University of Southern California

Institutional Animal Care and Use Committee

Euthanasia

A. Background

The purpose of this policy is to establish standard methods for the humane euthanasia of animals used in biomedical research and teaching at USC.

B. Definitions

Euthanasia: a "good death" in which death occurs rapidly with minimal pain and distress

IC**:** intracelomic injection route

IP: intraperitoneal injection route

IV: intravenous injection route

Large animals: animals include dogs, ferrets, goats, rabbits, sheep, swine, tree shrews

Rodent: animals include mice of the genus *Mus* and *Acomys*, rats of the genus *Rattus* and *Dipodomys*, gerbils, guinea pigs, hamsters

SC: subcutaneous injection route

C. Applicability

This policy applies to all individuals performing animal euthanasia to ensure humane methods are used in accordance with the AVMA Guidelines for the Euthanasia of Animals, USDA Animal Welfare Regulations, Public Health Service Policy on Humane Care and Use of Laboratory Animals, and The Guide for the Care and Use of Laboratory Animals.

The AVMA Guidelines state that animal euthanasia must be performed with the highest degree of respect. Euthanasia is generally performed when animals experience severe or chronic pain or distress that cannot be relieved, or at the end of the experimental procedure or project. The method of euthanasia must be described in the animal protocol, approved by the IACUC, and must be consistent with the recommendations of the AVMA Guidelines. Deviations from the methods described in this policy may be considered with scientific justification and approval by the IACUC.

Exceptions to this policy may only be made by DAR animal care and veterinary staff who may perform a primary method only in cases when using the Euthanex machine or under veterinary guidance.

D. Policy

Euthanasia should be performed quickly and effectively in a non-public area. Agents used for anesthesia and euthanasia must be pharmaceutical grade unless otherwise approved on the IACUC protocol.

The training of the personnel performing the euthanasia is critical. If any staff member needs assistance with the euthanasia, they should contact one of the Department of Animal Resources veterinarians for guidance.

The following methods are acceptable at USC. In general, euthanasia includes a primary chemical method that **must** be followed by a physical method unless otherwise indicated below or approved in the IACUC protocol. Other methods not listed below may be approved on the IACUC protocol with scientific justification and in accordance with the AVMA Guidelines.

See Appendix for a table summary of the information below.

Rodents

The primary method may include one of the following chemical methods:

CO2 narcosis

- Must be delivered from a pressurized tank source equipped with a regulator and flow meter set to a displacement rate between 30% to 70% of the chamber volume/min.
- The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO2, is unacceptable.
- Recommend performing euthanasia in animal's home cage to reduce stress from handling and novel environments.
- Whenever gradual displacement methods are used, CO2 flow should be maintained for at least 1 minute after respiratory arrest.
- If CO2 euthanasia is approved by the IACUC to be performed in the laboratory space, you must contact DAR to ensure appropriate chamber setup and flow rates (daradm@med.usc.edu).

Anesthetic overdose

- Pentobarbital +/- phenytoin (e.g. Euthasol®) dose of >200 mg/kg IP
- Ketamine/Xylazine SC or IP (2-3 times anesthetic dose recommended)

- Isoflurane delivered from a calibrated vaporizer dosed 5% or higher
- Isoflurane open drop is acceptable for euthanasia only with scientific justification and approval in the IACUC protocol.
  - Animals must be protected from direct contact with the anesthetic.
  - The procedure must be conducted in a fume hood for personnel safety.
  - Open drop is unacceptable in situations where anesthesia needs to be maintained (survival surgery, non-survival surgeries, and perfusions).
- Note: all vaporizers must have calibration checked every 2 years to ensure proper dosing

The secondary method may include one of the following physical methods. The animal must be non-responsive to noxious stimuli (firm toe pinch) or cessation of breathing confirmed before the physical method is performed.

- Cervical dislocation[+,*]
- Decapitation*

  Note: Specialized rodent guillotines are commercially available and must be kept clean, in good condition with sharp blades. Refer to the Guillotine Use and Maintenance section at the bottom of the policy
- Bilateral thoracotomy
- Dissection that ensures death (i.e. removal of major organs such as brain, heart, lung)
- Tissue perfusion
- Exsanguination

[+] Cervical dislocation is acceptable for mice only. Cervical dislocation is not an acceptable euthanasia method for other rodents of any size.
* Cervical dislocation (mice only) or decapitation may be used as a primary euthanasia method without anesthesia only with scientific justification and approval in the IACUC protocol. If used as a primary euthanasia method, each researcher to perform this method must contact DAR veterinary staff to demonstrate competency (vetmed@med.usc.edu).

Neonates

Precocial neonates should be euthanized as adults (e.g. *Acomys* or Guinea pigs).

Altricial neonatal rodents (pinkies) 0-6 days old must be euthanized by one of the following methods:

- Anesthesia followed by decapitation with sharp scissors
  - Hypothermia may be induced by laying pups with indirect contact (e.g. on a weigh boat) over ice-filled bucket until movement ceases.
  - Exposure to CO2 prior to decapitation

- Injection of species-appropriate anesthetic prior to decapitation
- Decapitation alone with sharp scissors

Large Animals

Species in this category are typically euthanized with pentobarbital +/- phenytoin (e.g. Euthasol®) at >100 mg/kg body weight IV under species-appropriate general anesthesia. Euthanasia is confirmed by thoracic auscultation to confirm cessation of heartbeat and lung sounds. A secondary physical method is not required, but may be performed (e.g. exsanguination, removal of major organs, etc.)

It is a requirement to contact DAR veterinary staff prior to performing euthanasia to demonstrate competency in techniques. Generally, a DAR veterinarian will perform the euthanasia unless a researcher has been sufficiently trained in and supervised by the veterinary staff.

Birds

Methods may include one of the following below the appropriate sub-heading.

Adults and hatched chicks:
Each of the below methods must be followed by a physical method to ensure death such as cervical dislocation or decapitation.

- IV injection of pentobarbital +/- phenytoin (e.g. Euthasol®) at 120 mg/kg while awake, under sedation, or species-appropriate general anesthesia
  IC route may be used under species-appropriate general anesthesia. Caution must be used to avoid injection into air sacs.
- Anesthetic overdose (2-3 times anesthetic dose)
- CO2 delivered via a pressurized tank source until cessation of breathing. Chambers must be of sufficient size for normal bird posture.
  Immersion into 100% CO2 is unacceptable.

Embryonated eggs:

- Prolonged exposure to CO2 for at least 20 minutes followed by freezing

Fish, Amphibians, Reptiles

Euthanasia methods may include one of the following below the appropriate sub-heading. Note that tricaine methanesulfonate (MS-222) is the anesthetic commonly used in this group of animals. Care must be taken when handling this chemical as it can pose a safety hazard to personnel.

Pharmaceutical-grade MS-222 must be used (source: https://syndel.com/product/syncaine/). Additionally, the compound must be buffered to a neutral pH (see below) prior to use for animals. This can be accomplished by mixing 1 part of MS-222 to 2 parts of sodium bicarbonate.

Fish

Adult and larvae (> 7 days post fertilization (dpf))

- Immersion in 250-500 mg/L MS-222 buffered with sodium bicarbonate to a pH 7-7.5. Fish must remain immersed for minimum 30 minutes following loss of opercular movements then frozen as secondary method.
- Rapid chilling (2°-4°C) for minimum 10 minutes following loss of opercular movements followed by freezing
  Ensure fish do not come in direct contact with the ice, but come in full contact with the chilled water for optimal exposure

Embryos and larvae (≤ 7 dpf)

- Rapid chilling (2°-4°C) for minimum 20 minutes following loss of opercular movements followed by freezing or addition of diluted sodium hypochlorite solution (500 mg/L)
  Ensure fish do not come in direct contact with the ice, but come in full contact with the chilled water for optimal exposure
- Immersion in diluted sodium hypochlorite solution (500 mg/L) for minimum 5 minutes

Amphibians

Each of the below methods must be followed by a physical method to ensure death such as double-pithing or decapitation followed by pithing. Decapitation alone is not an adequate secondary method.

- Immersion in 5g/L MS-222 buffered with sodium bicarbonate to a pH 7-7.5 for minimum 20 minutes
  Up to 1 hour maybe required to ensure death. Ensure sufficient water to cover the animal completely.
- IC or SC lymph sac injection of MS-222 at ≥ 250 mg/kg
- IC or SC lymph sac injection of pentobarbital +/- phenytoin (e.g. Euthasol®) at 100 mg/kg (Axolotl) or 1110 mg/kg (*Xenopus*)

Reptiles

Each of the below methods must be followed by a physical method to ensure death such as double-pithing or decapitation followed by pithing. Decapitation alone is not an adequate secondary method.

- IC or IV injection of pentobarbital +/- phenytoin (e.g. Euthasol®) at 100 mg/kg while animal is under species-appropriate general anesthesia
- Anesthetic overdose (2-3 times anesthetic dose)

- 2-stage administration of MS-222.
  1. IC injection of MS-222 (pH-neutralized) at 250-500 mg/kg
  2. Once unconscious, second IC injection of unbuffered 50% (v/v) MS-222

Other Species

For species not covered specifically in this policy, please contact the DAR veterinary staff for information regarding more specific euthanasia procedures (vetmed@med.usc.edu).

Verification of Death

For all species, it is important to verify death prior to final carcass disposition. Regardless of which method of euthanasia is performed, personnel must ensure that death has occurred. A combination of criteria is most reliable in confirming death, including lack of pulse, cessation of breathing, lack of corneal reflex, lack of response to firm toe pinch, inability to hear respiratory sounds and heartbeat by use of a stethoscope, graying of the mucous membranes, and rigor mortis. None of these signs alone, except rigor mortis, confirms death. Death can also be ensured by a secondary method such as cervical dislocation, bilateral thoracotomy, or pithing, depending on the species.

Secondary physical methods of euthanasia are also means to confirm death in reptilian, amphibian, and aquatic species. For fish, loss of movement, loss of reactivity to stimuli, initial flaccidity prior to rigor mortis as well as cessation of rhythmic opercular movement for at least 30 minutes, and loss of the vestibulo-ocular reflex are indicators that verify death. If there is any doubt that death has not occurred, a secondary method should be used or the physical exam should be repeated.

Carcass disposal

After death has been confirmed, place the carcass in a paper bag and into a designated carcass freezer. DAR will collect and incinerate non-hazardous animal carcasses.

Animals treated with biohazardous or chemical hazard substances have special bagging requirements. Please refer to EH&S' Animal Research Biosafety Manual for proper procedures. https://ehs.usc.edu/research/bio/animal-research-biosafety-program/

Guillotine, Sharp Scissors Usage and Maintenance

When euthanizing awake/conscious animals using either a guillotine or sharp scissors please observe the following criteria

- The guillotine or scissors operate in a smooth manner and is sufficiently sharp to work in one clean stroke
- Prior to operation, the blade is clean and rust free
- After use ensure to properly clean to remove any blood and organic material

Documentation

- Labs are required to demonstrate when scissors or guillotine blades are replaced or demonstrated to be in good working order
- Demonstration of good working order of the guillotine/scissors can be achieved by using a fresh carcass of the same species and operating the instrument in one smooth motion
- DAR provided logs maybe used, see Appendix

Each lab personnel will need to be individually assessed and approved for live decapitation of adult rodents

E. References

1. *The AVMA Guidelines* for the *Euthanasia* of Animals: *2020* Edition
https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf

2. ACLAM Task Force Statement on Rodent Euthanasia
https://www.aaalac.org/pub/?id=DA493B29-D28D-9B8A-3E64-142F58D51546

F. Appendix (next page)

Table Summary of Euthanasia Methods by Species

Species	Primary Method (select one)	Secondary Method (select at least one)
Mouse, Spiny Mouse	▪ CO2 overdose ▪ Anesthetic overdose	▪ Cervical dislocation ▪ Decapitation ▪ Bilateral thoracotomy ▪ Dissection that ensures death ▪ Tissue perfusion ▪ Exsanguination
Rat, Kangaroo Rat, Gerbil, Guinea Pig, Hamster, Chinchilla	▪ CO2 overdose ▪ Anesthetic overdose	▪ Decapitation ▪ Bilateral thoracotomy ▪ Dissection that ensures death ▪ Tissue perfusion ▪ Exsanguination
Dog, Ferret, Goat, Rabbit, Sheep, Swine, Tree Shrew	▪ IV pentobarbital +/- phenytoin while animal is under general anesthesia	▪ Not required, but must auscultate chest to confirm cessation of heartbeat
Birds	▪ IV pentobarbital +/- phenytoin while animal is awake, sedated, or under general anesthesia ▪ IC pentobarbital +/- phenytoin while animal is under general anesthesia ▪ Anesthetic overdose ▪ CO2 overdose	▪ Cervical dislocation ▪ Decapitation ▪ Dissection that ensures death ▪ Tissue perfusion ▪ Exsanguination
Fish	▪ MS-222 overdose ▪ Rapid chilling (2º-4ºC)	▪ Freezing ▪ Exposure to sodium hypochlorite solution (embryos and larvae ≤ 7 dpf only)
Amphibians	▪ MS-222 overdose - Immersion - IC or SC lymph sac injection ▪ IC or SC lymph sac pentobarbital +/- phenytoin	▪ Double-pith ▪ Decapitation followed by pithing
Reptiles	▪ IC or IV pentobarbital +/- phenytoin while animal is under general anesthesia ▪ Anesthetic overdose ▪ 2-stage administration of MS-222	▪ Double-pith ▪ Decapitation followed by pithing

Guillotine Usage and Maintenance Log

Refer to IACUC Policy for Euthanasia

this template is available on the DAR website.

Lab Location: _____ Principal Investigator: _____

Date Used	Species	# Euthanized	Personnel Initials	Date of Blade Sharpening/Blade Replacement