chambers are not acceptable).

- CO_2 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

- Neonatal rodents are resistant to the effects of CO2, thus adjunctive physical methods must be performed after the neonate is unresponsive to stimuli if CO_2 is used.
- Neonates up to 10 days of age:
  - Decapitation without anesthesia using scissors or a sharp blade is acceptable with conditions for altricial neonates up to 10 days of age.
    - Personnel performing decapitation without anesthesia of neonates must still have their technique verified by AEC or an AEC-approved designated trainer per Section C.
    - Some rodent neonates may have a tissue mass that is too large for scissors, so appropriate decapitation tools should be selected

Rabbits*:

Acceptable	Acceptable With Conditions
• Intravenous barbiturates	• Inhaled anesthetic overdose • CO_2** • Cervical Dislocation

*Confirmation of death must include a combination of criteria, including lack of pulse or lack of ausculted heartbeat, breathing, or corneal reflex. A secondary physical method such as pneumothorax or exsanguination may also be used

**CO_2 must be delivered with flow rate of 50-60% of the chamber volume per minute. Sedation is strongly recommended before exposure to CO_2.

Dogs and Cats*:

Acceptable	Acceptable With Conditions

• Intravenous barbiturates • Injected anesthetic overdose	• Barbiturates (alternate routes of administration) • Inhaled anesthetic overdose

*Confirmation of death must have combination of criteria including lack of pulse or lack of ausculted heartbeat, breathing, corneal reflex or a secondary physical method such as pneumothorax or exsanguination.

Finfish*:

Acceptable	Acceptable with Conditions

• Injected barbiturates
• Immersion in:**
  ○ Buffered benzocaine or benzocaine HCl
  ○ Ethanol
  ○ Buffered tricaine Methanesulfonate (MS222) (250-500 mg/L)
  ○ 2- phenoxyethanol
• Rapid chilling (2° to 4°C)***
  ○ tropical fish - up to 3.8cm in length
	• Eugenol, isoeugenol, Clove oil**
	• CO_2-saturated water (aquarium-fish facilities/fisheries)
	• Decapitation
	• Cervical transection
	• Manually applied blunt force trauma followed by pithing
	• Rapid chilling followed by adjunctive method (aquarium-fish facilities)

*Confirmation of death must include secondary method such as decapitation, pithing, exsanguination, or chemical methods for destroying brain function.
**Fish should be left in anesthetic solution for a minimum of 30 minutes after cessation of opercular movement.
***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*:

Acceptable Methods (as appropriate by species)	Acceptable with Conditions (as appropriate by species)
• Injected barbiturates • Injected anesthetic overdose	• Inhaled anesthetics • CO_2 • Penetrating Captive Bolt

• Topical buffered MS-222 • Benzocaine hydrochloride	• Blunt force trauma to the head

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

Reptiles:

Acceptable (as appropriate by species)	Acceptable With Conditions (as appropriate by species)
• Injected barbiturates • Injected anesthetic overdose	• Inhaled anesthetics as specified • CO_2 • Penetrating captive bolt or firearm Manually applied blunt force trauma to the head • Rapid freezing for animals < 4 g

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.

Bovids and Small Ruminants*:

Acceptable Methods	Acceptable with Conditions
• Injected barbiturates	• Penetrating captive bolt • Non-penetrating captive bolt • Potassium Chloride (KCl)** Magnesium sulfate ($MgSO_4$)***

*Confirmation of death must have combination of criteria including lack of pulse or lack of ausculted heartbeat, breathing, and corneal reflex or a secondary physical method such as pneumothorax or exsanguination

**(Adjunctive) Saturated KCl can be administered ONLY to a deeply anesthetized animal IV or IC

***(Adjunctive) Saturated $MgSO_4$ can be administered ONLY to a deeply anesthetized animal IV

Equine*:

Acceptable Methods	Acceptable with Conditions
• Injected barbiturates	• Penetrating captive bolt • Potassium Chloride (KCl)** • Magnesium sulfate ($MgSO_4$)*** • Lidocaine 2%****

*Confirmation of death must have combination of criteria including lack of pulse or lack of ausculted heartbeat, breathing, and corneal reflex or a secondary physical method such as pneumothorax or exsanguination

**(Adjunctive) Saturated KCl can be administered ONLY to an anesthetized animal IV or IC

***(Adjunctive) Saturated $MgSO_4$ can be administered ONLY to an anesthetized animal IV

****(Adjunctive) 2% lidocaine injected intrathecally (60mls)

Swine (Mature sows, Boars, and Grower-Finisher Pigs) *:

Acceptable Methods	Acceptable with Conditions
• Injectable barbiturates	• CO_2 • Penetrating captive bolt**

*Confirmation of death must have combination of criteria including lack of pulse or lack of ausculted heartbeat, breathing, corneal reflex or a secondary physical method such as pneumothorax or exsanguination

**Must be appropriately selected for the size of the animal. Secondary step should be performed (exsanguination, pithing)

Avian species*:

Acceptable Methods	Acceptable with Conditions
• Injected barbiturates	• CO_2** • Inhaled anesthetics

	• Cervical dislocation • Decapitation

*Confirmation of death must have a secondary method such as decapitation, exsanguination or cervical dislocation.

**Pre-fill the euthanasia chamber with CO_2, 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_2, cooling (<4°C for 4 hours) or freezing. If the egg is over 80% incubation, the methods specified above for older birds must be used.

For finfish, amphibian, and reptile eggs, larvae and embryos, acceptable euthanasia methods vary based on age and life stage. It is recommended to view the AVMA Guidelines for guidance on acceptable methods.

Tissue Collection Following CO_2 or Inhalant Anesthesia Exposure

If necessary, the animal can be removed from the CO_2 or inhaled anesthetic before complete cessation of breathing and euthanized by an appropriate physical method (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.

F. Mass Depopulation and Euthanasia in Emergency Situations

Mass depopulation refers to methods by which large numbers of animals must be destroyed quickly and efficiently with as much consideration given to the welfare of the animals as practicable, but where the circumstances and tasks facing those doing the depopulation are understood to be extenuating. The AVMA Guidelines for Depopulation of Animals can be found here[2].

In the event of an emergency requiring mass animal depopulation for reasons of animal welfare and/or public health, the AEC animal care community including veterinary,

research and animal care personnel will attempt to follow this policy and the AVMA Guidelines. If circumstances of an emergency occur such that following the euthanasia guidelines are unsafe and/or unachievable, methods of mass depopulation may be utilized.

G. 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) http://grants.nih.gov/grants/olaw/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.asas.org/docs/default-source/default-document-library/ag_guide_3rded.pdf?sfvrsn=4
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/arac-guidelines/b4_rodent_euthanasia_pup.pdf