



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

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Responsible University Office:
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Vice President for Research

Policy Owner:
*Bloomington Institutional Animal Care and Use
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Policy Statement

The following policy outlines the approved procedures for humanely euthanizing any laboratory animal 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@iu.edu if training is needed on these techniques.

Responsibilities

It is the responsibility of each person engaged in administering anesthetics and/or 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

BLOOMINGTON INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

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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 the BIACUC 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 IACUC. Regarding euthanasia for rodents, euthanasia may be performed with the appropriate number of animals in a single cage and not in an animal holding room unless approved by the IACUC. Contact a LAR veterinarian for further information on the appropriate number of rodents to place in a single cage for euthanasia.

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

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

METHOD ^{1,18}	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	ACJ	AC	AC
Carbon dioxide ⁵	U	AC	U	ACJ	ACJ	U	AC
Microwave Irradiation ⁶	U	U	U	U	U	U	ACJ
Tricaine methanesulfonate ⁷	A	U	A	U	U	U	U
Benzocaine hydrochloride ⁸	A	U	A	U	U	U	U
Cervical dislocation ⁹	U	AC	U	ACJ	ACJ	U	ACJ
Decapitation ¹⁰	ACJ	ACJ	ACJ	ACJ	ACJ	ACJ	ACJ
Penetrating Captive bolt ¹¹	ACJ	U	U	U	ACJ	ACJ	U
Gunshot ¹²	U	ACJ	U	U	U	ACJ	U
Pithing ¹³	ACJ	U	U	U	U	ACJ	U
Stunning ¹⁴	ACJ	U	ACJ	U	U	ACJ	U
Rapid chilling/Hypothermia ¹⁵	U	U	A	U	U	U	ACJ
The methods listed below require that animals be anesthetized before euthanasia is performed.							
Electrocution	U	U	U	U	U	U	U
Exsanguination under anesthesia	A	A	A	A	A	A	A
Potassium chloride under anesthesia ¹⁶	A	A	A	A	A	A	A
Terminal procedures (incl. removal of a vital organ and pneumothorax) ¹⁷	A	A	A	A	A	A	A
Rapid freezing under anesthesia	AC	U	U	U	U	AC	ACJ

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;
- ACJ = Acceptable with Conditions requiring Justification**- may not consistently produce a humane death as sole means and requires IACUC approval;
- 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: 2013 Edition

<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>

2 Intravenous (IV) injection is the preferred method for euthanasia. Intraperitoneal (IP) 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.

3 Chloral Hydrate is unacceptable for euthanasia alone but can be used as an anesthetic followed up with a 2nd method (e.g., removal of vital organ) to ensure death.

4 Either of equal preference: isoflurane, or sevoflurane. In rabbits and other species that show aversive reactions to inhaled anesthetics exposure to high concentrations resulting in rapid loss of consciousness is preferred.

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 10-30% of chamber volume/minute. 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).

6 For use with small laboratory rodents only. Must be justified to ensure specially designed equipment is used.

7 Fish and amphibians may be euthanized by immersion in a tank containing tricaine methanesulfonate at a concentration of > 250 mg/liter of water for 10-20 minutes. The solution should be buffered to a pH of 7.0-7.5 with sodium bicarbonate.

8 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 10-20 minutes.

9 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.

10 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.

- 11 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.
- 12 This method is only recommended for wild or free-ranging species. If animals can be restrained, penetrating captive bolt should be used instead. 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. Hypothermia is most effective way of euthanizing zebrafish 14 d after fertilization (larval). 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.
- 16 The only acceptable routes of administration are IC and IV.
- 17 Rapid freezing (e.g. liquid nitrogen) should only be used for reptiles, amphibians, and <5 day old altricial rodents. In all cases, animals must be anesthetized or rendered unconscious prior to freezing.
- 18 Unacceptable methods include: chloroform, carbon monoxide, thoracic decompression, formaldehyde, 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: 2013 Edition

<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>

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