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UC Davis Institutional Animal Care and Use Committee (IACUC)

Title: Euthanasia Guidelines for Research and Teaching Animals

I. <u>Purpose:</u>

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

II. <u>Background:</u>

According to the *Guide for the Care and Use of Laboratory Animals, PHS Policy on the Humane Care and Use of Laboratory Animals,* and the *Animal Welfare Regulations,* methods of euthanasia must be consistent with the current American Veterinary Medical Association Guidelines (AVMA Guidelines) for Euthanasia of Animals, unless a deviation is justified for scientific or medical reasons. In addition, methods must be specified and approved in Animal Care and Use Protocols. Methods are chosen to minimize pain and distress to the animals as well as meet the needs of the research or teaching protocol.

III. Policy Statement:

Euthanasia is the procedure of ending the life of an animal rapidly, painlessly, and without distress. Euthanasia must be carried out by trained personnel using acceptable techniques in accordance with applicable regulations and policies. Euthanasia techniques should result in rapid unconsciousness followed by cardiac and/or respiratory arrest, lack of corneal reflex, and the ultimate loss of brain function.

The method of euthanasia must be an acceptable method as outlined in the AVMA Guidelines; deviation from the AVMA Guidelines require IACUC approval prior to implementation. If unique circumstances arise that require deviation from these guidelines the Principal Investigator (PI) must consult with Campus Veterinary Services (CVS) (530-752-0514, lahc@ucdavis.edu) or the veterinary care designee noted on the approved protocol.

Chemical methods of euthanasia (CO₂ chamber, inhalant or chemical anesthetics, sodium pentobarbital) must be confirmed by a secondary physical method and/or

through direct assessment (e.g., thoracic auscultation, ECG, observation; see below).

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, absence of respiratory sounds and heartbeat confirmed via 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 a 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 must be used or the physical exam should be repeated. Death must be confirmed before disposal.

Personnel performing euthanasia must be trained, knowledgeable, and proficient in the chosen technique(s), trained on how to ensure that euthanasia was successful, and training must be documented. Personnel using physical methods of euthanasia must have demonstrated proficiency for each type of physical method used to ensure euthanasia is conducted appropriately.

Individuals who need to perform cervical dislocation and/or decapitation without anesthesia in select adult species (see V. C., below), for research or teaching, must be certified to perform these techniques. Certification must be verified by an approved Designated Trainer. Individuals can obtain assistance in identifying an approved Designated Trainer by contacting CVS or the IACUC Office. Designated Trainers will provide training and assessment of the participant's skill level and verify the individual's proficiency in use of the technique(s). Once an individual has been certified as proficient in a particular technique they may be listed as certified to perform the technique on an approved Animal Care and Use Protocol.

Ideally, euthanasia should be performed in procedure rooms or laboratory space away from other animals. Death must be confirmed (see above) prior to bagging the carcass for disposal.

NOTE: Please refer to the <u>AVMA Guidelines for Euthanasia of Animals: 2020 or</u> <u>subsequent most recent version</u> for information on other species and additional euthanasia methods not covered in this policy.

IV. <u>Euthanasia of Mice and Rat Fetuses and Neonates:</u>

Rodent fetuses and other mammals 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 if fetuses are not collected.

- A. Chemical:
 - 1. Injection with an overdose of anesthetic or euthanasia solution should be used whenever possible.
- B. Hypothermia:
 - 1. Gradual cooling of fetuses and altricial neonates is acceptable with the following conditions:
 - i. This method can be used for fetuses and neonates ≤ 10 days of age.
 - ii. Gradual cooling must induce an anesthetized state. Preliminary data suggests approximately 15 minutes.
 - iii. The animal cannot be in direct contact with the ice or precooled surface used for hypothermia (i.e., place ≤10-day old neonates in a latex glove, plastic bag, or on a cloth first, and then place on a frozen surface).
 - iv. A secondary method must be performed after the animal is anesthetized. Secondary methods include:
 - 1. Decapitation
 - 2. Bilateral thoracotomy
 - 3. Removal of major organs
- C. Rapid freezing (only in fetuses and neonates <5 days of age):
 - Altricial neonates less than 5 days of age do not have sufficient central nervous system development to perceive pain and may be quickly euthanized by rapidly freezing in liquid nitrogen (N₂).
- D. Decapitation (acceptable for fetuses and altricial neonates):
 - 1. Documented training for this procedure is required.
 - 2. Use of sharp scissors or sharp blades is required.
 - 3. Depending on the developmental stage of animal and tissue mass, this technique may require the use of a guillotine.
- E. Cervical Dislocation (acceptable for fetus and neonates):
 - 1. Documented training and proficiency are required to perform this technique.
 - 2. Appropriate disruption of the spinal cord in the high cervical region is required.

Neonatal rodents are resistant to the effects of CO_2 , thus an adjunctive method (e.g., cervical dislocation or decapitation) should be performed after a neonate is nonresponsive to painful stimuli. The use of CO_2 as a sole method of euthanasia in neonates is strongly discouraged and must be justified and approved in an IACUC protocol.

Mice and rats older than 10 days of age should be euthanized following the guidelines for adult rodents.

*Precocious neonates (i.e., guinea pigs) should be treated as adults *Opossums should be treated as neonates until 36 days of age

V. Other Euthanasia Methods:

A. Carbon dioxide (CO₂)

A CO_2 chamber is the most common method of euthanasia for small rodents. The chamber must allow viewing of the animal during euthanasia. Proper technique must be followed to ensure a humane death, as CO_2 has noxious properties that can cause unnecessary pain and distress.

- 1. Euthanasia in the home cage is recommended. If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses. Do not overcrowd the chamber; all animals in the chamber must be able to make normal postural adjustments in a single level. If combining animals from multiple cages for euthanasia, euthanasia must be performed immediately to prevent animals from fighting. Only animals of the same species may be euthanized in the same chamber at the same time.
- 2. The flow rate for CO₂ euthanasia systems should displace 30% to 70% of the chamber or cage volume/min. Directions and signage detailing displacement rate and chamber size are available in areas where CO₂ euthanasia is used. The higher range is recommended and should be continued at least one minute after respiratory arrest.
- 3. Prefilled chambers are unacceptable.
- 4. An appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size of the container utilized are required.
- 5. Remove the animal from the chamber and confirm the absence of respiration. It is important to verify that an animal is dead after exposure to CO₂. Death must be confirmed by a secondary physical method (i.e., bilateral thoracotomy, cervical dislocation, decapitation) or through a combination of other criteria (see above re: confirmation of death).
- 6. Clean the chamber with disinfectant to remove all urine, feces, and fur.
- 7. **CO**₂ generated from dry ice is <u>NOT</u> an acceptable method of euthanasia.

B. Potassium Chloride (KCl) saturated solution.

Personnel performing this technique must be trained and knowledgeable in anesthetic techniques and be competent in assessing the level of unconsciousness that is required prior to administration of potassium chloride solution intravenously (IV). Administration of potassium chloride solution IV requires animals to be within a surgical plane of anesthesia characterized by loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli.

- C. Cervical dislocation (without anesthesia) of rodents, small rabbits, chickens, and other birds:
 - 1. Cervical dislocation is acceptable for mice and rats <200 g.
 - 2. The use of cervical dislocation for euthanasia is limited to small birds (<200 g), chickens, mice, immature rats (< 200 g), and rabbits (< 1 kg).
 - 3. All users approved to perform cervical dislocation (without anesthesia in nonneonates described above) as the method of euthanasia must be certified by one of the approved campus trainers. *Personnel will be trained using at least two anesthetized/euthanized animals and must then demonstrate proficiency on two live, conscious animals to receive certification*.
- D. Decapitation (without anesthesia) of rodents, small rabbits, poultry, and other birds:
 - 1. Decapitation is acceptable for mice and rats.
 - Decapitation is justified for studies where undamaged and uncontaminated brain tissue is required. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure it is effective.
 - 3. All users approved to perform decapitation (without anesthesia in non-neonates described above) as the method of euthanasia must be certified by one of the approved campus trainers. *Personnel will be trained using at least two anesthetized/euthanized animals and must then demonstrate proficiency on two live, conscious animals to receive certification.*
- E. Perfusion or exsanguination.
 - 1. When animals to be euthanized are fully anesthetized, adjunctive methods such as bilateral thoracotomy, exsanguination, perfusion, and IV or intracardiac injection of potassium chloride are acceptable.

- 2. While an incision may be made to carry out these tasks, exsanguination and perfusion are not considered terminal surgery, but are instead adjunctive methods of euthanasia.
- F. Euthanasia of amphibians, reptiles, and fish.
 - 1. Pithing of amphibians:

Double pithing (destroying both the brainstem *and* spinal cord) can be used as a second-step euthanasia method in unconscious animals when performed by properly trained individuals.

- Decapitation of amphibians and reptiles: After an animal has been anesthetized, decapitation must be followed by double pithing or another method to destroy the brain and spinal cord. Decapitation can only be used as part of a 3-step euthanasia process (anesthesia, decapitation, pithing).
- 3. Buffered Tricaine Methanesulfonate (MS222) in amphibians and fish: The solution must be buffered with sodium bicarbonate resulting in a pH between 7.0-7.5. A 1:2 ratio of MS222 with sodium bicarbonate is recommended (e.g., 2 g of MS222 with 4 g of sodium bicarbonate in a 1 L solution). Due to species differences in response to MS222, a secondary method of euthanasia is recommended in some finfish and amphibians to ensure death (see above).
 - Amphibians MS222 dose ≥ 2-5 g/L (immersion for at least one hour). If immersion time is less a secondary euthanasia method must be used.
 NOTE: Overdose of MS222 in frogs must be followed by pithing with or without decapitation.
 - b. Finfish MS222 dose = 500 mg/L (immersion for at least 30 minutes). If immersion time is less a secondary euthanasia method must be used.
 - c. Please review the Standard of Care for MS-222 Preparation and Use.
- 4. Rapid chilling (hypothermic shock) in finfish: Rapid chilling is acceptable for small-bodied (3.8-cm-long or smaller; measured from the tip of the snout to the posterior end of the last vertebrae) tropical and subtropical stenothermic finfish, for which the lower lethal temperature is above 4°C.
- 5. Perfusion with fixative of a deeply anesthetized animal can be used to euthanize amphibians and reptiles when scientifically justified. Adequate depth of anesthesia must be verified prior to perfusion taking place.

Because it is often difficult to confirm that an amphibian or reptile is dead, the application of two or more euthanasia procedures is recommended.

VI. Equipment Maintenance:

In accordance with the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition "The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades."

- A. Decapitation may be accomplished by use of a commercial guillotine, dedicated scissors or razor/scalpel blades.
 - 1. Scissors, razors, or scalpel blades may only be used for neonatal rodents (altricial neonates less than 10 days of age) and small amphibians/fish.
 - 2. Dedicated scissors must be clean, in good condition, sharp, and move freely.
 - 3. A new razor and/or scalpel blade must be used for each animal to ensure it is clean, in good condition, and sharp.
 - 4. Guillotine:
 - a. Guillotines used to perform decapitation must be maintained in good working condition, serviced on a regular basis to ensure sharpness of blades, and cleaned after each use.
 - b. Before each use of a guillotine, it should be checked for rust, lack of visible nicks or other damage to the cutting edges, and cleanliness. The operator should ensure that the action is smooth with no perceptible binding or resistance.
 - c. A record certifying maintenance/sharpening of a guillotine must be maintained. The IACUC recommends sharpening at least annually, however the species involved, the number of animals, and manufacturer guidelines will dictate how often the blades need to be sharpened.
 - d. Professional blade sharpening services may be used.
 - e. If lubrication of the guillotine is necessary, the use of a Teflon or Silicone containing compound is recommended.
 - f. Old guillotine blades that are no longer serviceable must be discarded in a sharps container.
 - g. The responsibility for sharpening the guillotine rests with the PI.
 - h. Guillotines and their maintenance records will be inspected as part of the IACUC semi-annual inspections.
 - i. The IACUC requires that all individuals using guillotines be trained and certified on proper use. Training must be documented.

VII. <u>Animal Carcass Disposal:</u>

After death has been verified, place the carcass in a disposable waterproof bag. Seal the bag and place the bagged carcass in an area designated for carcass disposal. This may include a barrel or bin inside a designated cold room, refrigerator, or freezer. Research animal carcasses must never be placed in the regular trash. Some untreated wild type rodents and other animal carcasses are used as food for predator species (for example,

raptors or snakes). Transgenic and/or genetically modified animals must not be used as food for other animals. The method of euthanasia must be compatible for use as food stuff. For example, animals euthanized with a barbiturate overdose should not be used as food for other animals.

Carcasses that are radioactive must be disposed of according to the procedures stated in the Principal Investigator's Radioactive Use Authorization. Carcasses that are infectious must be disposed of according to the procedures stated in the Principal Investigator's Biological Use Authorization. Carcasses that have chemical contamination must be disposed of according to procedure established during review of the protocol by the Chemical Hygiene Officer.

VIII. <u>Resources:</u>

- 1. 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
- ILAR, Guide for the Care and Use of Laboratory Animals <u>http://nap.edu/12910</u>
- 3. PHS Policy https://olaw.nih.gov/policies-laws/phs-policy.htm
- SC-40-406 MS-222 Preparation and Use <u>https://research.ucdavis.edu/wp-content/uploads/SC-40-406.pdf</u>

	Acceptable	Acceptable with	
Species	Methods	Conditions	Notes
Amphibians	 Injected barbiturates Dissociative agents and anesthetics as specified Topical or injected buffered MS-222 Topical Benzocaine hydrochloride 	 Inhaled anesthetics as specified CO₂ Penetrating captive bolt or firearm Manually-applied blunt force trauma to the head Rapid freezing of small (<4 g) animals where immediate death occurs 	Because it is often difficult to confirm that an amphibian is dead, the application of two or more euthanasia procedures is usually recommended. Consulting multiple references on amphibian euthanasia is advised as a means of identifying methods that are most appropriate for a given species and set of circumstances. (AVMA, 2020)
Aquatic Invertebrates (Cephalopods)	 Immersion in anesthetic solution (magnesium salts, clove oil, eugenol, ethanol) 	 Adjunctive methods include 70% alcohol and neutral-buffered 10% formalin, pithing, freezing, boiling 	
Avian Species (non-poultry)	 Intravenous barbiturates 	 Inhaled anesthetics Other gas inhalation (CO₂, CO, N₂, Ar) Cervical dislocation (small birds) Decapitation (small birds) Gunshot (free-ranging birds) 	
Dogs and Cats	 Intravenous barbiturates Injected anesthetic overdose Tributame T-61 	 Barbiturates (alternate routes of administration) Inhaled anesthetic overdose CO* CO₂* Gunshot* Penetrating captive bolt** 	*Not recommended for routine use **Acceptable with conditions in dogs only; not recommended for routine use
Finfish	 Immersion in buffered benzocaine or 	 Eugenol Isoeugenol Clove oil CO₂-saturated water) 	

Appendix A: Table of Acceptable and Acceptable with Conditions Methods by Species from the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

	benzocaine hydrochloride Isoflurane Sevoflurane Quinaldine sulfate Buffered MS- 222 2- phenoxyethanol Injected pentobarbital Rapid chilling (appropriate species) Ethanol	 Decapitation/cervical transection/manually applied blunt force trauma followed by pithing or exsanguination Maceration (research setting) Captive bolt (large fish) 	
Nonhuman Primates	 Intravenous barbiturates Anesthetic overdose 	 Inhaled anesthetics CO₂ CO 	When animals to be euthanized are fully anesthetized, adjunctive methods such as bilateral thoracotomy, exsanguination, perfusion, and IV or intracardiac injection of potassium chloride are acceptable (AVMA, 2020).
Poultry	 Injected barbiturates Anesthetic overdose 	 Other gas inhalation (CO₂, CO, N₂, Ar) Low-atmospheric- pressure stunning Cervical dislocation (as appropriate) Decapitation Manual blunt-force trauma Electrocution Gunshot Captive Bolt 	
Rabbits	 Intravenous barbiturates 	 Inhaled anesthetic overdose CO₂ Cervical dislocation (as anatomically appropriate) Penetrating captive bolt 	

		 Non-penetrating captive bolt 	
Reptiles	 Injected barbiturates/MS -222 Dissociative agents with adjunctive methods and anesthetics as specified 	 Inhaled anesthetics as specified CO₂ Penetrating captive bolt or firearm Manually applied blunt force trauma Rapid freezing for animals < 4 g where immediate death occurs Spinal cord severance/ destruction of brain (crocodilians) 	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 (AVMA, 2020).
Rodents and Opossums	 Injected barbiturates and barbiturate combinations Injected dissociative agent combinations 	 Inhaled anesthetics CO₂* CO Tribromoethanol Cervical dislocation Decapitation Focused beam microwave irradiation 	 *Conditions required for CO₂ euthanasia Source is from a compressed gas CO₂ cylinder (dry ice and other sources not acceptable) Flow rate displaces 30%- 70% of the chamber volume per minute (prefilled chambers are not acceptable) Flow should be maintained for one minute after respiratory arrest Death is verified by physical exam or ensured by an adjunctive physical method Animals should be euthanized in their home cages whenever possible Chamber should be cleaned after each use
Ruminants, Camelids, and Horses	 Injected barbiturates 	 Penetrating captive bolt Gunshot to the head CO2* Non-penetrating captive bolt* 	*Goat kids only

Swine (Mature	 Injected 	 Other gas inhalation 	*Piglets only
sows, Boars,	barbiturates	(CO ₂ , CO, NO, N ₂ , Ar)	
and Grower-		 Gunshot 	
Finisher Pigs)		 Electrocution 	
		 Penetrating captive 	
		bolt	
		 Non-penetrating 	
		captive bolt*	
		 Manually-applied 	
		blunt force trauma	