

Purdue University Laboratory Animal Program Standards of Care

Title: Euthanasia Procedures for the Purdue University Animal Care Program

I. Purpose:

The purpose of this policy is to establish minimum standards for euthanasia for species in the lab animal setting.

II. Policy:

All units providing animal care must meet or exceed these minimum requirements for euthanasia based on the Guide for the Care and Use of Laboratory Animals, the Ag Guide, and the AVMA Guidelines for the Euthanasia of Animals. Research staff shall use the methods approved in their animal protocol. Veterinary Staff and Husbandry staff may use an AVMA-approved method as described below, provided the appropriate training and certification have been completed.

III. Procedure:

Refer to the most current AVMA Guidelines on Euthanasia for approved euthanasia methods:

The agents and methods of euthanasia appropriate for rodent species are available in the most current AVMA Guidelines for the Euthanasia of Animals. Euthanasia is the procedure of killing 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. The method used should not interfere with postmortem evaluations. Proper euthanasia involves skilled personnel to help ensure that the technique is performed humanely and effectively and to minimize risk of injury to people. Personnel who perform euthanasia must have training and experience with the techniques to be used. The equipment and materials required to perform euthanasia should be readily available, and the attending veterinarian or a qualified animal scientist/trainer should ensure that all personnel performing euthanasia have demonstrated proficiency in the use of the techniques selected.

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. In addition, the technique used should minimize any stress and anxiety experienced by the animal before unconsciousness. For this reason, anesthetic agents are generally acceptable, and animals of most species can be quickly and humanely euthanized with the appropriate injection of an overdose of a barbiturate. Certain other methods may be used for euthanasia of anesthetized animals because the major criterion (insensibility) has been fulfilled.

Physical methods of euthanasia may be used if persons performing the procedure are properly trained. If physical methods such as cervical dislocation are used, anesthetizing the rodent prior to this procedure is recommended. Every attempt should be made to minimize stress to the animal before euthanasia.

If euthanizing more than one animal at a time in the same chamber, the chamber must not be overcrowded. Animals must be able to move freely and make normal postural adjustments. Only animals of the same species may be euthanized in the same chamber at the same time.

Regardless of the method of euthanasia that is performed, personnel must ensure that death has occurred. A combination of criteria is most reliable in confirming death, including lack of pulse, breathing, corneal reflex, and 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.

Personnel shall be trained on how to assure death in animals.

2020 AVMA Guidelines for the Euthanasia of Animals

Amphibians:

Acceptable Methods (as appropriate by species)	Acceptable with Conditions (as appropriate by species)
Injected barbiturates Dissociative agents and anesthetics as specified Topical or injected buffered MS-222 Topical Benzocaine hydrochloride	Inhaled anesthetics as specified CO ₂ Penetrating captive bolt or firearm Manually-applied blunt force trauma to the head Rapid freezing of small (<4 g) individuals 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 reptile 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:

Acceptable Methods	Acceptable with Conditions
Immersion in anesthetic solution (magnesium salts, clove oil, eugenol, ethanol)	Adjunctive methods (second step) include 70% alcohol and neutral-buffered 10% formalin, pithing, freezing, boiling

Avian species (see Poultry below):

Acceptable Methods	Acceptable with Conditions
Intravenous barbiturates	Inhaled anesthetics CO ₂ CO Ar N ₂ Cervical dislocation (small birds) Decapitation (small birds) Gunshot (free-ranging birds)

Dogs and Cats:

Acceptable Methods	Acceptable With Conditions
Intravenous barbiturates Injected anesthetic overdose Tributame T-61	Barbiturates (alternate routes of administration) Inhaled anesthetic overdose CO* CO ₂ * Gunshot* Penetrating captive bolt**

*Not recommended for routine use

**Acceptable with conditions in dogs only; not recommended for routine use

Finfish:

Acceptable Methods	Acceptable with Conditions
Immersion in buffered benzocaine or benzocaine hydrochloride Isoflurane Sevoflurane Quinaldine sulfate Buffered tricaine methanesulfonate 2-phenoxyethanol Injected pentobarbital Rapid chilling (appropriate species) Ethanol	Eugenol Isoeugenol Clove oil CO ₂ -saturated water) Decapitation/cervical transection/manually applied blunt force trauma followed by pithing or exsanguination, Maceration (research setting) Captive bolt (large fish)

Poultry:

Acceptable Methods	Acceptable with Conditions
Injected barbiturates Anesthetic overdose	CO ₂ CO N ₂ Ar Low-atmospheric-pressure stunning Cervical dislocation (as anatomically appropriate) Decapitation Manual blunt-force trauma Electrocutation Gunshot Captive Bolt

Rabbits:

Acceptable Methods	Acceptable With Conditions
Intravenous barbiturates	Inhaled anesthetic overdose CO ₂ Cervical dislocation (as anatomically appropriate) Penetrating captive bolt Non-penetrating captive bolt

Reptiles:

Acceptable Methods (as appropriate by species)	Acceptable With Conditions (as appropriate by species)
Injected barbiturates/MS-222 Dissociative agents with adjunctive methods and anesthetics as specified	Inhaled anesthetics as specified CO ₂ 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:

Acceptable Methods	Acceptable with Conditions
Injected barbiturates and barbiturate combinations Injected dissociative agent combinations	Inhaled anesthetics CO ₂ * CO Tribromoethanol Cervical dislocation Decapitation Focused beam microwave irradiation

*Conditions required for CO₂ euthanasia provided

- 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 and Horses:

Acceptable Methods	Acceptable with Conditions
Injected barbiturates	Penetrating captive bolt Gunshot to the head CO ₂ * Non-penetrating captive bolt**

*CO₂ (goat kids only)

**Non-penetrative captive bolt (goat kids only)

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

Acceptable Methods	Acceptable with Conditions
Injected barbiturates	CO ₂ CO NO
	N ₂ Ar Gunshot Electrocution Penetrating captive bolt Non-penetrating captive bolt* Manually-applied blunt force trauma

*Non-penetrating captive bolt (piglets)