



INDIANA UNIVERSITY

Office of the Vice President for Research
Office of Research Compliance

Institutional Animal Care and Use Committee (IACUC) Office of Research Compliance (ORC)

Euthanasia of Laboratory Animals

FULL POLICY CONTENTS

Policy Statement
Responsibilities
Procedures
Sanctions
Contacts
References

Effective: 11-27-2017
Last Updated: 9-28-2020

Responsible University Office:
Fred H. Cate
Vice President for Research

Policy Owner:
*Bloomington Institutional Animal Care and Use
Committee (BIACUC)*

Policy Contact:
IACUC Administrator

Policy Statement

The following policy outlines the approved procedures for humanely euthanizing laboratory animals used at Indiana University-Bloomington. This document applies to all personnel, including research staff and husbandry staff, engaged in or associated with euthanizing animals at IUB. Contact the LAR office (extension 52356) or lar@indiana.edu, if training is needed on these techniques.

Responsibilities

It is the responsibility of each person engaged in performing euthanasia of animals to follow all procedures and guidelines as stated in this policy as well as current standards set by regulating agencies including but not limited to the United States Department of Agriculture (USDA), Office of Laboratory Animal Welfare (OLAW), and AAALAC. All

pertinent information can be referenced within the current version of the *AVMA Guidelines for Euthanasia of Animals*.

Procedures

Definitions

AVMA- American Veterinary Medical Association

Euthanasia- painless death

General

(1) *Finding a suitable agent*: Identify the suitable anesthetic or euthanasia agent for the species in use and the route of administration that is suitable for that species, as per Section E of the BIAUCUC protocol.

(2) *Gas scavenging*: Anesthetic gases must be used in an approved anesthesia machine with appropriate gas scavenging capability, in a fume hood, or with a snorkel scavenging system nearby. This equipment will be used according to the manufacturer's recommendations.

(3) *Proper restraint*: Animals will be handled gently and restrained in a manner appropriate for the species. Mechanical restraint devices suitable for the species may be used when appropriate.

(4) *Euthanasia in isolation*: If possible, euthanasia will not be performed on an animal while another animal is in close proximity, or in animal holding rooms unless approved by the BIAUCUC.

(5) *Personnel Training*: Personnel must have appropriate training and guidance from the veterinary staff or other qualified personnel prior to performing anesthesia or euthanasia.

Note: Personnel performing decapitation or cervical dislocation without the use of anesthetics must demonstrate their proficiency to IUB veterinarians before performing these procedures.

Summary Table of Allowable Methods of Euthanasia for IUB Animal Species

METHOD ^{1,19}	Species						
	Amphibians	Birds	Fish	Ferrets/Bats	Rabbits	Reptiles	Rodents and Small Animals
Barbiturates ²	A	A	A	A	A	A	A
Injectable anesthetics ³	A	A	U	A	A	A	A
Inhalant anesthetics ⁴	U	AC	U	AC	AC	AC	AC
Carbon dioxide ⁵	U	AC	U	AC	AC	U	AC
Clove oil, Eugenol or Isoeugenol ⁶	U	U	AC	U	U	U	U
Microwave Irradiation ⁷	U	U	U	U	U	U	AC
Tricaine methanesulfonate ⁸	A	U	A	U	U	U	U
Benzocaine hydrochloride ⁹	A	U	A	U	U	U	U
Cervical dislocation ¹⁰	U	AC	U	AC	AC	U	AC
Decapitation ¹¹	AC	AC	AC	AC	AC	AC	AC
Penetrating Captive bolt ¹²	AC	U	U	U	AC	AC	U
Pithing ¹³	AC	U	U	U	U	AC	U
Stunning ¹⁴	AC	U	AC	U	U	AC	U
Rapid chilling/Hypothermia ¹⁵	U	U	A	U	U	U	AC
The methods listed below require that animals be anesthetized before euthanasia is performed.							
Electrocution ¹⁶	U	U	U	U	U	U	U
Exsanguination under anesthesia	A	A	A	A	A	A	A
Potassium chloride under anesthesia ¹⁷	A	A	A	A	A	A	A
Terminal procedures (incl. removal of a vital organ and pneumothorax) ¹⁸	A	A	A	A	A	A	A
Rapid freezing under anesthesia	AC	U	U	U	U	AC	AC

Abbreviation codes key:

- A = Acceptable**- those which consistently produce a humane death when used as the sole means of euthanasia;
AC = Acceptable with Conditions- those which consistently produce a humane death when specific conditions are met;
U = Unacceptable- deemed inhumane under any conditions, or found to pose substantial risk to humans applying the technique.

- 1 These guidelines are based on 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 Intravenous (IV) injection is the preferred method for euthanasia. Intraperitoneal (IP) or intracoelemic injection may be used in situations when IV injection would be distressful or dangerous. **Intracardiac (IC) injection must only be used if the animal is unconscious or anesthetized.** Euthanasia dose is 3 times the anesthetic dose. Urethane must be used as an anesthetic before application of an approved adjunctive method.
- 3 Dissociative anesthetics can be used in combination with an α_2 -adrenergic receptor agonist or benzodiazepine.
- 4 Inhalant anesthetics can be used for rodents when physical restraint is difficult and require longer exposure times if used alone. It is recommended to use a secondary method to ensure death. Either of equal preference: isoflurane, or sevoflurane, **exposure to high concentrations resulting in rapid loss of consciousness is preferred.** In rabbits and other species that show aversive reactions to inhaled anesthetics, it is best to pre-anesthetize the animal with a sedative prior to removal from the home cage.
- 5 **Compressed gas in cylinders** is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. Flow rate should be **30-70% of chamber volume/minute in rodents.** For neonates and immature animals, the time required for euthanasia may be prolonged. In small rodents, **carbon dioxide exposure must be followed by an active method to ensure death (e.g. bilateral pneumothorax, exsanguination, decapitation, removal of a vital organ).** Prefilled chambers are not recommended. In rabbits, a carefully controlled displacement rate of 50-60% must be used. This method is not preferred and must be scientifically justified.
- 6 Clove oil, eugenol, or isoeugenol based products must be standardized, with known concentrations of essential oils so that accurate dosing can occur. Fish should be immersed in a tank containing >1.2 g/L anesthetic solution in water for a minimum of 10 minutes following cessation of opercular movement.
- 7 For use with small laboratory rodents only. Must be justified to ensure specially designed equipment is used.
- 8 **Fish >14 days of age** may be euthanized by immersion in a tank containing **tricaine methanesulfonate at a concentration of \geq 250 mg/liter of water for for 30 minutes after cessation of opercular movement followed by a secondary physical method to ensure death.** Amphibians require a higher concentration of 5 g/L and must be immersed for at least 1 hour. In all species, the solution should be buffered to a pH of 7.0-7.5 with sodium bicarbonate. If using an IACUC approved secondary method of euthanasia,

- fish and frogs may be immersed until they reach a surgical plane of anesthesia (no reflex responses, no response to stimuli), then the secondary method may be applied. It may take 10 minutes or more to reach an appropriate plane.
- 9 Fish and amphibians may be euthanized by immersion in a tank or recirculation system containing **benzocaine hydrochloride at a concentration of >250 mg/liter of water for at least 30 minutes after cessation of opercular movements**. If using an IACUC approved secondary method of euthanasia, fish and frogs may be immersed until they reach a surgical plane of anesthesia (no reflex responses, no response to stimuli), then the secondary method may be applied. It may take 10 minutes or more to reach an appropriate plane.
 - 10 Manual cervical dislocation is conditionally acceptable in mice, gerbils, hamsters, and other small rodents, bats, **rats weighing less than 200 g**, and rabbits or ferrets weighing less than 1 kg. Cervical dislocation may be performed on larger rats and rabbits manually by an individual with demonstrated proficiency or if a mechanical dislocator is utilized.
 - 11 **In amphibians, fish, and reptiles, decapitation should be followed by pithing of both the brain and spinal cord.** Decapitation is also acceptable for neonates less than 10 days of age. This method provides uncontaminated tissues.
 - 12 This method requires a secondary method to confirm euthanasia (e.g. exsanguination, etc.). For fish and reptiles, this should only be used in large species.
 - 13 May be used as only as secondary method of euthanasia in species with anatomic features that facilitate easy access to the central nervous system (e.g. frogs).
 - 14 **Stunning is unacceptable as a sole method of euthanasia.** If performed properly, stunning will produce unconsciousness but must be followed by decapitation or pithing to ensure the animal's death.
 - 15 Rapid chilling of zebrafish is acceptable as long as transfer **to water at temperatures of 2° to 4°C** occurs rapidly with as little transfer of warmer water as possible. Adult fish (>14 days of age) should be left immersed for **10 minutes after loss of opercular movement. Fry (4-14 days of age)** should be left immersed for **20 minutes after cessation of opercular movements**. Hypothermia is an acceptable method of anesthesia for neonatal rodents, however it requires a secondary method of euthanasia to ensure death and cannot be used as a sole means of euthanasia. **Animals should not come in contact with ice or precooled surfaces. Hypothermia is not recommended after 7 days of age for rodents.**
 - 16 Electrodes must be placed such that the animal is rendered unconscious prior to or simultaneously with cardiac fibrillation. Dogs must first be rendered unconscious by an acceptable means (e.g. general anesthesia).
 - 17 The only acceptable routes of administration are IC and IV.
 - 18 Rapid freezing (e.g. liquid nitrogen) should only be used for reptiles, amphibians, weighing <4 g and <5 day old altricial rodents. In all cases, animals must be anesthetized or rendered unconscious prior to freezing.
 - 19 Unacceptable methods for any species include: chloroform, carbon monoxide, formaldehyde, neuromuscular blocking agents, and non-penetrating captive bolt.

Sanctions

Failure to comply with IACUC policies may result in noncompliance reports to the Institutional Official, the Office of Laboratory Animal Welfare (OLAW), the U. S. Department of Agriculture (USDA), and/or the suspension of animal use privileges. In addition, the availability of sponsored research funds may be affected when an Investigator is found to be in violation of these policies.

Contacts

Subject	Contact	Phone	Email
Veterinary Concerns	LAR Veterinarians	855-2356	lar@indiana.edu
Policy	IACUC Administrator	855-5138	biacuc@indiana.edu

References

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>

Strykowski JL, Schech JM. Effectiveness of Recommended Euthanasia Methods in Larval Zebrafish (*Danio rerio*). *JAALAS*. Jan. 2015, 54(1): 81-84.