FLORIDA INTERNATIONAL UNIVERSITY

SOP #: 107.04

Title: **SOP -** Euthanasia

Approvals:

Attending Veterinarian _____ **Date:** 10/18/22

IACUC Chairman REL **Date:** 10/19/22

1. Purpose

1.1 The intent of this standard operating procedure (SOP) is to outline the humane methods of euthanizing laboratory animals at Florida International University. Most commonly used methods will be described. The appropriate method for commonly used animal species will be indicated.

2. Responsibility

2.1 It is the responsibility of all personnel using animals in research and teaching to abide by this policy. It is the responsibility of the IACUC to review for approval properly justified requests for an exception to this policy.

3. Definitions

3.1 The term, euthanasia, is derived from the Greek terms eu meaning good and thanatos meaning death. Euthanasia therefore is a "good death" which would be one with minimal pain and distress.

3.2 Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia.

3.3 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.

3.4 Unacceptable methods: are those methods deemed inhumane under any conditions or that the Panel on Euthanasia found posed a substantial risk to the human applying the technique.

3.5 Adjunctive methods: are those that should not be used as a sole method of euthanasia, but that can be used in conjunction with other methods to bring about euthanasia.

4. Guidelines

4.1 The Guide for the Care and Use of Laboratory Animals and the American Veterinary Medical Association (AVMA) Guideline for the Euthanasia of Animals specifies that "...other animals should not be present when euthanasia is performed" which precludes euthanasia of any individual or group of animals in the presence of other animals or in the animal rooms unless written scientific justification has been submitted to and accepted by the IACUC.

4.2 All euthanasia procedures must be conducted in the allocated spaces in the ACF. If a PI must euthanize the animal in the Investigator's lab to perform necropsy, a valid scientific justification must be provided to the IACUC and approved prior to transporting animals outside of the ACF.

4.3 Acceptable methods

4.3.1 Barbiturate overdose - Overdosing with a commercial injectable euthanasia solution can be used to euthanize all animal species.

4.3.1.1 Amphibians, Fish, and Reptiles: Sodium pentobarbital (60 to 100mg/kg of body weight) can be administered intravenously, intra-abdominally, or intra-pleuroperitoneally in most ectothermic animals, depending on anatomic features. Subcutaneous lymph spaces may also be used in frogs and toads. Time to effect may be variable, with death occurring in up to30minutes.

4.3.1.2 Mice: For an average mouse inject a total of 11-20 mg Sodium pentobarbital intra-peritoneally (IP). Use additional quantity if necessary. Record volume of drug used (controlled drug).

4.3.1.3 Rats: Inject 120 mg/kg Sodium pentobarbital IP. Use more if necessary. Record volume of drug used (controlled drug).

4.3.1.4 Rabbits: inject 100 mg/kg Sodium pentobarbital IP or IV. Use more if necessary. Record volume of drug used (controlled drug).

4.3.1.5 Pigs and Ferrets: A 2-stage euthanasia process involving the use of a tranquilizer Ketamine/Xylazine/Butorphanol will be administered at least 15 minutes before euthanasia. Inject 100 mg/kg of sodium pentobarbital intravenously into the cephalic vein.

4.3.1.6 Birds: Inject Sodium pentobarbital, or commercially available euthanasia solution, intravenous (IV), or intracoelomic (IC) into the bird at a dose of 100-150 mg/kg.

4.3.1.7 When the IC route of administration is used, place the bird in a small cage in a quiet area to minimize excitement and trauma, as birds may be slow to become sedated.

4.3.2 MS-222 (Finquel and Tricaine-S)

4.3.2.1 Acceptable method for euthanasia for finfish and some reptiles and amphibians (for some reptiles, amphibians and large finfish, a secondary method should be used to ensure death).

4.3.2.2 Preparation of solution:

4.3.2.2.1 A 10 g/L stock solution can be made, and sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5 for the solution. Preparation of the stock solution should take place in a certified fume hood.

4.3.2.2.2 The stock solution should be protected from light and refrigerated or frozen if possible.

4.3.2.2.3 The solution should be replaced monthly and any time a brown color is observed.

4.3.2.2.4 Potency is increased in warm water and decreased in cold water.

4.3.2.3 Amphibian Euthanasia

4.3.2.3.1 Studies with Xenopus laevis (African clawed frog or platanna) have shown that the concentrations of MS 222 traditionally used for amphibian euthanasia (0.25 to 0.5 g/L) are not sufficient to induce reliable euthanasia in this species. Immersion of frogs in 5 g/L of MS 222 resulted in deep anesthesia within 4 minutes, but at least 1 hour of immersion at this concentration was required to reliably euthanize 100% of frogs. The authors of that study recommended that if a concentration of MS 222 < 5 g/L or a shorter time frame than 1 hour is allowed, a secondary euthanasia method should be used for X laevis.

4.3.2.4 Reptile Euthanasia

4.3.2.4.1 A 2-stage euthanasia method for reptiles using MS 222 has been described. The first stage entails intracoelomic injection of 250 to 500 mg/kg (113.6 to 227.3 mg/lb) of a pH-neutralized solution (0.7% to 1.0%

MS 222), which results in rapid loss of consciousness (< 30 seconds to 4 minutes). Once unconsciousness occurs, a second intracoelomic injection of unbuffered 50% MS 222 is administered.

4.3.2.5 Finfish Euthanasia

4.3.2.5.1 A fish is typically placed into a dedicated anesthetic container. The swimming movements of the fish will slow down gradually (approximately 30 seconds, depending on the size of the animal). It will lose its ability to stay upright in the water and keel over on its side or totally lie on the bottom upside down. The mouth and gill arches will still be moving, albeit very slowly. It will take ~10 minutes for the fish to become fully over-anesthetized.

4.3.2.5.2 Due to species differences in response to MS 222, a secondary method of euthanasia is recommended in some finfish and amphibians to ensure death

4.3.2.5.3 Ensure death by use of an adjunctive method such as removal of the heart or exposure of the coelomic cavity.

4.3.2.5.4 Proper notes regarding age, phenotypic information etc. must be recorded at this time.

4.3.3 Rapid chilling (hypothermic shock; 1 step or 2step).

4.3.3.1 It is acceptable for zebrafish (D rerio) to be euthanized by rapid chilling (2° to 4°C) until loss of orientation and operculum movements and subsequent holding times in ice-chilled water, specific to finfish size and age.

4.3.3.2 Zebrafish adults (approx 3.8 cm long) can be rapidly killed (10 to 20 seconds) by immersion in 2°to 4°C (36° to 39°F) water. Adult zebrafish should be exposed for a minimum of 10 minutes and fry 4 to 7 dpf for at least 20 minutes following loss of operculum movement.

4.3.3.3 Zebrafish embryos < 3 dpf.

4.3.3.3.1 To ensure embryonic lethality these methods should be followed with an adjunctive method such as use of dilute sodium or calcium hypochlorite solution at 500 mg/L

4.3.3.3.2 If necessary to ensure death of other life stages, rapid chilling may be followed by either an approved adjunctive euthanasia method or a humane killing method.

4.3.3.4 Until further research is conducted, rapid chilling is acceptable with conditions for other small-bodied, similarly sized tropical and subtropical stenothermic species.

4.3.3.4.1 Species-specific thermal tolerance and body size will determine the appropriateness and effectiveness of rapid chilling for euthanasia of finfish. Finfish size is important because the rate of heat loss via thermal conduction from a body is proportional to its surface area. Based on these 2 factors, it has been suggested that rapid chilling in water associated with an ice slurry is a suitable killing method for small tropical and subtropical finfish species 3.8 cm in length (tip of the snout to the posterior end of the last vertebra) or smaller, having lower lethal temperatures above 4°C.

4.4 **Acceptable with Conditions Methods**

4.4.1 Carbon Dioxide (CO2) overdose for rodent and bird euthanasia

4.4.1.1 Compressed CO2 gas in cylinders (medical grade) must be the source of CO2 because the inflow into the euthanasia chamber can be precisely regulated.

4.4.1.2 Concentrations of 30% and higher cause deep anesthesia and death with prolonged exposure.

4.4.1.3 Carbon dioxide has the potential to cause distress in animals via three different mechanisms:

4.4.1.3.1 pain due to formation of carbonic acid on respiratory and ocular membranes,

4.4.1.3.2 production of so-called air hunger and a feeling of breathlessness, and

4.4.1.3.3 direct stimulation of ion channels within the amygdala associated with the fear response.

4.4.1.4 As a general rule, an optimal flow rate for CO2 euthanasia systems should displace 30% to 70% of the chamber or cage volume/min.

4.4.1.5 Procedure

4.4.1.5.1 The chamber should be slowly filled but NOT pre-filled. With an animal in the chamber, an optimal flow rate should displace at least **30%** of the chamber volume per minute.

4.4.1.5.2 Do not overcrowd the animals in the chamber. Once placed in the chamber, the animal(s) should quickly succumb. Gas flow should be

maintained for at least 1 minute after apparent clinical death. To minimize distress, it is preferably that the animals are euthanized in their own cage.

4.4.1.5.3 Pneumothorax after apparent death from CO2 is one way to ensure the irreversibility of the procedure.

4.4.1.6 Acceptable for use in mice, rats, rabbits, hamsters and birds (Fill the chamber slowly to minimize nasal/ocular irritation & aversion to CO2).

4.4.1.7 Neonatal and diving birds are tolerant of high concentrations of CO2; therefore, prolonged exposure to high concentrations of CO2 will be required to produce death (e.g., in excess of 5 minutes in 60–70% CO2 for 1-day old chicks).

4.4.2 Inhalant anesthetic overdose

4.4.2.1 Acceptable with conditions for animals < 7kg, where the following contingencies can be met:

4.4.2.1.1 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 that chamber volume, flow rate, and anesthetic concentration will have on the time constant and rate of rise of anesthetic concentration. 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 killed by a secondary method.

4.4.2.1.2 Order of preference is isoflurane, halothane, sevoflurane, enflurane, methoxyflurane, and desflurane, with or without N2O. 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.

4.4.2.1.3 Although acceptable, inhaled anesthetics are generally not used for larger animals because of cost and difficulty of administration.

4.4.2.1.4 Exposure of workers to anesthetics must comply with state and federal occupational health and safety regulations.

4.4.2.2 Expose the animal to a high gas concentration can be performed using an anesthetic vaporizer or soaked gauze in a closed container.

4.4.2.2.1 If this latter method is used, because the liquid state of most inhaled anesthetics is irritating, animals should be exposed only to vapors.

4.4.2.3 Vapors are inhaled until respiration ceases and death ensues. Gas flow should be maintained for at least 3 minutes after apparent clinical death.

4.4.2.4 Note: Inhaled anesthetics are aversive to rabbits and laboratory rodents and the same may be true for other species. Animals may struggle and become anxious during induction of anesthesia, with some animals exhibiting escape behaviors prior to onset of unconsciousness. Should apnea or excitement occur, time to loss of consciousness may be prolonged.

4.4.3 Cervical Dislocation

4.4.3.1 Manual cervical dislocation is acceptable with conditions for euthanasia of small birds, poultry, mice, rats weighing < 200 g, and rabbits when performed by individuals with a demonstrated high degree of technical proficiency.

4.4.3.2 In lieu of demonstrated technical competency, animals must be unconscious or anesthetized prior to cervical dislocation.

4.4.3.3 For heavy rats and rabbits, the large muscle mass in the cervical region makes manual cervical dislocation physically more difficult. This procedure is only for use in rodents and birds under 200 grams body weight.

4.4.3.4 This method is humane when applied by individuals with a demonstrated high degree of technical proficiency. In lieu of demonstrated technical competence, animals must be sedated or anesthetized prior to cervical dislocation.

4.4.3.5 To perform on rodents, place a closed scissor or other similar object firmly across the animal's neck at the base of the skull and quickly perform cervical dislocation by grasping the tail near the base and sharply pulling away from the body. The animal should immediately become unresponsive, although some involuntary muscle activity may persist.

4.4.3.6 This method is best conducted on sedated or anesthetized animals.

4.5 Adjunctive Methods

4.5.1 Exsanguination - Death can be assured by the removal of a large volume of blood. This technique is never performed on a conscious animal. Animals may be exsanguinated to obtain blood products or for optimal fixation of neural tissue, but only when they are sedated or anesthetized.

4.5.2 Pneumothorax - To create a pneumothorax on an anesthetized or unconscious animal, a cut is made through the abdominal wall using scissors or a scalpel blade

and the diaphragm is lacerated. The heart can also be cut or removed to ensure death.

4.5.3 Pithing – in amphibians is used as an adjunctive procedure to ensure death in an animal that has been rendered unconscious by other means.

4.6 Embryos/Eggs

4.6.1 Pipped eggs (Pipping is the process of cracking open an eggshell by the neonate) marks the initiation of hatching. At this point, the embryo is considered a live animal by regulatory agencies).

4.6.1.1 Use acceptable methods appropriate for hatched birds (e.g., barbiturate) or Acceptable With Conditions Methods (CO_2 or inhalant overdose).

4.6.1.2 Neonates are tolerant of high concentrations of CO2; therefore, prolonged exposure to high concentrations of CO2 will be required to produce death (e.g., in excess of 5 minutes in 60–70% CO2 for 1-day old chicks).

4.6.2 Non-pipped eggs

4.6.2.1 Embryonated eggs may be destroyed by prolonged exposure (20 minutes) to CO2, cooling (4 hours at 40°F), or freezing. In some cases inhaled anesthetics can be administered through the air cell at the large end of the egg. Egg addling can also be used. Embryos in eggs that may have been opened may be decapitated.

5. References

5.1 The Guide for Care and Use of Laboratory Animals 8th Ed. (p.123-124).

5.2 American Veterinary Medical Association Guidelines for the Euthanasia of Animals 2020 Ed.

5.3 PHS Policy on Humane Care and Use of Laboratory Animals Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small laboratory Animals - 2002 (NOTICE: NOT-OD-02-062). (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html)

5.4 Guidelines to the Use of Wild Birds in Research (http://www.nmnh.si.edu/BIRDNET/guide/guidelines.html)

6. Revisions

6.1 Revision 0.2: Barbiturate overdose of birds (4.2) - paragraphs 4.2.16, and 4.2.17 were added to the section. Inhalant overdose section (4.23) was added as a new entry. Carbon Dioxide (CO2) overdose for rodent euthanasia section (4.22) was modified to reference also hamsters and birds. Also included 4.2.2.6 and 4.2.2.7 as new entries. Cervical Dislocation section (4.3.1) was updated to include birds. Updated the reference section to include Guidelines to the Use of Wild Birds in Research (5.4). Embryos/Eggs Section (4.5) was added as new section to the document.

6.2 Revision 0.3: Paragraphs 3.2-3.4, the description of euthanasia methods were modified to be congruent with the new AVMA Guidelines on Euthanasia 2013 Ed. Multiple paragraphs were revised, such as moving CO_2 overdose and inhalant anesthetic overdose under acceptable with conditions section, updating the CO_2 euthanasia section, updating MS-222 section that requires now that the stock solution preparation needs to take place in a fume hood and it needs to be buffered.

6.3 Revision 0.4: paragraphs 4.3.1.4 and 4.3.1.5.1 the description of euthanasia methods were modified to be congruent with the new AVMA Guidelines on Euthanasia 2020 Ed. Paragraph 4.2 is clarifying that all euthanasia procedures must be conducted in the allocated spaces in the ACF. If a PI must euthanize the animal in the Investigator's lab, a valid scientific justification must be provided to the IACUC and approved prior to transporting animals outside of the ACF. Paragraph 4.3.1.5 the euthanasia of cats and dogs was switched to pigs and ferrets and the 2 step procedure was updated.