



IACUC Guidance:	<b>TAMU-G-028</b>	Title:	<b>IACUC Guidelines on Euthanasia Using Carbon Dioxide</b>	
	Location	Effective Date	Review By	
	<b>College Station/Dallas/Galveston/Kingsville</b>	12/01/2023	11/30/2026	
	<b>Houston</b>	01/01/2024	11/30/2026	

**1. PURPOSE**

- 1.1. To ensure the proper application of carbon dioxide for the humane euthanasia of applicable research animals at Texas A&M University.

**2. SCOPE**

- 2.1. There is ongoing controversy about the aversive characteristics of CO<sub>2</sub> as an inhalant euthanasia agent. This is an area of active research and subject to change.
- 2.2. Does not include discussion of gas mixtures (CO<sub>2</sub>, Argon, Nitrogen).
- 2.3. Does not apply to mass depopulation.

**3. RESPONSIBILITY**

- 3.1. The **PI** is responsible for:
  - 3.1.1. Ensuring that any euthanasia method that deviates from the AVMA Guidelines on Euthanasia is justified for scientific or medical reasons and is described in the approved animal use protocol.
  - 3.1.2. Ensuring that animals are euthanized using appropriate technique, equipment and agents, as outlined in the approved animal use protocol.
  - 3.1.3. Training personnel to adhere to animal care and use protocols, SOPs, and guidelines.
  - 3.1.4. Training all personnel administering CO<sub>2</sub> to recognize and confirm death.
  - 3.1.5. Documenting training according to TAMU-G-029.
  - 3.1.6. Maintaining equipment and monitoring personnel as narcosis is a human safety risk associated with the use of CO<sub>2</sub>.
  - 3.1.7. Posting this guideline and/or any SOPs for CO<sub>2</sub> euthanasia where CO<sub>2</sub> euthanasia occurs.

**4. DEFINITIONS AND/OR ACRONYMS**

- 4.1. **Adjunctive Method:** A method of assuring death that may be used after an animal has been made unconscious.
- 4.2. **Aversion:** A desire to avoid or retreat from a stimulus
- 4.3. **AV:** Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly to, and have delegated authority from, the AV.
- 4.4. **AVMA:** American Veterinary Medical Association. Nation’s leading advocate for the veterinary profession through a variety of avenues including education programs and the provision of position statements on key issues including humane euthanasia, i.e. AVMA Guidelines for the Euthanasia of Animals.
- 4.5. **CO<sub>2</sub>:** Carbon dioxide
- 4.6. **CO<sub>2</sub> Narcosis:** A state of stupor, unconsciousness, or arrested activity produced by the influence of carbon dioxide.
- 4.7. **EHS:** Environmental Health and Safety Department
- 4.8. **Euthanasia:** A method of humane destruction that minimizes pain, distress, and anxiety experienced by the animal prior to loss of consciousness, and causes rapid loss of consciousness followed by cardiac or respiratory arrest and death.
- 4.9. **Exsanguination:** The action of draining an animal of blood.
- 4.10. **Flowmeter:** An instrument used to deliver a precise volume of gas, calibrated for a specific gas. A pressure regulator cuts off the flow of the gas at a certain pressure and cannot be used to deliver a precise volume of gas; however, some flow meters have regulators incorporated and do not require a separate regulator.
- 4.11. **Neonate:** An animal in the first week of life.

- 4.12. **PI:** Principal Investigator. The individual who has ultimate administrative and programmatic responsibility for the design, execution, and management of a project utilizing vertebrate animals.
- 4.13. **Precocious:** Capable of a high degree of independent activity (ie, mobility, feeding) from birth.
- 4.14. **Rodent:** Of the order Rodentia
- 4.15. **Secondary Method:** A euthanasia method employed subsequent to a primary method to ensure death of an unconscious animal before it can recover consciousness. Also, see Adjunctive Method.
- 4.16. **Small Laboratory and Wild-Caught Rodents:** Mice, rats, hamsters, guinea pigs, gerbils, degus, cotton rats, monodelphis
- 4.17. **SOP:** Standard Operating Procedure.
- 4.18. **Unconsciousness:** Loss of individual awareness. This occurs when the brain's ability to integrate information is blocked or disrupted. Onset of unconsciousness is associated with loss of the righting reflex. An unconscious animal is therefore recumbent and, by definition, unable to perceive pain; however, unconscious animals may respond to noxious stimulation with spinally mediated involuntary movements depending on the degree of CNS depression present.
- 4.19. **USDA:** United States Department of Agriculture. USDA Animal Care, a unit under the Animal and Plant Health Inspection Service, administers the Animal Welfare Act (AWA) and associated Animal Welfare Act Regulations (AWAR).
- 4.20. **USDA Regulated:** Species or activities which fall under the AWA/AWAR.
- 4.21. **Vital Organs:** The main organs inside the body that are necessary for life, e.g. heart, lungs, brain.
- 4.22. **Wild Species:** A free-roaming animal of a nondomestic, native species.

## 5. GUIDELINES OR PROCEDURE

- 5.1. In evaluating the appropriateness of euthanasia methods, some of the criteria that should be considered are:
  - 5.1.1. Ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety;
  - 5.1.2. Reliability;
  - 5.1.3. Irreversibility;
  - 5.1.4. Time required to induce unconsciousness;
  - 5.1.5. Appropriateness for the species and age of the animal;
  - 5.1.6. Compatibility with research objectives; and
  - 5.1.7. The safety of and emotional effect on personnel.
  - 5.1.8. Appropriateness of euthanasia methods should be discussed with the AV, or designee.
- 5.2. General Recommendations:
  - 5.2.1. When gradual displacement rates are used, CO<sub>2</sub> flow should be maintained for at least 1 minute after respiratory arrest.
  - 5.2.2. Only one species is allowed in the chamber at a time.
  - 5.2.3. The chamber must allow for visibility of the animal(s).
  - 5.2.4. Animals are not to be left in the chamber unattended.
  - 5.2.5. Overcrowding of the euthanasia chamber has been noted to lead to inadequate asphyxiation. The number of animals must be limited to allow free flow of CO<sub>2</sub> to each animal and allow animals to turn around.
    - 5.2.5.1. Appropriate techniques for assuring death must be applied individually, regardless of the number of animals being euthanized.
  - 5.2.6. As gas displacement rate is critical to the humane application of CO<sub>2</sub>, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size container being utilized is absolutely necessary.
  - 5.2.7. Immature animals must be exposed to high concentrations of CO<sub>2</sub> for an extended period of time to ensure death.
  - 5.2.8. Carbon dioxide containers should never be placed in an unventilated area due to risks associated with an overdose of gaseous CO<sub>2</sub> for humans.

- 5.2.9. Compressed gas cylinders are the only acceptable source of carbon dioxide for euthanasia.
  - 5.3.7.1. Contact [EHS](#) for instruction on the safe use of compressed gases, including: storage, transport and proper security.
- 5.3. CO<sub>2</sub> for Small Rodent Euthanasia
  - 5.3.1. CO<sub>2</sub> euthanasia is recognized as a humane form of euthanasia for adult rats and mice.
  - 5.3.2. Conscious animals must be placed in uncharged chambers and flow rates must be used which displace **30-70%** of the chamber volume per minute. (For a 10-liter volume chamber, use a flow rate of approximately 3-7 liter(s) per minute.)
    - 5.3.2.1. The practice of immersion of conscious animals into a container pre-filled or precharged with CO<sub>2</sub>, is **unacceptable**.
    - 5.3.2.2. There is potential for increased distress due to dyspnea at lower flow rates or mucous membrane pain associated with flow rates at the high ends of this range.
    - 5.3.2.3. As there is no clear evidence of a flow rate that optimally minimizes both pain and distress for all species, sexes, and genetic backgrounds, consult the AV, or designee to determine which flow rate is appropriate for the circumstance.
  - 5.3.3. There is no apparent welfare advantage to euthanizing animals with CO<sub>2</sub> when prior exposure to inhaled anesthetics has occurred.
  - 5.3.4. Consideration should be given to the benefits of using a darkened home cage, while also keeping in mind the need to have the animal under observation.
  - 5.3.5. Use of the home cage as the euthanasia chamber reduces stress and is the preferred method to employ.
    - 5.4.8.1. Mixing of unfamiliar or incompatible animals is discouraged.
  - 5.3.6. Euthanasia should occur in a procedure room or laboratory, away from other rodent housing.
  - 5.3.7. CO<sub>2</sub> is denser than room air, thus the chamber will need to be “purged” (dumped) between groups of cages.
  - 5.3.8. Because CO<sub>2</sub> first anesthetizes animals and then, only after adequate exposure time, will result in death, rodents must be exposed to the gas until respiration has ceased, (at least one additional minute) within the euthanasia chamber with CO<sub>2</sub> continuing to flow.
  - 5.3.9. Death of the animal must be ensured prior to disposal of the carcass. Therefore, the IACUC is requiring that a secondary, physical method that ensures irreversibility of the procedure is used.
    - 5.3.9.1. Acceptable physical methods include:
      - 5.3.9.1.1. exsanguination
      - 5.3.9.1.2. decapitation
      - 5.3.9.1.3. cervical dislocation
      - 5.3.9.1.4. bilateral thoracotomy
      - 5.3.9.1.5. removal of vital organs
- 5.4. Unintended recovery of animals after apparent death from CO<sub>2</sub> is a documented occurrence. Such incidents constitute noncompliance with the PHS policy and serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals. See TAMU-G-015 Guidelines for Reporting Unanticipated or Adverse Events and Protocol Drift.
- 5.5. CO<sub>2</sub> Euthanasia for Rodent Fetuses and Neonates
  - 5.5.1. Rodents with altricial young, such as mice and rats, must be differentiated from rodents with precocial young, such as guinea pigs. Precocial young should be treated as adults.
  - 5.5.2. Neonatal mice may take up to 50 minutes to die from CO<sub>2</sub> exposure, and neonatal rats may take as long as 35 minutes.
  - 5.5.3. Adequate exposure time should be provided, or an adjunctive method (eg, cervical dislocation, or decapitation) should be performed after a neonate is nonresponsive to painful stimuli. Rodent fetuses

along with other mammals are unconscious in utero and hypoxia does not evoke a response. Therefore, it is unnecessary to remove fetuses for euthanasia after the dam is euthanized.

5.6. Amphibians and Reptiles

5.6.1. Carbon dioxide may be considered for euthanasia of amphibians and reptiles if alternate methods are not practical and where the limitations of this method are understood and addressed. Due to the potential lack of response to this method by many species and the requirement for a prolonged exposure time, other methods are preferable. Death by CO<sub>2</sub> must be verified, and preferably, assured by application of a secondary lethal procedure.

5.7. Rabbits

5.7.1. While CO<sub>2</sub> is an effective method of euthanasia, its use as the sole agent in rabbits may cause distress. Premedication with sedative agents may reduce potential aversion responses.

5.7.2. The recommended CO<sub>2</sub> displacement rate for rabbits is 50% to 60% of the chamber or cage volume/min.

5.8. Avian

5.8.1. Variability of species in this taxa requires consultation with the AV for choice of the most appropriate method of euthanasia.

5.8.2. If CO<sub>2</sub> is an appropriate method of euthanasia, High (> 40%) concentrations of CO<sub>2</sub> induce anesthesia initially followed by loss of consciousness.

5.8.3. Application rate of CO<sub>2</sub> needs to be balanced with situational needs as rapid increases in CO<sub>2</sub> concentration decrease the amount of time to loss of posture and consciousness, while slower increases in concentration may cause less aversion or reaction but increase time of exposure.

5.8.4. Neonatal birds and diving birds may require higher concentrations of CO<sub>2</sub> to cause death.

5.9. Poultry

5.9.1. When relatively large numbers are involved, exposure to carbon dioxide in enclosed containers may be used.

5.9.2. It is important that the process be observed and carbon dioxide added, if necessary, to ensure that death is attained without undue delay.

5.9.3. Birds should be checked to verify death.

5.9.4. Immersion of poultry in CO<sub>2</sub> concentrations less than 100% is acceptable with conditions as it does not appear to be distressing.

5.9.5. Although euthanasia of poultry in high concentrations of carbon dioxide is relatively rapid, it also tends to promote vigorous convulsive wing flapping after loss of posture.

5.9.6. There is no flow rate requirement at this time for the use of carbon dioxide in poultry; however, adjustments are required if time to death is more prolonged than expected.

5.9.6.1. In tests where it took 8 seconds to achieve the target gas concentration, broilers and mature hens collapsed in 19 to 21 seconds at 65% CO<sub>2</sub> and 25 to 28 seconds at 35% CO<sub>2</sub>.

5.9.6.2. In a gradual-fill study, ducks and turkeys lost consciousness before 25% CO<sub>2</sub> was reached and died after the concentration reached 45%.

5.9.7. Nonhatched eggs (pips), newly hatched chicks and poults have a greater tolerance to carbon dioxide.

5.9.7.1. Concentrations as high as 80% to 90% than for adults of the same species may be needed.

5.9.7.2. Follow-up exposure to hypoxemia, or a secondary euthanasia method, may be required to ensure unconsciousness and death.

5.10. Food Animal

5.10.1. Carbon dioxide is the only chemical currently used in euthanasia of food animals (primarily swine) that does not lead to tissue residues. Use of carbon dioxide is generally not recommended for euthanasia of larger animals.

- 5.10.2. Carbon dioxide is a suitable method for euthanizing pigs less than 10 weeks of age (nursery pigs <70lbs).
  - 5.10.2.1. Approach can be gradual or pre-fill. In both methods, exposure of pigs with normal respiration to a constant supply of 80% to 90% CO<sub>2</sub> for a minimum of 5 minutes is necessary for effective euthanasia.
- 5.11. Goat Kids
  - 5.11.1. Young goat kids < 3 weeks of age. Euthanasia by CO<sub>2</sub> inhalation has not been evaluated in older goats or sheep.
  - 5.11.2. It is recommended that once kids are placed in a euthanasia chamber it should be filled at a rate sufficient to achieve a CO<sub>2</sub> concentration of > 70% by 5 minutes and to use a dwell time of 10 minutes to assure euthanasia.
- 5.12. Dogs and Cats
  - 5.12.1. Carbon dioxide is acceptable with conditions for use in institutional situations where appropriately designed and maintained equipment and trained and monitored personnel are available to administer it, but it is not recommended for routine euthanasia of cats and dogs.
- 5.13. Small Mammals Euthanized in the Field
  - 5.13.1. Small scale CO<sub>2</sub> delivery systems have been developed for use by trained biologists working with wildlife in the field.
  - 5.13.2. Small scale systems should discharge CO<sub>2</sub> at a rate that satisfies the AVMA guidelines on euthanasia.

## 6. EXCEPTIONS

- 6.1. The PI may request an exception to the above standards by describing the departure in the AUP
- 6.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013

## 7. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

- 7.1. References
  - 7.1.1. Guide for the Care and Use of Laboratory Animals, 8th Edition
  - 7.1.2. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.
  - 7.1.3. [OLAW FAQ F.1. Is the use of carbon dioxide an acceptable euthanasia agent?](#)
  - 7.1.4. NIH Guidelines for the Euthanasia of Rodent Fetuses and Neonates
  - 7.1.5. Murray V Ellis, Development of a compact system for field euthanasia of small mammals, Journal of Mammalogy, Volume 98, Issue 4, 1 August 2017, Pages 1211–1214, <https://doi.org/10.1093/jmammal/gyx072>
  - 7.1.6. B.I. Baker, et.al., Defining characteristics of immersion carbon dioxide gas for successful euthanasia of neonatal and young broilers, Poultry Science, Volume 99, Issue 9, 2020, Pages 4408-4416, <https://doi.org/10.1016/j.psj.2020.05.039>.
- 7.2. Resources
  - 7.2.1. EHS:
    - 7.2.1.1. <https://ehs.tamu.edu/>
    - 7.2.1.2. 979-845-2132
    - 7.2.1.3. [ehsd@tamu.edu](mailto:ehsd@tamu.edu)
- 7.3. [IACUC/AWO Referenced Documents](#): (requires TAMU NetID authentication)
  - 7.3.1. AWO-O-014 CO<sub>2</sub> Euthanasia of Rodents
  - 7.3.2. TAMU-G-015 Guidelines for Reporting Animal Concerns, Unanticipated or Adverse Events, and Potential Noncompliance
  - 7.3.3. TAMU-G-025 Guidelines on Physical Methods of Euthanasia in Warm Blooded Species
  - 7.3.4. TAMU-G-029 Guidelines for Animal Protocol Participation and Handling
- 7.4. Acknowledgements



7.4.1. This document was partially adapted using materials obtained from the University of Georgia and Marquette and Indiana Universities.

**8. HISTORY**

Effective Date	Version #	Description
12/19/2019	000	College Station/Galveston: New document
05/21/2020	001	College Station/Galveston: Content updated per the AVMA 2020 Guidelines on Euthanasia. Reviewed and approved via email.
07/21/2020	002	Dallas: New document, updated content. Partially replaced CD-205.03.
08/03/2020	003	Houston/Kingsville: New document, updated content. Partially replaced IBT-205.02. Reviewed and approved via email.
03/01/2021	004	College Station/Galveston: Renewal document, clarification to definitions and additional reference.
04/01/2021	005	Houston/Kingsville: Renewal document, clarification to definitions and additional reference.
04/20/2021	006	Dallas: Renewal document, clarification to definitions and additional reference.
03/24/2022	007	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
10/20/2022	008	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.
12/01/2023	009	College Station/Dallas/Galveston/Kingsville: Renewal; minor changes to definitions and resources, addition of Exception section. Reviewed and approved via TEAMS.
01/01/2024	010	Houston: Renewal; minor changes to definitions and resources, addition of Exception section. Reviewed and approved via TEAMS.