

<b>Institutional Animal Care Program (IACP)</b>	
<b>Title: Rodent, Rabbit, Bird, Fish, and Amphibian Euthanasia</b>	
<b>Policy number IACP 008</b>	<b>Date in Effect:</b> 01/11/08
<b>Revision 5, 08/14/20</b>	<b>Re-reviewed Date:</b> 02/14/25, no changes
<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Rescinded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Date Rescinded: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 investigators using animals in research or teaching at UT San Antonio (UTSA) and the animal care staff to abide by and enforce this policy.

B) BACKGROUND INFORMATION

Euthanasia techniques should result in a rapid loss of consciousness followed by cardiac or 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 Institutional Animal Care and Use Committee (IACUC) protocol. Overdosing with an inhalant agent overdose 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 Principal Investigator's (PI) 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 from within the lab group if the existing staff has adequate expertise. Additional training in these techniques is available from the Laboratory Animal Resources Center (LARC}. Personnel who will be performing these techniques (or their PIs) can arrange training by contacting the LARC at x-6692 or [larc@utsa.edu](mailto:larc@utsa.edu).

C) APPLICATION

This policy is based on the 2020 AVMA Guidelines for the Euthanasia of Animals.

D) DEFINITIONS

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

E) PROCEDURES

1) **Pentobarbital sodium**

A single lethal intraperitoneal (IP) (poultry, birds, mice, rats, hamsters and guinea pigs) or intravenous (IV) (rabbits) administration of a pharmaceutical grade veterinary euthanasia solution containing pentobarbital (390 mg/ml) (e.g., Fatal Plus, Beuthanasia-D, SleepAway, Somnosol, Euthasol) is considered the preferred agent/method of euthanasia and administered at a dose of 1 ml/10 lb. of body weight. If Pentobarbital sodium anesthetic is used, 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



injection may be used if the animal is heavily sedated, unconscious, or anesthetized.

2) **Tricaine methane sulfonate (MS 222) for fish and amphibians**

Tricaine methane sulfonate (MS 222) can be used either as an injectable agent (200-300 mg/kg of a 1% buffered solution) or as an immersion bath (2 mg/ml in H<sub>2</sub>O) for amphibians and fish. Tricaine is a benzoic acid derivative and generally should be buffered with sodium bicarbonate. The immersion time needed to assure death can range from 20 minutes to three hours, so it may be advantageous to use MS222 as an anesthetic followed by a physical method. In other words, once in a surgical plane of anesthesia, euthanasia may be accomplished by a physical method described under E) 2) c) Benzocaine immersion (100-200 mg/liter H<sub>2</sub>O) is also acceptable.

**Safe Preparation of MS 222 Solution:** MS 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, facemasks and safety glasses should be worn. If a hood that is exhausted to the outside is not available, a fit-tested N-95 mask should be worn, and the procedure conducted in a well-ventilated area. 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.

a) **Fish:** Fish should be left in the MS 222 solution for at least 10 minutes following cessation of opercular movement. Large fish may be removed from the water, a gill cover lifted, and a concentrated solution from a syringe flushed over the gills.



- b) **Amphibians:** Amphibians should be left in this solution for at least 10 minutes following cessation of movement. MS 222 may also be injected into lymph spaces and pleuroperitoneal cavities. Amphibians may also be fully anesthetized followed by a physical method described under E) 2) c).
- c) Death must be assured by using one of the following methods as a secondary method to ensure effective euthanasia of aquatics:
  - (1) Pithing
  - (2) Decapitation
  - (3) Removal of multiple organs for tissue procurement
  - (4) Exsanguination

3) **Carbon dioxide (CO<sub>2</sub>)**

CO<sub>2</sub> inhalation is a common method of euthanasia used for poultry, birds, mice, rats, guinea pigs and hamsters and must be used as follows:

- a) Euthanasia should be conducted in the home cage (mice, rats, hamsters) whenever possible.
  - (1) Place any remaining animals (mice, rats, hamsters), 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.
  - (3) Empty 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. DO NOT EXCEED A FLOW RATE OF 70%. The formula for the flow rate calculation is:
- Chamber/cage volume calculation: Chamber/cage volume (in<sup>3</sup>) = (height inches) x (width inches) x (length inches).
  - Flow rate calculation: Multiply total chamber/cage volume in<sup>3</sup> volume x 0.3 or 0.7 to find flow rate (30%-70%).
  - Delivery flow rate calculation: The delivery flow rate setting in liters (L) per minute (min) is calculated by dividing the flow rate by 61.024. Please refer to table in Appendix A (below) for recommended flow rates.
- f) For example; an 18-liter volume chamber (large standard rat cage), will require a flow rate of approximately 5-6 L per minute to achieve a 30 % or 13 L per minute to achieve 70% concentration of CO<sub>2</sub>. The pressure reduction regulator should never be set above 30 pounds per square inch



- (psi). After the animals become unconscious, the flow rate can be increased (if using rate  $\leq 70\%$ ) to minimize the time to death.
- 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, mice, hamsters, rats (<200 grams), 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 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., fresh pancreas 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) **Halogenated gaseous agents (e.g., isoflurane, sevoflurane)**
- a) Halogenated agents 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 halogenated agent.
  - 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, mice, rats, guinea pigs and hamsters is followed by a secondary method of euthanasia such as:

- (1) Cervical dislocation in poultry, birds, mice, rats (<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 the chest with a scalpel or sharp scissors to open up the lung cavity).

5) **Euthanasia of mouse, rat, hamster and guinea pig 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 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 pigs 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 adults are. Thus, decapitation with surgical scissors is the preferred method

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<sup>1</sup> & <sup>2</sup> NIH Guidelines for the Euthanasia of Rodent Fetuses and Neonates.



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 into the second week of postnatal life. 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. Death must be verified after euthanasia and prior to disposal by one of the methods listed above.

a) **Mouse, Rat and Hamster 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 E) 1) 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 prolonged exposure may be necessary. Immersion in liquid nitrogen may be used only if preceded by anesthesia. Similarly, anesthesia should precede immersion or perfusion with chemical fixatives. 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) **Mouse, rat and hamster 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.
- 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 UTSA 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, mice, rats 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 and serviced on a regular basis to ensure sharpness of blades. Sharpness of blades must be verified before decapitating animals. The use of plastic cones (known as decapicones) to restrain animals appear to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency

## 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 -16.25 L/min
Rat Standard		30%-70%	5.64 -13.16 L/min
Mouse Standard (Allentown, Lab Products)		30%-70%	1.90 – 4.42 L/min
Mouse Tecniplast		30%-70%	2.58-6.02 L/min-
Mouse Lab Products Zytem (Amber)		30%-70%	2.35-5.48 L/min-