



POLICY

TITLE:	Euthanasia
Policy Number:	2014-015
Responsible Department:	Institutional Animal Care and Use Committee
Policy Contact:	IACUCOffice@westernu.edu
Approval Date:	8/13/14
Reviewed:	6/7/17; 3/11/2020
Revised Date:	1/5/16 (Expanded scope beyond rodents); 8/10/16 (Added euthanasia methods for zebrafish and birds); 6/7/17 (refined euthanasia methods); 3/11/2020 (Added procedure-specific training required; documentation of confirmation of death for USDA-covered species; updated link to 2020 AVMA Guidelines; pre-emptive analgesia w/ IP pentobarbital; refined euthanasia of birds)

Purpose of Policy: To ensure that euthanasia is performed in as humane a manner as possible that does not interfere with the scientific objectives.

Policy Information: The Institutional Animal Care and Use Committee (IACUC) has approved this policy. Any deviation must be justified and authorized by the IACUC prior to its implementation.

In accordance with the [2020 AVMA Guidelines for the Euthanasia of Animals](#), anesthesia must be administered prior to euthanasia by a physical method unless scientific evidence is provided that such agents would interfere with the experimental endpoints.

Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function. The technique should minimize distress and anxiety experienced by the animal prior to loss of consciousness. Documentation of confirmation of death is required for animals covered by the United States Department of Agriculture (USDA) and confirmation by a second person is recommended when feasible. This policy provides general guidance on humane euthanasia using acceptable methods. For specific dosages and procedures, consult the appropriate Standard Operating Procedure or contact the Attending Veterinarian (AV) by e-mailing dwolf@westernu.edu for more information.

Qualifications of Personnel and General Considerations

1. All individuals performing euthanasia must be appropriately qualified. Minimal required training is: IACUC 101 and species- and procedurally-specific training as determined by the IACUC.
 2. Adhere to all IACUC-approved protocols and institutional policies.
 3. In consultation with the Attending Veterinarian (AV), select the method of euthanasia based on the species, location and objectives of the protocol.
 4. Properly maintain reagents, supplies and equipment to ensure optimal performance.
 5. Minimize distress to the animal, as well as to the operator, by handling the animals gently and carefully.
-

Species-Specific Considerations

EUTHANASIA of RODENTS and LEAST SHREWS

Non-Physical Methods

A. Overdose of inhalant anesthetic (32% isoflurane)

NOTES: This method is not approved as a sole means of euthanasia for neonates up to 7 days of age; see section below on "Euthanasia of Neonatal Rodent and Least Shrews". For safety reasons, **ether** is not permitted for anesthesia or euthanasia, as it is highly flammable, potentially explosive and hepatotoxic to laboratory animals and humans.

1. If euthanizing more than one animal at a time, do not overcrowd the container.
2. Do not mix different species in a single chamber. When combining animals from different cages, carry out euthanasia immediately upon mixing populations to minimize stress and opportunities for fighting.
3. Expose the animal(s) to a maximum concentration of isoflurane gas (32%) using soaked gauze or a cotton ball inside a tea infuser and placed in a tightly closed container (e.g., Bell jar, closed induction chamber, Tupperware) in a properly vented enclosure (e.g., chemical fume hood, induction chamber equipped with an exhaust port). Avoid direct contact of the liquid anesthetic agent with the skin as this can be injurious to the animal. This is accomplished by use of a tea infuser, tea ball or a mesh separating the soaked gauze or cotton ball from the animal(s).
4. Vapors are inhaled until respiration ceases and death ensues.
5. If the animal is deeply unconscious but respiration has not ceased (or for additional security after respirations have ceased), follow the anesthesia with a physical method of euthanasia, e.g., cervical dislocation, decapitation, bilateral thoracotomy.

IMPORTANT: Verify that an animal is dead before disposing of the carcass by making sure that there are no respiratory movements or a palpable heartbeat **for at least 5 minutes**.

Although the [2020 AVMA Guidelines for the Euthanasia of Animals](#) lists carbon dioxide (CO₂) asphyxiation as "acceptable with conditions", the IACUC does not consider this use of CO₂ to be humane and, therefore, will not approve this use of CO₂ without rigorous scientific justification (Refer to IACUC Policy No. 2014-005; Use of Carbon Dioxide for Euthanasia). Any means of euthanasia not described in this policy must be approved in writing by the IACUC.

- B. Overdose of injectable barbiturate (e.g., pentobarbital, at least 100 mg/kg; Euthasol (390 mg pentobarbital + 50 mg phenytoin per ml, at least 5 ml/kg IP or IV)). However, as IP pentobarbital can cause pain, it is recommended that a pre-emptive analgesic be given.
1. Inject euthanasia solution intravenously (IV). Intraperitoneally (IP) injection is acceptable if IV administration is not practical or possible. However, IP injection can be painful. Prior anesthesia is recommended for the IP route of administration.
 2. If the animal is deeply unconscious but respiration has not ceased (or for additional security after respirations have ceased), follow the anesthesia with a physical method of euthanasia, e.g., cervical dislocation, decapitation, bilateral thoracotomy.

IMPORTANT: Verify that an animal is dead before disposing of the carcass by making sure that there are no respiratory movements or a palpable heartbeat **for at least 5 minutes prior to disposal.**

Physical Methods

All staff utilizing physical methods of euthanasia must be trained and have demonstrated proficiency to the veterinary staff. Apply prior anesthesia or sedation whenever possible. Physical methods of euthanasia may be accompanied by ultrasonic vocalizations and pheromones released during the process and odors released in the blood afterwards. Such stimuli may induce stress in other animals housed nearby. Therefore, euthanasia using physical methods should be performed outside the animal housing room and away from other animals. If multiple animals are to be euthanized, they should be brought into the euthanasia location one at a time.

A. Cervical dislocation

NOTE: Cervical dislocation can cause serious injury and suffering to an animal if done incorrectly. Therefore, those carrying out the method must be properly trained to do so. In accordance with the [AVMA Guidelines](#), cervical dislocation cannot be used as a sole means of euthanasia of rodents weighing more than 200 grams since the large physical mass in the cervical region makes the procedure physically more difficult. In such cases, decapitation is recommended.

1. Place the thumb and index finger on either side of the neck or at the base of the skull or, alternatively, press a hard object (e.g., rod or metal cage card holder) at the base of the skull.
2. With the other hand, grasp the base of the tail between the thumb and forefinger and pinch firmly. Slightly elevate the hindquarters, no more than 20-30 degrees. Quickly and firmly pull the hindquarters by the tail base away from the head and neck while simultaneously driving a thumbnail or hard object down behind the base of the skull with the other hand.
3. Confirm cervical vertebrae separation by palpating the neck.

IMPORTANT: Verify that an animal is dead before disposing of the carcass by making sure that there are no respiratory movements or a palpable heartbeat **for at least 5 minutes.**

B. Decapitation

NOTE: This method may only be used if required by the experimental design.

1. Ensure blade sharpness and proper function of scissors, guillotines or other devices prior to their being used for decapitation. Document when the equipment was checked and by whom.
2. Use appropriately sized instruments for the species.

3. Decapitation using scissors or a sharp blade is acceptable for altricial neonates less than 7 days of age. For precocial neonates (e.g., Guinea pigs), follow guidelines for an adult. Proficiency must be demonstrated prior to the use of surgical scissors for decapitation of adult, conscious mice or shrews.
4. Maintain the equipment used for decapitation in good working order and service on a regular basis to ensure sharpness of the blades. Refer to SOP, *Maintenance of Decapitation Equipment*.
5. Quickly separate the head from the body at the cervical level.

NOTE: The use of plastic cones (i.e., DecapiCone; Braintree Scientific, Inc. P.O. Box 850498 Braintree, MA 02185) to restrain animals is recommended because it appears to reduce distress from handling, minimizes the chance of injury to personnel and improves positioning of the animal in the guillotine.

C. Exsanguination

1. Deeply anesthetize the animal.
2. Verify that the pedal withdrawal reflex is absent by pinching the toes.
3. For cardiac puncture, insert an appropriately sized needle into the chest via a lateral or ventral approach.
4. For abdominal aorta puncture, open the abdomen and retract viscera to expose the aorta. Then, insert an appropriately sized needle into the vessel.
5. Withdraw the maximal volume of blood.
6. If the animal is deeply unconscious but respiration has not ceased (or for additional security after respirations have ceased), follow the exsanguination by a physical method of euthanasia, e.g., cervical dislocation, decapitation, bilateral thoracotomy.

IMPORTANT: Verify that an animal is dead before disposing of the carcass by making sure that there are no respiratory movements or a palpable heartbeat **for at least 5 minutes**.

Euthanasia of Fetal Rodents and Least Shrews

Neural development in fetuses up to 14 days gestation is minimal and pain perception is unlikely. Euthanasia of the mother followed by removal of the fetus should ensure rapid death from loss of blood supply. Neural development in fetuses from 15 days gestation to birth is such that fetuses might experience pain. Fetuses at this age are resistant to inhalational anesthetics, however, and it is preferable to euthanize them with parenteral anesthetics, cervical dislocation or decapitation with surgical scissors.

If chemically fixing whole fetuses, they should be fully anesthetized prior to immersion in or perfusion with fixative solutions.

If fetuses are not required for the study, euthanasia of a pregnant animal with a rapid acting anesthetic (e.g. pentobarbital) will ensure rapid death of the fetuses.

Euthanasia of Neonatal Rodents and Least Shrews

1. Generally, neonates are more resistant to hypoxia than adults of the same species.
2. Non-physical methods of euthanasia utilizing inhalant anesthetics should be followed-up with a

secondary method (e.g., decapitation) in order to ensure death, as all inhalant agents ultimately induce death via hypoxia.

Acceptable procedures for euthanasia of neonatal mice, rats, least shrews and hamsters less than 10 days of age:

1. Decapitation; see Physical Methods, part B, above.
2. Decapitation, preceded by loss of consciousness via inhalant anesthetic administration, or hypothermia (see also SOP on Rodent Anesthesia or consult with the veterinarian).
3. Overdose of injectable barbiturate IP, see Non-Physical Methods, part B., above.
4. For euthanasia of mice, rats, least shrews and hamsters greater than 10 days old, follow the guidelines for adults.
5. For euthanasia of precocial rodent neonates (e.g., Guinea pigs), follow the guidelines for adults.

NOTE: Near-term fetuses should be confirmed non-viable by monitoring for inactivity prior to disposal or processing (e.g., tissue collection/fixation). For additional security, follow the guidelines for euthanasia of neonates.

REFERENCES

1. AVMA Guidelines on Euthanasia, June 2013:
<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
2. Artwohl J, Brown P, Corning B, Stein S. 2006. Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia. JAALAS. 45(1): 98-105.
http://www.aclam.org/content/files/files/public/active/report_rodent_euth.pdf
3. PHS Implementation of the Revised AVMA Guidelines on Euthanasia
<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-005.html>
4. USDA Care Policy (Euthanasia on page 3.5)
https://www.aphis.usda.gov/animal_welfare/downloads/Animal%20Care%20Policy%20Manual.pdf
5. PHS Policy on Humane Care and Use of Laboratory Animals Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small Laboratory Animals; Notice: NOT-OD-02-062; National Institutes of Health (NIH), Bethesda, MD. July 17, 2002. <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html>

EUTHANASIA of ZEBRAFISH

Non-Physical Methods

A. Overdose of External / Topical Chemical Agents

Tricaine methane sulfonate (MS-222) or Benzocaine hydrochloride (HCl): These agents are acidic in solution and must be buffered with sodium bicarbonate.

A. Tank immersion method:

1. Place the animal in a buffered solution of MS-222 (>250 mg/L) or Benzocaine HCl (>250 mg/L).
2. Immerse animal until death results. Time to effect is proportional to solution concentration.

B. Alternative methods:

1. Remove fish from water and flush gills with a buffered, concentrated, solution of MS-222 or Benzocaine HCl.
2. 2-phenoxyethanol: Place fish into a bath of 2-phenoxyethanol solution at a concentration of 0.5-0.6 mL/L or 0.3-0.4 mg/L; immerse until death results.

NOTE: Chemical methods of euthanasia may require up to 3 hours before death results. A physical method (described below) can be applied once unconsciousness is achieved.

For zebrafish \geq 8 days post-fertilization (dpf):

1. Overdose of tricaine methane sulfonate (MS222, 250-300 mg/l) by immersion in the solution for at least 10 minutes following cessation of opercular (i.e., gill) movement.
2. Anesthesia with MS222 (168 mg/l) followed by rapid freezing in liquid nitrogen.
3. Immobilization by submersion in ice water (5 parts ice/1 part water, 0-4°C) for at least 10 minutes following cessation of opercular movement. In any fish where it is difficult to visualize opercular movement, fish should be left in the ice water for **at least 20 minutes** after cessation of all movement to ensure death by hypoxia under full unconsciousness.

For zebrafish 4-7 dpf:

1. Immobilization by submersion in ice water (5 parts ice/1 part water, 0-4°C) for at least 20 minutes to ensure death by hypoxia.
2. Addition of bleach solution (sodium hypochlorite 6.15%) to the culture system water at 1 part bleach to 5 parts water. They should remain in this solution at least five minutes prior to disposal to ensure death. This step is also suitable for embryos <3 dpf and is especially important for larvae up to 15 dpf.

Physical Methods

NOTE: Anesthesia or heavy sedation must be applied prior to the use of physical techniques unless otherwise approved by the IACUC.

Use sharp equipment of the appropriate size for the species to be euthanized to ensure that the head is separated from the body rapidly and completely. Follow decapitation with pithing via the open spinal canal.

Thermal shock:

Rapid freezing: Anesthetize animal as per SOP, *Euthanasia of Zebrafish*, and then immerse in liquid nitrogen.

Rapid Chilling (hypothermic shock) in tropical fresh water fish: Anesthetize animal as per SOP, *Rodent Anesthesia*, and then immerse in an ice bucket or cooler containing ice slush.

REFERENCES

1. [NIH Guidelines for use of zebrafish](http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf): <http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf>
2. Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). JM Wilson, et al. JAALAS. 2009. Nov; 48(6), 785-789.
3. Neiffer, D.L., Stamper, M.A. 2009. Fish sedation, anesthesia, analgesia, and euthanasia: Considerations, methods, and types of drugs. *ILAR Journal*, 50(4), 343-360.

4. [AVMA Guidelines on Euthanasia, June 2013](https://www.avma.org/KB/Policies/Documents/euthanasia.pdf): <https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
5. Guidelines for Euthanasia of Nondomestic Animals, American Association of Zoo Veterinarians; 2006.
6. [Recommendation for euthanasia of experimental animals: Part 2. Lab. Anim. 31:14-15](http://la.rsmjournals.com/cgi/reprint/31/1/1.pdf): <http://la.rsmjournals.com/cgi/reprint/31/1/1.pdf>
7. [Guidelines for the Use of Fishes in Research \(2004\)](http://fisheries.org/docs/policy_useoffishes.pdf), 2004 by the American Fisheries Society: http://fisheries.org/docs/policy_useoffishes.pdf
8. Formulary for Laboratory Animals. CT Hawk, et al. 3rd ed., 2005

EUTHANASIA of BIRDS

Non-Physical Methods

A. Overdose of inhalant anesthetic (32% isoflurane)

1. Expose the animal(s) to a maximum concentration of isoflurane gas (32%) using an anesthesia mask suited for birds or, if unavailable, use soaked gauze or a cotton ball inside an appropriately sized, tightly closed container (e.g., Bell jar, closed induction chamber, Tupperware) in a properly vented enclosure (e.g., chemical fume hood, induction chamber equipped with an exhaust port).
2. Vapors are inhaled until respiration ceases and death ensues.
3. Vapor exposure should be maintained for at least 3 minutes after apparent clinical death.
4. If the animal is deeply unconscious but respiration has not ceased (or for additional security after respirations have ceased), follow the anesthesia with a physical method of euthanasia, e.g., cervical dislocation or decapitation.

IMPORTANT: Verify that an animal is dead before disposing of the carcass by making sure that there are no respiratory movements or a palpable heartbeat **for at least 5 minutes**.

Although the [AVMA Guidelines](#) lists carbon dioxide (CO₂) asphyxiation as "acceptable with conditions", the IACUC does not consider this use of CO₂ to be humane and, therefore, will not approve this use of CO₂ without rigorous scientific justification (Refer to IACUC Policy No. 2014-005; Use of Carbon Dioxide for Euthanasia). Any means of euthanasia not described in this policy must be approved in writing by the IACUC.

- B. Barbiturate overdose: Inject sodium pentobarbital, or commercially available euthanasia solution (pentobarbital sodium and phenytoin sodium), for intravenous (IV) administration at a dose of 100-150 mg/kg of pentobarbital sodium.

NOTE: When IV administration is not possible, the pentobarbital can be injected by other routes (for intracardiac, consult with the Attending Veterinarian) so long as the bird is fully anesthetized (e.g., via isoflurane inhalation).

IMPORTANT: Verify that an animal is dead before disposal of the body by confirming that there are no respiratory movements or corneal reflex and a palpable heartbeat **for at least 5 minutes prior to disposal**.

Physical Methods

Cervical dislocation

1. Acceptable for poultry and other birds less than 3 kg.

2. When feasible, a secondary method such as decapitation or exsanguination should be employed to ensure death.

Decapitation

1. Perform with dedicated and clean equipment that will ensure that the head is separated from the body rapidly and completely.
2. Refer to SOP, *Maintenance of Decapitation Equipment*, for proper compliance and care.

Euthanasia of Embryos / Eggs / Hatchlings

Non-pipped eggs

1. For eggs that are less than 50% of the total incubation period, destroy the viability of the eggs prior to disposal (e.g., freezing, coating eggs with oil, or maceration).
2. Bird embryos that have attained at least 50% incubation have developed a neural tube sufficient for pain perception; therefore they should be euthanized by similar methods used in avian neonates (see below). Eggs of unknown incubation time should be assumed to be at least 50% incubated.

Pipped eggs and hatchlings up to 72 hours old.

Anesthetic overdose or decapitation is acceptable.

Chicken eggs that hatch prematurely (18-21 days) must be euthanized using adult guidelines.

REFERENCES

1. [AVMA Guidelines for the Euthanasia of Animals: 2013 Edition:](https://www.avma.org/KB/Policies/Documents/euthanasia.pdf)
<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
2. [Guide for the Care and Use of Laboratory Animals.](https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf) National Research Council; National Academy Press, Washington, DC, 2011: <https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf>
3. [Recommendation for euthanasia of experimental animals: Part 2. Lab. Anim. 31:14-15:](http://la.rsmjournals.com/cgi/reprint/31/1/1.pdf)
<http://la.rsmjournals.com/cgi/reprint/31/1/1.pdf>