TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER
EL PASO

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
Policy #9
Rodent Euthanasia
In concurrence with
TTUHSC El Paso Assurance #D19-01056
and Federal Regulations and Guidelines

Purpose
To define the regulations for rodent euthanasia at TTIHSC El Paso.

Overview
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[1] serve as the standard for acceptable methods on euthanasia.

Recommended euthanasia methods for rodents are described in the table below. Any other method of euthanasia requires justification in the Animal Protocol. For other species, the Principal Investigator should consult with the Institutional Veterinarian for recommendations.

The following guidelines provide important criteria for the successful implementation of euthanasia.
- 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 an inhalant or injectable euthanasia procedure, death must be confirmed by a secondary method as noted below.
- Euthanasia may not be performed in the animal housing room.
- The euthanasia method must be appropriate to the species, approved in the animal study proposal, and conform to the most recent *AVMA guidelines for the euthanasia of animals*[1]. The euthanasia method must observe the conditions and precautions in the pertinent sections of that report, and to NIH guidelines in their latest revisions[2-4].

Recommended Euthanasia Methods

Species	Age	Methods	Notes
Mouse Rat Hamster Gerbil	Pregnant dam	Barbiturates CO_2 inhalation Isoflurane/Sevoflurane inhalation Cervical dislocation Decapitation	Cervical dislocation is appropriate for rats and mice less than 200g when performed under anesthesia. Decapitation is acceptable when justified by experimental conditions in the IACUC protocol.
	pups in utero	Euthanize dam	According to the AVMA guidelines, rodent fetuses 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.
	0-10 days old	Anesthetic overdose (Ketamine/Xylazine, Barbiturates)	At this stage, neonates are resistant to carbon dioxide euthanasia.

		Cervical dislocation/Decapitation under anesthesia Isoflurane/sevoflurane	
	Adult (>10 days old)	Barbiturates CO_2 inhalation Isoflurane/sevoflurane inhalation Cervical Dislocation Decapitation	Cervical dislocation is appropriate for rats and mice less than 200g when performed under anesthesia. Decapitation is acceptable when justified by experimental conditions in the IACUC protocol.
Guinea pig	Pregnant dam	Anesthetic overdose (Ketamine/Xylazine, Barbiturates) Carbon dioxide	
	pups in utero	Euthanize dam	According to the AVMA guidelines, rodent fetuses 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.
	Birth → adult	Anesthetic overdose (Ketamine/Xylazine, Barbiturates) Carbon dioxide	-
Other Species	-	-	Consult the Institutional Veterinarian

Secondary Methods to Confirm Death after Inhalant on Injectable Euthanasia

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, administration of an inhalant on injectable overdose must be followed by one of the following secondary procedures:

- Cervical dislocation
- Decapitation
- Exsanguination
- Bilateral thoracotomy
- Major organ removal

Guidelines for Euthanasia of Rodents Using Carbon Dioxide

CO_2 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 CO_2 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 carbon dioxide at a rate of 30-70% (liters per minute). Appropriate flow rate for each species and cage type will be posted in the LARC.
- Animals should be left in the container until breathing has ceased. Observe for one additional minute and use a secondary method to ensure clinical death
- The use of dry ice for CO_2 euthanasia is not permitted.

Guidelines for Euthanasia by Cervical Dislocation

The IACUC allows cervical dislocation to be used as a primary method for euthanasia only for mice and rats (under 200g body weight), and only after demonstration by appropriate lab members of proficiency in the technique. In most cases the IACUC will require anesthesia or sedation prior to cervical dislocation, and bypassing this requirement must be justified and approved in the IACUC protocol.

In heavier animals (e.g., rats over 200g body weight and rabbits over 1 kg body weight), the greater muscle mass in the cervical region makes manual cervical dislocation physically more difficult. Therefore, other methods of euthanasia must be performed on these types of animals.

Guidelines for Euthanasia by Decapitation

The IACUC generally discourages the practice euthanizing adult animals by decapitation, but recognizes that for some studies this method may be necessary. Decapitation must be performed by trained personnel, in a safe manner, and using sharp instruments. In most cases the IACUC will require anesthesia or sedation prior to decapitation, and bypassing this requirement must be justified and approved in the IACUC protocol.

The PI is responsible for maintaining their decapitation apparatus in good working order, including maintenance of blade sharpness. The use of plastic cones to restrain unanesthetized animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal for decapitation. Therefore, the use of plastic cones prior to decapitation is strongly encouraged in cases where anesthesia will not be used.

A. Rodents: Euthanasia by decapitation should normally be performed while the animal is under general anesthesia and may be used in research settings only when its use is required by the experimental design and is approved by the IACUC. Although it has been demonstrated that electrical activity in the rodent brain persists for 13 to 14 seconds following decapitation [5], more recent studies indicate that this activity does not infer the ability to perceive pain, and conclude that loss of consciousness develops rapidly [6]. Therefore, decapitation of rodents and small rabbits (<1 kg) is conditionally acceptable if performed correctly by trained personnel.

Training personnel to perform euthanasia

It is the PI's responsibility to determine that all personnel have been trained to perform the techniques listed in the protocol, and to monitor that personnel consistently apply it humanely and effectively. PIs who need help training their personnel should reach out to the Institutional Veterinarian and LARC staff.

Exceptions

Exceptions to these guidelines will be considered by the IACUC on a case-by-case basis.

Related policies

Investigators must comply with all other institutional policies at TTUHSC El Paso and Federal Guidelines. This list includes, but is not limited to, the following:

IACUC policy 23: Use of Zebrafish for Research and Teaching

References

1. AVMA Guidelines for the Euthanasia of Animals, 2020
2. https://www.avma.org/sites/default/files/2020-01/2020_Euthanasia_Final_1-15-20.pdf 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
3. NIH Office of Animal Care and Use. Guidelines for the Euthanasia of Rodents Using Carbon Dioxide, rev 01/25/17 http://oacu.od.nih.gov/ARAC/index.htm
4. NIH Notice NOT-OD-02-062 released 7/27/2002

5. Cooper JE, Ewbank R, Platt C, et al. Euthanasia of amphibians and reptiles. London: UFAW/WSPA, 1989.
6. Holson RR. Euthanasia by decapitation: evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. Neurotoxicol Teratol 1992; 14:253-257.
7. Vanderwolf CH, Buzak DP, Cain RK, et al. Neocortical and hippocampal electrical activity following decapitation in the rat. Brain Research 1988; 451:340-344.