



Division of Research

SUBJECT: Euthanasia Methods	Effective Date: 8/28/2020	Policy Number: 10.4.11	
	Supersedes: 3/6/2020 12/13/2019 12/15/2016 12/13/2013 6/26/2009	Page 1	Of 9
	Responsible Authorities: Principal Investigator Vice President, Research Institutional Animal Care and Use Committee Assistant Vice President for Research, Research Integrity Assistant Vice President for Research, Comparative Medicine		

- I. Background
Public Health Policy (PHS) requires IACUCs to determine that methods of euthanasia proposed in protocols meet criteria consistent with the recommendations of the American Veterinary Medical Association (AVMA). The NIH Office of Laboratory Animal Welfare (OLAW) requires that the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition will be implemented by PHS Assured Institutions no later than October 1, 2020. AAALAC, Int. has adopted the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition as well. It can be accessed at <https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>. Unintended recovery of animals after apparent death constitutes serious noncompliance with PHS Policy and serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals.

- II. Purpose
To establish a policy that will provide guidance to Animal Care and Research Staff in regard to proper procedures for euthanasia of laboratory animals and wildlife compliant with laws and regulations governing animals in research and teaching as well as state of the art veterinary medical techniques.

- III. General Statement
When required for scientific data collection or clinical or operational requirements, animals will be humanely euthanized, as detailed in the approved protocol. All individuals who perform or are expected to perform euthanasia have to be trained in the relevant

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technique(s) and need to be familiar with the details of the particular animal care and use protocol as well as the provisions of this policy.

IV. Policy

1. Any method listed in this policy may be used for humane euthanasia as long as the method has been approved on the protocol.
2. If methods not mentioned in this policy need to be employed for scientific reasons the responsible researcher needs to discuss it with the veterinarian prior submitting the protocol to the IACUC for review and approval.
3. **Humane Euthanasia**, as defined in this policy, consists of a primary method and a secondary method whenever possible. **Death must be ensured by a second physical form of euthanasia** in rodents, amphibians and reptiles unless otherwise approved by the IACUC. In all other species, at a minimum respiratory and cardiac arrest must be ensured by a trained technician.
4. A secondary physical method of euthanasia must be performed before carcass disposal whenever possible:
 - a. Bilateral thoracotomy
 - b. Collection of vital tissues to assure the animal will not recover
 - c. Exsanguination
 - d. Decapitation
 - e. Cervical dislocation
 - f. Pithing (destruction of brain tissue) in fish, amphibians, and reptiles.
5. Chambers for delivery of inhalant euthanasia agents to terrestrial animals must not be overcrowded and unfamiliar animals must not be combined to avoid anxiety and/or aggression shortly before euthanasia.
6. Preferably, animals should be euthanized in their home cages. However, since this is not always possible due to different euthanasia systems delivering an inhalant agent, animals might be placed directly in the inhalation chamber. This requires sanitization of the chamber after euthanasia and before other animals will be transferred into the chamber.
7. All individuals responsible for euthanasia of research animals must be qualified and trained to perform euthanasia humanely. Documentation of personnel qualifications and training will be reviewed by the IACUC and is reflected in the approved protocol.
8. Species must be separated for euthanasia.
9. Placing live animals in chambers with recently deceased animals is prohibited.
10. Disposal of any carcass or parts (all animals) that are not immediately incinerated or otherwise disposed requires:
 - a. Placing the carcass (or parts) in a plastic bag
 - b. Labeling the bag with the date of euthanasia/death, profile number and if applicable hazardous agents used and placed in the appropriate freezer in the vivarium or research lab.

V. Definitions

Euthanasia - The term euthanasia is derived from the Greek terms eu meaning "good" and thanatos meaning "death." A "good death" would be one that occurs without pain and distress. In the context of this policy, euthanasia is the act of inducing humane death in an animal. Euthanasia techniques should result in rapid unconsciousness followed by cardiac or respiratory arrest and ultimately loss of brain function. In addition, the technique should minimize any stress and anxiety experienced by the animal prior to unconsciousness.

Stress can be minimized by technical proficiency and humane handling of the animals to be euthanized.

Humane is defined as "Characterized by kindness, mercy, or compassion, marked by an emphasis on humanistic values and concerns; *Synonyms: compassionate, humanitarian, merciful.*

VI. Accountability

The Principal Investigator (PI) will be responsible for:

- § Assuring that procedures are performed as described in the corresponding IACUC protocol and if necessary, submitting an amendment to the protocol and awaiting approval before new method(s) will be introduced.
- § Training or requesting training of personnel in euthanasia methods approved in the relevant IACUC protocol(s), regulations related to euthanasia and other pertinent topics, and confirming proficiency of personnel in specific euthanasia techniques
- § Maintaining euthanasia equipment so that it is always in proper working order.

The IACUC will be responsible for:

- § Reviewing and approving, requiring modifications in (to secure approval) or withholding approval of IACUC protocols and/or amendments
- § Providing oversight for all animal procedures conducted including euthanasia, and developing and directing an appropriate training program
- § Assuring that conditions such as adequate training, availability of appropriate equipment or minimizing risks for the handler have been met by the relevant research personnel when approving euthanasia methods that are acceptable with conditions.

The Research Integrity office will be responsible for:

- § Providing administrative support of the IACUC members to facilitate their regulatory function
- § Maintaining policy and assuring regular review and update as necessary by the IACUC
- § Keeping relevant training records and providing them to the IACUC for review.

The Office of Comparative Medicine (CM) will be responsible for:

- § Ensuring that Comparative Medicine personnel abide to this policy when performing euthanasia
- § Providing veterinary review of IACUC protocol and giving advice to the PI on appropriate method of euthanasia
- § Providing support and training for all personnel regarding euthanasia including hands-on training, verification of proficiency of personnel in particular euthanasia methods via observation, and support regarding proper maintenance of equipment.

VII. Procedures

1. Acceptable Methods of Euthanasia

Acceptable methods are those that consistently produce humane death.

- a. **Barbiturates:** The advantage of using barbiturates for euthanasia far outweighs the disadvantages, and barbiturate overdose is a common euthanasia method for mammals (including marine mammals), reptiles, amphibians, fish, and birds.

- i. It can be used as combination product with lidocaine or phenytoin or as pure sodium pentobarbital.
 - ii. It can be administered intravenously or intraperitoneally in rodents or intravenously or intra-coelomically in birds.
 - iii. In amphibians, fish and reptiles, sodium pentobarbital may also be administered intra-coelomically depending on anatomic features of the particular species.
 - iv. Barbituates may be injected into subcutaneous lymph sacs in frogs and toads. Time to effect may be variable, with death occurring in up to 30 minutes.
- b. **Ketamine (with Xylazine, Acepromazine or Diazepam)** is considered acceptable for euthanasia of amphibians, reptiles and rodents. However, reliable euthanasia dosages have not been established for all species and therefore should not be used as sole agent. These agents may be used to provide a deep surgical plane of anesthesia prior to other methods of euthanasia (e.g. cervical dislocation, decapitation, exsanguination).
- c. **MS-222 (Tricaine Methane Sulfonate, TMS):** MS-222 can be used for the euthanasia of fish and some amphibians.
- i. Tricaine is a benzoic acid derivative and generally should be buffered with sodium bicarbonate. The products *Finquel* and *Tricaine S* are FDA-approved. Please refer to the relevant guidelines on preparation, dosages and other pertinent information.
 - ii. For euthanasia via immersion in MS-222, a concentration ≥ 250 mg/L is recommended and can be much higher in certain species:
 - 1. Fish: Large fish may be removed from the water, a gill cover lifted, and a concentrated solution from a syringe flushed over the gills until 10 minutes after loss of opercular movement.
 - 2. Amphibians: MS-222 may also be injected into lymph spaces and pleuroperitoneal cavities.
 - 3. Animals should be left in this solution for at least 10 minutes and in some species up to 30 min (e.g. zebrafish) following cessation of movement.
- d. **Benzocaine:** Benzocaine hydrochloride is a pharmacological compound similar to MS-222 and may be used as a bath for euthanasia of amphibians and fish.
- i. Benzocaine hydrochloride is water soluble and can be used in concentrations ≥ 250 mg/L for euthanasia.
 - ii. Animals should be maintained in the solution for at least 10 minutes following cessation of any movement to ensure death.
 - iii. It can also be used as a 7.5% or 20% gel administered topically to the ventrum of amphibians.
- e. **Buffered quinaldine sulfate** at a dosage of ≥ 100 mg/L is used and acceptable for euthanasia in fish.
- f. **Rapid Chilling** of Zebrafish at 2-4 °F. Placement of adult zebrafish in a solution of 2-4 °F ice water with separation of the fish from the ice until there is a loss of orientation and opercular movements.
- i. Adult zebrafish are maintained for a minimum of 10 additional minutes to ensure death.

- ii. Zebrafish fry 4 to 14 days post fertilization should be exposed for at least 20 additional minutes after cessation of opercular movement.
- iii. Rapid chilling is not an approved method for zebrafish embryos less than 3 dpf and therefore should not be used for these.

2. Acceptable with Conditions Methods of Euthanasia

Methods in this category are equivalent to acceptable methods when all criteria for application can be met. Those methods have either greater potential for error or safety hazards, are not well covered in the scientific literature, and/or require secondary method(s) to ensure death.

It is not required to provide scientific justification in the IACUC protocol as long as evidence can be provided that the specific conditions required have been met. Only then will IACUC approval be granted for a particular method in this category.

- a. **Carbon Dioxide** provides rapid cardiac and respiratory depression with analgesic and anesthetic effects (CO₂ narcosis) and is the most common agent used for euthanasia in rodents. It can also be used for fish via CO₂ saturated water. Proper training has to be documented before this method can be employed by any faculty member or technician and will be reviewed by the IACUC.
 - i. Compressed CO₂ gas in cylinders is the only acceptable source of carbon dioxide because the inflow to the chamber or tank can be exactly regulated. Carbon dioxide generated by other methods such as from dry ice or chemical means is not acceptable.
 - ii. The cage or chamber for euthanasia of rodents should not be prefilled. Slowly fill the enclosure with CO₂ to displace 30-70% of the air volume per minute while the animal is inside the cage/chamber. Flow meters which effectively regulate the displacement rate may be purchased from a vendor (e.g., VWR Catalog Item No. 401740). Flow meters are available in the Veterinary Services Vivaria and should be set for:
 1. Standard mouse cage: 4 liters per minute
 2. Standard rat cage: 9 liters per minute
 3. If other chambers are provided in the animal facilities, a sign will indicate the appropriate flow rate.
 4. Use this formula to calculate the flow rate:

$$\frac{\text{height} \times \text{width} \times \text{length}}{61} = \text{liters} \times .30 = \text{flow rate/minute}$$
 - iii. Expose animals to CO₂ until complete cessation of breathing is observed plus an additional 2 minutes or for up to 30 min for fish. Follow up with a secondary method of euthanasia.
 - iv. CM offers various automated Euthanasia Chambers with different settings for Mouse, Rat or Pups. However, death still must be confirmed prior to carcass disposal.
- b. **Halogenated agents (e.g. isoflurane, sevoflurane):** Halogenated agents can be used with or without nitrous oxide in rodents and birds when proper training of personnel has been accomplished and documented, safety measures are followed, and a secondary method of euthanasia is applied.
 - i. Halogenated agents may be provided by a closed pre-charged container (i.e., bell jar) or by a continuous flow vaporizer (i.e., anesthesia machine). Those agents are volatile and represent a hazard to exposed humans and therefore

must be used within a fume hood while administered in a bell jar or similar container, except under field situations.

- ii. Animals must never come into direct contact with the halogenated agent.
- iii. Loss of consciousness should be induced rapidly by exposing animals to the maximum agent concentration possible.

Gas flow (if using a vaporizer) should be maintained for at least 2 minutes after apparent clinical death, or animals should be left in the chamber (if using a bell jar) for an additional 2-3 minutes after apparent clinical death, followed with a secondary method of euthanasia.

- c. **Cervical Dislocation (without Anesthesia):** Cervical dislocation is considered a humane technique for euthanasia of small birds, mice and rats (latter weighing <200 g) when performed by well trained individuals. Technical proficiency has to be demonstrated. The IACUC authorizes the Attending Veterinarian and his/her designee to evaluate proficiency of personnel performing cervical dislocation as the sole euthanasia method. This needs to be documented.
- d. **Decapitation (without Anesthesia):** This technique is considered acceptable with conditions for rodents and small birds. The IACUC will oversee proper training of personnel and ensure that the conditions listed below are followed. Proficiency of performing decapitation as the sole euthanasia method has to be demonstrated to the Attending Veterinarian or his/her designee experienced in the method and documented.
 - i. The equipment used to perform decapitation must be cleaned and sanitized after each use, maintained in good working order, and serviced on a regular basis to ensure sharpness of blades. Service for guillotines is recommended on a yearly basis or more often depending on usage. The use and maintenance of heavy-duty scissors must be described in an SOP, reviewed and approved by the IACUC.
 - ii. The use of plastic cones to restrain small animals (i.e. mice and rats) appears to reduce distress from handling, minimizes the chance of injury to personnel and improves positioning of the animal in the guillotine.
- e. **Physical methods (without anesthesia) in amphibians and reptiles:**
 - i. Manually applied blunt force trauma to the head is acceptable with conditions if other options are not available (e.g. in field study situations). It must be performed by well-trained and skilled personnel followed by decapitation or pithing.
- f. **Physical methods (without anesthesia) in fish:**
 - i. Decapitation followed by pithing. Decapitation alone is not considered humane due to tolerance to hypoxia in many species.
 - ii. Cervical transection followed by pithing. A sharp instrument is inserted caudal to the skull to separate the spinal cord and cervical vertebrae, leaving the head attached to the body. Cervical transection alone is not considered humane due to tolerance to hypoxia in many species.
 - iii. Blunt force trauma to the head followed by pithing.
 - iv. Captive bolt, both penetrating and non-penetrating, are methods usually applied to large fish species. It must be performed with the proper equipment by trained personnel who are regularly monitored for proficiency.

3. Adjunctive Methods

Adjunctive methods are not to be used as a sole method of euthanasia, but may be used in conjunction with other methods to help effectively administer euthanasia. The IACUC will only approve these methods if it can be shown that proper equipment is available for use and personnel are trained appropriately. The IACUC will monitor regularly for proficiency.

- b. **Decapitation under Deep Anesthesia** with heavy shears or a guillotine is effective in amphibian and reptile species with appropriate anatomic features. Because their central nervous system is tolerant to hypoxic and hypotensive conditions, decapitation must be followed by pithing. Decapitation in amphibian and reptile species is always a 3-step process: anesthesia, decapitation, and pithing.
- c. **Exsanguination under Deep Anesthesia:** Animals may only be exsanguinated when sedated, stunned, or anesthetized. Exsanguination as a sole means of euthanasia is unacceptable because hypovolemia is associated with anxiety.

4. Unacceptable Methods

- a. **Carbon Dioxide generation of CO₂ using dry ice** is prohibited by virtue of NIH/PHS policy.
- b. **Barbiturates in combination with neuromuscular blocking agents** as a means of primary euthanasia is prohibited due to the inability to assess sentience under neuromuscular blockade.
- c. **Nitrous Oxide (N₂O)** may be used with other inhalants to speed the onset of anesthesia, but by itself does not induce anesthesia in animals, even at 100% concentration. When used alone, N₂O produces hypoxemia before respiratory or cardiac arrest. As a result, animals may become distressed prior to loss of consciousness.
- d. **Strychnine, Nicotine, Caffeine, Magnesium Sulfate, Potassium Chloride, Cleaning Agents, Solvents, Disinfectants** and other toxins or salts, and all neuromuscular blocking agents are unacceptable.
- e. **Thoracic (cardiopulmonary, cardiac) compression** used to euthanize wild small mammals and birds under field conditions is now considered unacceptable in conscious animals. However, the IACUC can grant approval when alternative methods are not feasible, or objectives of the study protocol provide a strong scientific justification. The IACUC will ascertain that training was provided and the individual(s) can show proficiency in this method. The continued approval will be re-evaluated by the IACUC as more evidence-based data become available

5. Special Considerations for Euthanasia of Marine Mammals

Euthanasia of marine mammals within the FAU animal care and use program is supervised by a clinical veterinarian specialized in marine mammal medicine who is knowledgeable and trained in applicable euthanasia methods.

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- a. AVMA considers **barbiturates** or other injectable **anesthetic agents** (latter only for free-ranging animals) acceptable for euthanasia of marine mammals.
 - b. Acceptable with conditions: For free-ranging wildlife, manually applied blunt force trauma or an accurately placed gunshot is acceptable with conditions.
6. Special Considerations for Euthanasia of Mouse and Rat Fetuses and Neonates:
 These special considerations apply only to rodents with altricial young (i.e. naked, blind and dependent) such as mice and rats. Precocial young (i.e., relatively mature and mobile newborn) such as guinea pigs must be treated as adults with regards to euthanasia.
- a. Acceptable methods:
 - i. Euthanasia of the dam does not require the removal of the fetuses from the womb since mammals are unconscious *in utero* and hypoxia does not evoke a response. However, if fetuses ≥ 15 days old need to be removed from utero for experimental purposes, they need to be individually euthanized. This is best accomplished by decapitation.
 - ii. Injectable barbiturates and dissociative agents combined with α_2 -adrenergic receptor agonist or benzodiazepines are acceptable for euthanasia of fetuses or neonates.
 - b. Acceptable with Conditions:
 As with adult animals, if acceptable with conditions methods are used the IACUC will only grant approval if proficiency can be demonstrated by the personnel employing the particular method listed below.
 - i. **Inhalant anesthetics** are effective for both fetuses and neonatal rodents. This method must be **followed by** a physical method such as **decapitation**.
 - ii. Fetuses that are believed to be unconscious and neonates ≤ 5 days of age may be quickly euthanized by **rapid freezing in liquid nitrogen** since the nervous system is not sufficiently developed to perceive pain.
 - iii. Neonates up to 10 days of age can be euthanized via **hypothermia** while precautions must be employed to avoid direct contact of the neonate with the ice or a precooled surface. It should be followed with a secondary method.
 - iv. Decapitation with scissors or sharp blades is acceptable with conditions depending on tissue mass and considering potential pain from tissue crushing as well as personnel safety. When appropriate, an adult decapitator should be used.

VIII. Policy Renewal Date
 8/28/2023

IX. References

1. PHS Policy on Humane Care and Use of Laboratory Animals
2. Animal Welfare Regulations, 9 CFR Ch. 1
3. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
4. Guide for the Care and Use of Laboratory Animals, 8th edition, 2011
5. American Association of Zoo Veterinarians (AAZV). Guidelines for Euthanasia of Nondomestic Animals. 2006, 111 pp.

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POLICY APPROVAL

Initiating Authority: Vice President for Research and Institutional Official

Signature _____ Date: _____

Name: Daniel C. Flynn, Ph.D., Vice President for Research

Executed signature pages are available in the Initiating Authority Office(s)