

| <b>Institutional Animal Care &amp; Use Program - UTEP</b>                                      |   |
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| <b>Title:</b> Rodent, Rabbit, Bird, Fish, Reptile & Amphibian Euthanasia                       |   |
| <b>Policy#:</b> 008  | <b>Date in Effect:</b> 21 November 2014 |
| <b>Version #:</b> B  | <b>Rev Date:</b> 27 January 2020        |
| <b>In Effect</b> <input checked="" type="checkbox"/> <b>Rescinded</b> <input type="checkbox"/> | <b>Date Rescinded:</b>                  |

A) RESPONSIBILITIES

It is the responsibility of all personnel using animals at UTEP to abide by this policy. Exceptions to this policy must be approved by the IACUC when good justification is provided to deviate from this policy.

B) APPLICATION

This policy applies to applicable animals used in research and teaching at UTEP.

C) BACKGROUND INFORMATION

Euthanasia techniques should result in a rapid loss of consciousness followed by cardiac and respiratory arrest and the ultimate loss of brain function. The technique should minimize distress and anxiety prior to loss of consciousness. Animals must be euthanized by trained personnel only. An important component of training is verification of technical proficiency in the method of euthanasia to be used. This is necessary to ensure a painless death that satisfies research requirements. Death should be induced as painlessly and quickly as possible. Euthanasia should not be performed in the animal holding room. The euthanasia method must be appropriate to the species approved in the IACUC protocol. Overdosing with an inhalant agent may result in deep depression of all life signs prior to death. It is possible that animals could revive from this state, which can be mistaken for death during a cursory examination. To prevent such an occurrence and to ensure effective euthanasia, the IACUC has instituted this policy.

It is the PI's responsibility to determine that all personnel have been trained to perform the protocol-approved method of euthanasia, and to monitor that personnel consistently apply it humanely and effectively. Training can be provided within the lab group if the existing staff has adequate expertise. Additional training in these techniques is available from LARC (Laboratory Animal Resource Center).

D) APPLICATION

This policy is based on the [2020 AVMA Guidelines for the Euthanasia of Animals](#).

E) DEFINITIONS

Euthanasia is the act of ending the life of animals by methods that induce rapid unconsciousness and death while minimizing pain and distress.

F) SECONDARY PHYSICAL METHODS OF EUTHANASIA

Death must be assured by using one of the following methods as a secondary method to ensure effective euthanasia:

- (1) Pithing
- (2) Decapitation
- (3) Bilateral thoracotomy (removal of multiple vital organs for tissue procurement).
- (4) Exsanguination

G) PROCEDURES

1) **Pentobarbital sodium**

A single lethal IP/ID or IV (poultry, birds, and rabbits) administration of a pharmaceutical grade veterinary euthanasia solution containing sodium pentobarbital (390 mg/ml is considered the preferred agent/method of euthanasia and administered at a dose of 1 ml/10 lb. of body weight (1mL/4.5 kg). If Pentobarbital sodium anesthetic is used as a sole active ingredient and not as a component of a prepared euthanasia solution as noted above, a pharmaceutical grade (and not a chemical grade) of the drug must be used for euthanasia and administered at a dose of 150 mg/kg or greater by the same routes mentioned above. Sedation may be necessary to gain venous (IV) access for administration of an injectable barbiturate or injectable barbiturate combination. Intracardiac, intracoelomic, or intraosseous injection may be used if the animal is heavily sedated, unconscious, or anesthetized. Euthanasia solutions (e.g., Fatal Plus, Beuthanasia-D, SLeepAway, Somnasol, Euthasol) have multiple pharmaceutical grade products available which can also contain sodium phenytoin (50 mg/ml) in addition to the sodium pentobarbital and should be administered at a dose of > 90 mg/kg.

## 2) **Tricaine methane sulfonate (TMS 222) for fish, some amphibians and reptiles**

Tricaine methane sulfonate (TMS 222) can be used either as an injectable agent (except intracoelomic injection in amphibians) → (200-300 mg/kg of a 1% buffered solution), or as an immersion bath (250-500 mg/L or 5-10x anesthetic dosage)) for fin fish and amphibians. Tricaine is a benzoic acid derivative and generally should be buffered with sodium bicarbonate. The immersion time needed to assure death can range from 30 minutes to three hours, so it is necessary to use TMS 222 as an anesthetic followed by a physical method for some amphibians (such as *Xenopus* spp.) and larger finfish. In other words, once in a surgical plane of anesthesia, euthanasia of these species must be accomplished by a physical method described under F. Consultation with the Attending Veterinarian should occur when considering TMS 222 use without also employing a physical method. Benzocaine hydrochloride (stock solution of 100g/L with or without acetone or ethanol is also acceptable when delivered to the ventral abdomen of amphibians in a 20% concentration gel to an area measuring 2.0cm X 1.0mm for some species.

**Safe Preparation of TMS 222 Solution:** TMS 222 comes as a powder, and it is considered a chemical irritant and associated with retinal toxicity in humans. The solution should be prepared in a chemical fume hood exhausted out of the room, and nitrile gloves, dust mask, and safety goggles should be worn. If a hood that is exhausted to the outside is not available, a biological safety cabinet can be used to weigh the powder and prepare the solution. Alternatively, a fit-tested N-95 mask should be worn with eye protection, and the procedure conducted in a well-ventilated area. In water of low alkalinity (>50mg/L as CaCO<sub>3</sub>), the solution must be buffered with sodium bicarbonate. A 10g/L stock solution can be made to which sodium bicarbonate is added to saturation and resulting in a final pH range of 7.0 - 7.5. The stock solution should be stored in a dark brown bottle or a clear bottle covered by a darkening material (e.g., aluminum foil), and refrigerated or frozen if possible. The solution should be replaced monthly and any time a brown

color is observed. Contact EH&S to dispose of used and unused TMS 222 solution.

- a) **Fish:** Fish should be left in the MS 222 solution for at least 30 minutes following cessation of opercular movement. Larger fish should also be subjected to a physical method of euthanasia as described above under F (1) and (2).
- b) **Amphibians:** Amphibians should be left in this solution for at least 1 hour following cessation of movement. Amphibians may also be fully anesthetized in a properly buffered TMS 222 bath with a minimum 4-minute immersion that results in cessation of movement prior to application of euthanasia via a physical method as described under F.
- c) **Reptiles:** This may be accomplished in two steps using TMS 222. An intracoelomic injection of 250-500mg/kg of neutral pH solution (0.7% - 1.0% MS 222) typically results in complete loss of consciousness by 4 minutes post-injection; unbuffered, 50% TMS 222 must be administered via intracoelomic injection to complete euthanasia.
- d) Death must be assured by using one of the following methods as a secondary method to ensure effective euthanasia:
  - (1) Pithing
  - (2) Decapitation
  - (3) Bilateral Thoracotomy (removal of multiple vital organs for tissue procurement).
  - (4) Exsanguination

### 3) **Carbon dioxide**

CO<sub>2</sub> inhalation is a common method of euthanasia used for poultry, birds, rodents and, to a lesser extent in rabbits. Premedication with a sedative is suggested prior to euthanizing with CO<sub>2</sub>. When euthanizing with CO<sub>2</sub> the following guidelines must be used:

- a) Euthanasia should be conducted in the home cage (rodents) whenever possible.

- (1) Place any remaining animals (rodents), which are not being euthanized, in a clean cage.
- b) If euthanasia cannot be conducted in the home cage, then a euthanasia chamber should be utilized with the following instructions:
  - (1) The chamber should allow ready visibility of the animals.
  - (2) Do not overcrowd the chamber. All animals in the chamber must be able to assume normal postural adjustments. To avoid overcrowding and undue stress, no more than five mice (or like-sized rodents of the same species) or two rats should be allowed in the chamber during the euthanasia process.
  - (3) Empty and clean the chamber between uses.
  - (4) Completely flush the chamber with room air for at least 1-2 minutes between each euthanasia event to dislodge captured CO<sub>2</sub> from the container (CO<sub>2</sub> is heavier than air). This is done by turning the chamber on its side for 1-2 minutes between each euthanasia event.
  - (5) Dump all debris into the trash.
  - (6) Mist/wipe chamber with LARC approved disinfectant.
- c) Compressed CO<sub>2</sub> gas in cylinders is the only acceptable source of CO<sub>2</sub> euthanasia. CO<sub>2</sub> generated from other sources, such as dry ice or fire extinguishers is unacceptable because gas flow cannot be regulated precisely in those circumstances.
- d) Since carbon dioxide is 50% heavier than air, chambers should be designed so that as they fill with gas they can vent from the top. This allows the air to exit at the top and be completely replaced by carbon dioxide.
- e) Place the animal(s) in the chamber and introduce 100% carbon dioxide at a rate of 30-70% of the chamber volume per minute.
- f) No flow rate formula is currently in place at this time, the new AVMA Guidelines for Euthanasia has stated the following: An optimal flow rate for CO<sub>2</sub> euthanasia systems should displace 30% to 70% of the chamber or cage volume/min, with the understanding that 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  
However, as there is no clear evidence of a flow rate that optimally minimizes both pain and distress for all species, sexes, and genetic backgrounds, veterinarians should use their professional judgment to determine which flow rate is appropriate for their circumstances.

- g) Animals should be left in the container until clinical death is observed/confirmed.
  - h) To ensure death, perform one of the following secondary methods:
    - (1) Cervical dislocation in poultry, birds, rodents (<200 g), and rabbits (<1 kg).
    - (2) Decapitation.
    - (3) Exsanguination.
    - (4) Exsanguination as part of perfusion or organ removal.
    - (5) Bilateral thoracotomy (making a stab incision into both sides of the chest with a scalpel or sharp scissors to open up the lung cavity).
  - i) If fresh tissue is required for laboratory tests (e.g., tissues for RNA analysis); animals may be removed from the CO<sub>2</sub> chamber following sustained cessation of breathing (approximately 4-5 min), provided a physical secondary method as listed above is performed immediately.
- 4) **Inhaled anesthetics (e.g., isoflurane, sevoflurane)**
- a) Inhaled anesthetics may be administered in a closed pre-charged container (e.g. bell jar pre-charged with anesthetic, a.k.a. as the Open Drop Method) or by a continuous flow vaporizer.
  - b) Animals must never come in direct contact with the liquid form of the inhaled anesthetic. These agents in the liquid form are irritating to the mucous membranes.

- c) Loss of consciousness should be induced rapidly by exposing animals to the maximum agent concentration possible. If using a vaporizer, gas flow may be turned off after cessation of breathing.
- d) To ensure death, administration of an inhalant anesthetic overdose in poultry, birds, rodents, and rabbits is followed by a secondary method of euthanasia such as:
  - (1) Cervical dislocation in poultry, birds, and rodents (<200 g), and rabbits (<1 kg)
    - (a) Recommendation for rabbits:
    - (b) It is best to preanesthetize the animal with a sedative before removing it from the home cage and relocating it to access restraint devices and a gas anesthesia machine. This method will reduce their natural tendency to breath-hold when confronted with unpleasant odors.
  - (2) Decapitation
  - (3) Exsanguination
  - (4) Exsanguination as part of perfusion or organ removal
  - (5) Bilateral thoracotomy (making a stab incision into the chest with a scalpel or sharp scissors to open up the lung cavity).

Order of Inhaled Anesthetic Preference: Isoflurane, halothane sevoflurane, enflurane, methoxyflurane, and desflurane, with or without N<sub>2</sub>O. Nitrous oxide should not be used alone. Methoxyflurane is acceptable with conditions only if other agents or methods are not available. Ether is not acceptable for euthanasia.

5) **Euthanasia of rodent fetuses<sup>1</sup>**

- a) **Fetuses in utero:** Euthanasia of the dam will cause euthanasia of fetuses that remain in utero. No additional actions are necessary when fetuses remain in the uterus.
- b) **Fetuses up to 14 days in gestation (guinea pigs fetuses up to 34 days gestation) when removed from the uterus:** Neural development at this stage is minimal and pain perception is considered unlikely. Euthanasia of the

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<sup>1</sup> NIH Guidelines for the Euthanasia of Rodent Fetuses and Neonates.

mother or removal of the fetus from the uterus ensures rapid death of the fetus due to non-viability of fetuses at this stage of development.

- c) **Fetuses 15 days in gestation to birth (guinea pig fetuses 35 days gestation to birth) when removed from the uterus:** The literature on the development of pain pathways suggests the possibility of pain perception at this time. Fetuses at this age are less sensitive to inhalant agents than are adults. Thus, decapitation with surgical scissors or sharp blades is the preferred method of euthanasia. When chemical fixation or rapid freezing (e.g., immersion in liquid nitrogen) of the whole fetus is required, fetuses should be anesthetized prior to immersion in or perfusion with fixative solutions. Anesthesia may be induced by hypothermia of the fetus, by injection of the fetus with a chemical anesthetic, or by deep anesthesia of the mother with a chemical agent that crosses the placenta, e.g., pentobarbital. When fetuses are not required for the study, the method chosen for euthanasia of a pregnant mother must ensure rapid death of the fetus.
- 6) **Euthanasia of rodent neonates<sup>2</sup>:** Maturation of nociceptors and the development of excitatory and inhibitory receptor systems occur during the period prior to birth and post-natal day 5-7. Resistance to hypoxia at this life stage results in a prolonged time to unconsciousness when CO<sub>2</sub> is used as a euthanasia agent. (Mice can take up to 50 minutes and rats up to 35 minutes to die from CO<sub>2</sub>). Death must be verified after euthanasia and prior to disposal by one of the methods listed above.
- a) **Rodent Neonates up to 10 days of age:** Acceptable methods of euthanasia include injection of chemical anesthetics (e.g., pentobarbital or pentobarbital containing euthanasia solution at the same doses and route listed under F) above), and decapitation or cervical dislocation in anesthetized or sedated animals. Decapitation or cervical dislocation in alert animals must be scientifically justified and approved by the IACUC. Additionally, these animals are sensitive to inhalant anesthetics; e.g., isoflurane, although

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<sup>2</sup> NIH Guidelines for the Euthanasia of Rodent Fetuses and Neonates.



prolonged exposure may be necessary. Immersion in liquid nitrogen or perfusion with chemical fixatives may be used only if preceded by surgical-plane anesthesia. Alternatively, when adequately justified, hypothermia may be used in pups 7 days of age or less. It is unacceptable in pups >7 days of age. When applying hypothermia, caution should be taken to avoid direct contact of the pup with ice or precooled surfaces.

- b) **Guinea pig neonates:** Follow guidelines for adults.
  - c) **Rodent (other than guinea pig) neonates 11 days of age or older:** Follow guidelines for adults.
- 7) **Alternate methods of euthanasia may be IACUC-approved for use under specific conditions.** Euthanasia methods are classified in The 2020 AVMA Guidelines as acceptable, acceptable with conditions, and unacceptable. Please reference the guidelines for further information.
- a) **Acceptable** methods are those that reliably and easily cause humane euthanasia.
  - b) **Unacceptable** techniques are those methods deemed inhumane under any conditions. These methods of euthanasia are prohibited by the IACUC.
  - c) Methods **acceptable with conditions** are those techniques that may require certain conditions to be met to consistently produce humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure death. Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of that method can be met. The IACUC has determined that these methods of euthanasia require scientific justification as well as assurance that the required special conditions are being engaged. The methods included in this category for which scientific justification and specified conditions are required include:
    - (1) **Cervical dislocation of conscious animals:** Manual cervical dislocation can be a humane technique for euthanasia of poultry, small birds, rodents weighing <200 g and rabbits weighing <1 kg, when performed by individuals with a demonstrated high degree of technical proficiency. A

secondary method such as decapitation or exsanguination should be employed to ensure death when feasible. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency.


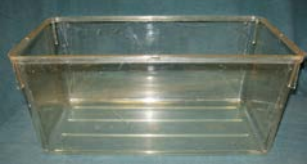

- (2) **Decapitation of conscious animals:** The equipment used to perform decapitation must be kept clean, in good working order with sharp blades. Sharpness of blades must be verified before decapitating animals and sharpening records maintained in the laboratory. The use of plastic cones (known as Decapicones) to restrain animals appear to reduce distress from handling, minimize the chance of injury to personnel, and improve positioning of the animal in the guillotine. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency

If using the following methods, please refer to the AVMA Guidelines for the Euthanasia of Animals 2020 for specific information.

- (1) Inhaled anesthetics
  - (a) Carbon dioxide
  - (b) Carbon monoxide
  - (c) Nitrous oxide
- (2) Non inhaled agents
  - (a) Tribromoethanol
  - (b) Ethanol
- (3) Focused beam microwave radiation

## Appendix A

### Rodent Euthanasia CO<sub>2</sub> (30 - 70% Concentration) Recommended Flow Rates per Cage Type and Size

| Cage Type        | Image of Cage   | Concentration of CO <sub>2</sub> | Flow Rate Setting on CO <sub>2</sub> Flow Meter |
|------------------|---|----------------------------------|---|
| Rat Tecniplast   |    | 30-70%                           | 6.96 -16.24<br>L/min-                           |
| Rat Standard     |   | 30-70%                           | 5.64 – 13.16<br>L/min                           |
| Mouse Tecniplast |  | 30-70%                           | 2.58 – 6.02<br>L/min                            |