IACUC Guidance:	**TAMU-G-047**	Title:	**Guidelines on Inhaled and Non-inhaled Agents of Euthanasia**

Location	Effective Date	Review By
College Station/Dallas/Galveston/Kingsville	10/20/2022	01/31/2025
Houston	03/01/2022	01/31/2025

1. PURPOSE

1.1. To describe common agents of inhaled and non-inhaled euthanasia of animals used for research, teaching, testing and other purposes at Texas A&M University.

2. SCOPE

2.1. Applies to commonly used inhaled and non-inhaled agents of euthanasia. For a complete review, see the current AVMA Guidelines on Euthanasia.

2.2. Does not apply to depopulation.

2.3. For guidance regarding:

2.3.1. Physical methods of euthanasia, see TAMU-G-009 and TAMU-G-025.

2.3.2. Use of MS222, see TAMU-G-021.

2.3.3. CO_2 asphyxiation, see TAMU-G-028.

2.3.4. Euthanasia method in fish, amphibians and reptiles, see TAMU-G-048

2.3.5. The safe use of inhalant anesthetics, see TAMU-G-003.

3. RESPONSIBILITY

3.1. The PI is responsible for:

3.1.1. Ensuring equipment used to deliver and maintain inhaled agents is in good working order and in compliance with state and federal regulations.

3.1.2. Instituting effective procedures to reduce animal worker exposure to anesthetic vapors in accordance with state and federal occupational health and safety regulations.

3.1.3. Using inhaled agents supplied in purified form without contaminants or adulterants, such that an effective displacement rate and/or concentration can be readily quantified.

3.1.4. 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.

3.1.5. Ensuring that animals are euthanized by trained personnel using appropriate technique, equipment and agents, as outlined in the approved animal use protocol.

3.1.6. Training personnel to minimize distress and to recognize and confirm death.

3.1.7. Documenting training according to TAMU-G-029.

3.1.8. Acquiring and maintaining licensure with the DEA for controlled substance use.

3.1.9. Securing approval for use of alternatives through the amendment or VVC process, as applicable, during times of pentobarbital shortage.

4. DEFINITIONS AND/OR ACRONYMS

4.1. **Acceptable**: A method considered to reliably meet the requirements of euthanasia. Also see EUTHANASIA.

4.2. **Acceptable With Conditions**: A method considered to reliably meet the requirements of euthanasia when specified conditions are met. Also see EUTHANASIA.

4.3. **Adjunctive Method**: A method of assuring death that may be used after an animal has been made unconscious.

4.4. **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.

4.5. **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.
4.6. **Aversion**: A desire to avoid or retreat from a stimulus
4.7. **DEA**: United States Drug Enforcement Agency
4.8. **Depopulation**: The rapid destruction of a population of animals in response to urgent circumstances with as much consideration given to the welfare of the animals as practicable.
4.9. **Distress**: The effect of stimuli that initiate adaptive responses that are not beneficial to the animal—thus, the animal's response to stimuli interferes with its welfare and comfort.
4.10. **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.
4.11. **Halogenated anesthetics**: Isoflurane, sevoflurane or desflurane.
4.12. **IC**: Intra-cardiac
4.13. **Inhaled Agents of Euthanasia**: include agents that are introduced into the body through direct delivery to the respiratory tract.
4.14. **IM**: Intramuscular
4.15. **IP**: Intraperitoneal
4.16. **IV**: Intravenous
4.17. **Non-inhaled Agents of Euthanasia**: Include chemical agents that are introduced into the body by means other than through direct delivery to the respiratory tract. The primary routes of their administration are parenteral injection, topical application, and immersion.
4.18. **Secondary Method**: A euthanasia method employed subsequent to a primary method to ensure death of an unconscious animal before it can recover consciousness. See ADJUNCTIVE METHOD.
4.19. **Unacceptable**: A method that does not meet the requirements of euthanasia.
4.20. **Verification of Death**: A requirement following euthanasia best accomplished using a combination of species-specific criteria. Methods that can be used for verification of death include: cessation of cardiac function (palpation of pulse in an appropriate anatomic location based on species, auscultation with a stethoscope, and use of Doppler ultrasound), cessation of breathing, lack of reflexes (corneal/firm toe pinch), graying of the mucous membranes, and confirmation by physical intervention (decapitation, cervical dislocation, bilateral thoracotomy, pithing).

5. GUIDELINES OR PROCEDURE

5.1. General Information – Inhaled Agents
5.1.1. Overdoses of inhaled anesthetics have been used to euthanize many species.
5.1.1.1. Order of preference is isoflurane and sevoflurane with or without N_2O. **Nitrous oxide should not be used alone**. **Ether is not acceptable for euthanasia**.
5.1.2. Inhaled anesthetics can be administered by several different methods depending on the circumstances and equipment available (e.g., face mask, open drop, precision vaporizer, rigid or non-rigid containers)
5.1.2.1. Because the liquid state of most inhaled anesthetics is irritating, the animal is **not** permitted to directly contact the anesthetic liquid.
5.1.2.2. Note: The use of halothane is highly discouraged due to severe hepatotoxicity and malignant hyperthermia that can occur in multiple species (including humans). Rigorous scientific justification would be required for its use. The IACUC would only consider halothane use in a closed system to minimize occupational exposure (i.e. never the open drop method).
5.1.3. All inhaled agents currently used for euthanasia have been identified as being aversive to varying degrees.

5.1.4. In sick or depressed animals where ventilation is decreased, agitation during induction is more likely.
5.1.5. Neonatal animals will require extended exposure times.
5.1.6. Rapid gas flows can produce noise or cold drafts leading to animal fright and escape behaviors.
5.1.7. In those species where aversion or overt escape behaviors have not been noted, exposure to high concentrations resulting in rapid loss of consciousness is preferred. Otherwise, gradual-fill methods can be used, keeping in mind the effect the time to unconsciousness with inhaled agents is dependent on the displacement rate, container volume, and concentration.
5.1.8. When possible, inhaled agents should be administered under conditions where animals are most comfortable (e.g., for rodents, in a darkened home cage; for pigs, in small groups). If animals need to be combined, they should be of the same species and compatible cohorts. If needed, animals should be restrained or separated so that they will not hurt themselves or others. Chambers should not be overloaded and need to be kept clean to minimize odors that might cause distress in animals subsequently euthanized.
5.1.9. Inhaled anesthetics can be administered as the sole euthanasia agent or as part of a 2-step process, where animals are first rendered unconscious through inhaled anesthetic agent exposure and then subsequently euthanized by a secondary method.
5.1.10. Death must be verified following administration of inhaled agents. If an animal is not dead, exposure must be repeated or followed with another method of euthanasia.
5.1.11. Inhaled anesthetics are generally used for euthanasia of smaller animals (< 7 kg [15.4 lb]) or for animals in which venipuncture may be difficult.

5.2. General Information – Non-inhaled Agents
5.2.1. Consideration needs to be given to the species involved, the pharmacodynamics of the chemical agent, degree of physical or chemical restraint required, potential hazards to personnel, consequences of intended or unintended consumption of the animal's remains by humans and other animals, and potential hazards to the environment from chemical residues.
5.2.2. Euthanasia solution is only to be used during humane termination of an animal's life. Any other use is contrary to the label instructions. **Euthanasia solution is not to be used as an anesthetic** for survival or non-survival procedures, unless justified in the AUP and approved by the IACUC.
5.2.3. Current federal drug regulations require strict accounting for barbiturates, and these must be used under the supervision of personnel registered with the United States DEA. See TAMU-G-031 Guidelines on DEA Licenses.
5.2.4. Non-pharmaceutical grade euthanasia solutions require scientific justification and approval by the IACUC. See TAMU-G-010.
5.2.5. Barbiturates (and barbituric acid derivatives)
5.2.5.1. FDA approved pentobarbital (or pentobarbital combination) drugs are a preferred method of euthanasia of dogs, cats, and other animals. During shortages, alternative drugs/methods may need to be considered. The 2020 AVMA Guidelines on Euthanasia provide such alternatives. The AV, or designee is also available for consultation.
5.2.5.1.1. Combining euthanasia solution with deep anesthesia may conserve pentobarbital during times of shortage; however, careful monitoring is required to confirm death and requires pre-planning with the office of the Attending Veterinarian.
5.2.5.2. IV Route
5.2.5.2.1. Barbiturates administered IV may be given alone as the sole agent of euthanasia or as the second step after sedation or general anesthesia. Refer to the product label or appropriate species references for recommended doses.
5.2.5.3. IP Route

5.2.5.3.1. When IV access would be distressful, dangerous, or impractical (e.g., small patient size such as puppies, kittens, small dogs and cats, rodents, and some other nondomestic species or behavioral considerations for some small exotic mammals and feral domestic animals), barbiturates may be administered IP (e.g., sodium pentobarbital, secobarbital; **not pentobarbital combination products** as these have only been approved for IV and intra-cardiac administration [pentobarbital/phenytoin (e.g., Euthasol)]

5.2.5.3.2. Because of the potential for peritoneal irritation and pain (observed in rats), lidocaine has been used with some success in rats to ameliorate discomfort. For applicability in other species, please consult the AV, or designee.

5.3. Species-Specific Recommendations:

5.3.1. **Companion Animals**

5.3.1.1. Overdoses of inhaled anesthetics administered via chamber (e.g., isoflurane, sevoflurane) are acceptable with conditions for euthanasia of small mammals and some other species < 7 kg. Because of the potential for recovery, care must be taken to ensure death has occurred prior to disposing of animal remains. Inhaled anesthetics may also be used to anesthetize small fractious animals prior to administration of an injectable euthanasia agent.

5.3.1.2. Intravenous injection of a barbituric acid derivative (e.g., pentobarbital, pentobarbital combination product) is the **preferred method** for euthanasia of dogs, cats, and other small companion animals

5.3.1.3. Nonbarbiturate anesthetic overdose—Injectable anesthetic overdose (e.g., combination of ketamine and xylazine given IV, IP, or IM or propofol given IV) is acceptable for euthanasia when animal size, restraint requirements, or other circumstances indicate these drugs are the best option for euthanasia.

5.3.1.4. When IV access is unachievable using manual restraint, general anesthesia followed by intra-organ (e.g., intraosseous, intra-cardiac, intrahepatic, and intra-renal) injection is recommended. Intra-cardiac is the **preferred** method.

5.3.2. **Small Laboratory and Wild-Caught Rodents** (mice, rats, hamsters, guinea pigs, gerbils, degus, cotton rats, etc.)

5.3.2.1. Isoflurane or sevoflurane with or without nitrous oxide may be useful in cases where physical restraint is difficult or impractical. When used as a sole euthanasia agent delivered via vaporizer for anesthetic induction and overdose, animals may need to be exposed for prolonged time periods to ensure death. Death can be rapid when using the open-drop technique, but care must be taken to ensure that the rodent does **not** come in direct contact with the anesthetic.

5.3.2.2. Anesthetic agents are effective for euthanasia of in utero fetuses.

5.3.2.3. An adjunctive method (e.g., cervical dislocation, decapitation) must be performed when anesthetics are used on neonatal rodents to avoid the possibility of recovery.

5.3.2.4. Barbiturates may be administered IP (e.g., sodium pentobarbital, secobarbital; **not** pentobarbital combination products as these have only been approved for IV and intra-cardiac administration [pentobarbital/phenytoin (e.g., Euthasol)]

5.3.2.5. **Unacceptable as a sole euthanasia agent**: Potassium chloride, Neuromuscular blocking agents, α-Chloralose, Opioids, urethane

5.3.2.6. **Also Unacceptable**: Combining injectable barbiturates and neuromuscular blocking agents in the same syringe for administration is unacceptable because the neuromuscular blocking agents may take effect before the animal is anesthetized.

5.3.2.7. Injectable barbiturates alone and in combination with local anesthetics and anticonvulsants; dissociative agents combined with α2-adrenergic receptor agonist or benzodiazepines—These agents are acceptable for use in fetuses or neonates.

5.3.3. **Laboratory Rabbits**

5.3.3.1. It is best to pre-anesthetize the animal with a sedative before removing it from the home cage to reduce their natural tendency to breath-hold when confronted with unpleasant odors. Animals already under anesthesia may be euthanized by an overdose of anesthetic.

5.3.3.2. If rabbits are conditioned to handling or restraint devices are available, venous access may be obtained via the ear. In the case of fractious rabbits, sedation may be necessary to gain venous access for administration of an injectable barbiturate or injectable barbiturate combination. Barbiturates may also be administered IP.

5.3.4. **Bovids, Small Ruminants, and Swine**

5.3.4.1. IV administration of euthanasia solutions containing barbiturates is acceptable.

5.3.4.2. Cost can be a deterrent to the use of barbiturates for euthanasia of large numbers of animals.

5.3.4.3. Animals must be anesthetized before administration of potassium chloride or magnesium sulfate.

5.3.5. **Avian** (pet, aviary, falconry, racing, research, and zoo birds)

5.3.5.1. Inhaled anesthetics may be used at high concentrations as a sole method of euthanasia or may be used to render birds unconscious prior to application of other methods of euthanasia. Euthanasia via inhaled gases may be more practical than use of an injectable agent if large numbers of birds, such as in flock or aviary situations, must be euthanized.

5.3.5.2. Euthanasia by exposure to gas anesthetics also induces minimal tissue damage and results in the least amount of tissue artifact for necropsy.

5.3.5.3. Barbiturates can be administered IV for euthanasia of anesthetized or properly restrained un-anesthetized birds. Barbiturates commonly used for injection are available as sodium salts that are alkaline and may be irritating and painful when injected directly into tissues, rather than IV. Therefore, when IV injection is impossible, injectable euthanasia agents can be administered via intra-coelomic, intra-cardiac, or intraosseous routes only if a bird is unconscious, or anesthetized.

5.3.6. **Poultry**

5.3.6.1. Poultry may be euthanized by IV injection of overdoses of anesthetics, including barbiturate and barbituric acid derivatives.

5.3.6.2. Potassium chloride or magnesium sulfate—Although IV or intra-cardiac administration of potassium chloride or magnesium sulfate to a conscious bird as a sole method of euthanasia is unacceptable, it is acceptable to administer these agents to a bird that is fully anesthetized or otherwise unconscious as a means to ensure death.

5.3.6.3. See TAMU-G-028 for recommendations regarding $C0_2$ asphyxiation.

5.3.7. **Equid**

5.3.7.1. Pentobarbital or a pentobarbital combination is the principal choice for equine euthanasia by chemical means.

5.3.8. **Fish**

5.3.8.1. Sodium pentobarbital (60 to 100 mg/kg [27.3 to 45.5 mg/lb]) can be administered by IV, intra-cardiac, or intra-coelomic routes for euthanasia.

5.3.8.2. Pentobarbital may also be administered via intra-cardiac injection for anesthetized animals as the second step of a 2-step euthanasia procedure. Death usually occurs within 30 minutes.

5.3.8.3. Ketamine may be administered at dosages from 66 to 88 mg/kg315 (30 to 40 mg/lb) via an IM injection followed by a lethal dose of pentobarbital. Observers should be advised about the possibility of ketamine-induced muscle spasms during induction.

5.3.8.4. A combination of ketamine, at dosages of 1 to 2 mg/kg, with medetomidine, at dosages of 0.05 to 0.1 mg/kg (0.02 to 0.05 mg/lb), may be administered via IM injection followed by a lethal dose of pentobarbital.

5.3.8.5. A dose of propofol, at 1.5 to 2.5 mg/kg (0.7 to 1.1 mg/lb) can be administered IV followed by an injection of a lethal dose of pentobarbital.

5.3.8.6. Concentrated liquid anesthetics (isoflurane, sevoflurane) can be added to water, although they are generally not very water soluble. Doses of > 5 to 20 mL/L can be used (10 times the upper range for anesthesia). However, because both anesthetics are highly volatile, human safety is of concern and use in a well-ventilated area is imperative.

5.3.9. **Captive Amphibians and Reptiles**

5.3.9.1. Many reptiles and amphibians are capable of breath holding and shunting of their blood, which permits conversion to anaerobic metabolism for survival during prolonged periods of anoxia (up to 27 hours for some species). Because of this, induction of anesthesia and time to loss of consciousness may be greatly prolonged when inhaled agents are used. Death may not occur even with prolonged exposure.

5.3.9.2. Lizards and most snakes do not hold their breath to the same extent as some of the chelonians, and are therefore more likely to have a clinical response to inhaled agents. Regardless of the species or taxonomic group, death must be verified prior to terminating the use of the inhaled agent, or a second, guaranteed lethal procedure (e.g., decapitation) should be performed to ensure death

5.3.9.3. Barbiturates are best administered intra-vascularly to minimize the discomfort upon injection. However, where intravascular administration is not possible or its benefits are outweighed by distress imposed by additional restraint, pain from alternate methods, risk to personnel, or other similar reasons, intra-coelomic administration is an acceptable route for administration of barbiturates.

5.3.9.4. Dissociative agents such as ketamine hydrochloride or combinations such as tiletamine and zolazepam; inhaled agents; and IV administered anesthetics, such as propofol, or other ultra–short-acting barbiturates, may be used for poikilotherms to induce rapid general anesthesia and subsequent euthanasia, although application of an adjunctive method to ensure death is recommended.

5.3.10. **Captive Nonmarine Mammals**

5.3.10.1. Inhaled anesthetics are most suitable for smaller species.

5.3.10.2. Inhaled anesthetics may be administered via face mask or chambers. Placing an animal's entire crate into a chamber will allow anesthesia to be induced with the least amount of distress.

5.3.10.3. Barbiturates may be administered IV or IP. Intra-cardiac administration must be limited to animals that are unconscious due to disease or the effects of anesthetics.

5.3.10.4. Opioids and other anesthetics may be administered IV or IM for euthanasia when animal size, restraint requirements, or other circumstances indicate these drugs are the best option for euthanasia.

5.3.10.5. Potassium chloride can be administered IV or intra-cardiac to stop the heart of animals that are deeply anesthetized or unconscious.

5.3.11. **Captive Marine Mammals**

5.3.11.1. Inhaled anesthetics are uncommonly used to euthanize marine mammals because these animals' ability to breath-hold means that extended periods of physical restraint are necessary for their administration.

5.3.11.2. Intravenous administration of barbiturates and their derivatives can be a rapid and reliable method of euthanasia for small pinnipeds, small odontocetes, and sirinids. Intraperitoneal administration is also acceptable where intravascular administration is not possible or is outweighed by distress from the requirement of additional restraint, pain from alternate methods, risk to personnel, or other similar reasons, although tissue irritation and variable absorption rates must be considered.

5.3.12. **Free Ranging Wildlife**

5.3.12.1. Smaller avian and mammalian species that can be confined in enclosed containers can be euthanized using open-drop methods of administration. Larger species may be restrained for face-mask administration, when animal distress associated with restraint can be minimized.

5.3.12.2. Portable equipment is available that can make these methods practical.

5.3.12.3. Preference should be given to the use of alternate methods for taxa that can breath-hold for extended periods of time.

5.3.12.4. Chemical methods of euthanasia applicable to free-ranging wildlife include overdoses of injectable anesthetic agents (including barbiturates).

5.3.12.4.1. The carcass should be removed from the field for disposal (e.g, incineration) or buried to prevent exposure of non-target species to anesthetics.

5.3.12.5. Potassium chloride may be administered IV or intra-cardiac to stop the heart of animals that are deeply anesthetized or unconscious.

5.3.13. **Free Ranging Marine Mammals**

5.3.13.1. While acceptable with conditions from an animal welfare standpoint, practical and human and environmental safety constraints generally prevent use of inhaled agents for euthanasia of marine mammals under field conditions.

5.3.13.2. Overdoses of injectable anesthetics can be used to euthanize marine mammals under field conditions. Anesthetics that can be used alone or in combination include tiletamine-zolazepam, ketamine, xylazine, meperidine, fentanyl, midazolam, diazepam, butorphanol, acepromazine, barbiturates, and etorphine. Intramuscular administration of anesthetics may be required to achieve restraint of conscious animals before personnel can safely perform euthanasia using injectable agents by an intravascular route.

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. AVMA Guidelines on Euthanasia 2020.

7.1.2. Conserving pentobarbital in times of shortage | American Veterinary Medical Association (avma.org)

7.1.3. Regulatory Considerations for Using Pharmaceutical Products in Research Involving Laboratory Animals 2015. https://olaw.nih.gov/sites/default/files/150604_seminar_transcript.pdf

7.1.4. OLAW webinar: Regulatory Considerations for Using Pharmaceutical Products in Research Involving Laboratory Animals - June 4, 2015. Transcripts: https://olaw.nih.gov/sites/default/files/150604_seminar_transcript.pdf

7.1.5. Pentobarbital back orders and potential alternatives | American Veterinary Medical Association (avma.org)

7.2. Resources:

7.2.1. For more information, please contact:

7.2.1.1. CMP at 979-845-7433

7.2.1.2. ARU: at (214) 828-8149

7.2.1.3. PAR: at (713) 677-7471

7.2.1.4. PRF: at (361) 221-0770

7.3. IACUC/AWO Referenced Documents: (requires TAMU NetID authentication)

7.3.1. TAMU-F-013 Request for Programmatic Exception from Animal Welfare Standards

7.3.2. TAMU-G-003 Guidelines on the safe use of inhalant anesthetics.

7.3.3. TAMU-G-009 Performance of Physical Methods of Euthanasia in Nonrodents

7.3.4. TAMU-G-010 Guidelines for the Use of Pharmaceutical and Non-Pharmaceutical Grade Drugs and Compounds

7.3.5. TAMU-G-021 Guideline for the Use of MS222

7.3.6. TAMU-G-025 Guidelines for the Performance of Physical Methods of Euthanasia in Mice and Rats

7.3.7. TAMU-G-028 Guidelines on Euthanasia Using Carbon Dioxide

7.3.8. TAMU-G-029 Guidelines for Animal Protocol Participation and Handling

7.3.9. TAMU-G-031 Guidelines on DEA Licenses

7.3.10. TAMU-G-048 Guidelines on Euthanasia of Fish, Amphibians and Reptiles

8. HISTORY

Effective Date	Version #	Description
07/01/2020	000	College Station/Galveston: New format and updated content; replaced unnumbered document titled “Euthanasia Guidelines”. Reviewed and approved by email.
08/03/2020	001	Houston/Kingsville: new document; partially replaced IBT-205. Reviewed and approved by email.
10/20/2020	002	Dallas: new document; partially replaced CD-205
02/01/2022	003	College Station/Galveston: Renewal; updated scope, definitions, and resources. Updated 5.2.5. due to pentobarbital shortage. Added Exceptions section. Reviewed and approved by email.
03/01/2022	004	Houston/Kingsville: Renewal; updated scope, definitions, and resources. Updated 5.2.5. due to pentobarbital shortage. Added Exceptions section.
03/24/2022	005	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
10/20/2022	006	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.