	UCSD INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE POLICY MANUAL Euthanasia	POLICY #13.05 Originally Issued: 7.17.02 Revised: 11.17.04 Revised: 05.20.15 Revised: 12.16.20 Revised: 03.15.21

I. Background and Purpose

Euthanasia is an integral part of many animal research and teaching protocols including but not limited to protocol specific end points and pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments. The humane use of euthanasia is required by federal regulations and must be appropriate for the species.

II. Who Should Read This Policy

All personnel engaged in or responsible for the euthanasia of animals.

III. Definitions

Term	Definition
Euthanasia	Euthanasia is derived from the Greek terms eu meaning good and thanatos meaning death. The term is usually used to describe ending the life of an individual animal in a way that minimizes or eliminates pain and distress. A good death is tantamount to the humane termination of an animal's life.

IV. Policy

The following rules apply to the euthanasia of animals used in research and teaching unless a scientifically justified exception has been submitted to and approved by the IACUC.

1. Euthanasia techniques must be consistent with the 2020 American Veterinary Medical Associations (AVMA) Guidelines for the Euthanasia of Animals.

2. All euthanasia methods and agents must be described in an approved UCSD Animal Protocol.

3. Only a trained and skilled person may perform euthanasia.

4. Animals must not be euthanized in an animal housing room OR euthanasia procedures must be planned to minimize distress to the other animals.

5. Anesthetic agents used for euthanasia must be given at a dose high enough to ensure death.

6. In rodents, chemical or gas euthanasia methods must be followed by a physical method from which the animal cannot recover, such as decapitation, cervical dislocation, bilateral thoracotomy, perfusion, or removal of a major organ. The animal must be completely non-responsive to a noxious stimulus (e.g. in a rodent, hind paw pinch) before any physical means are applied. In non-rodent species euthanized by chemical or gas euthanasia, confirmation of death is required by a method appropriate to the species as described in the protocol.

7. If the primary method of euthanasia is decapitation, cervical dislocation or exsanguination, it must be done in an anesthetized animal by personnel demonstrating technical proficiency.

8. When using carbon dioxide gas as a method of euthanasia,

    a. Investigator personnel must use an ACP approved apparatus (see additional information).
    b. If using alternative equipment or methods, it is the investigator's responsibility to document that they meet the specifications of the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition:
        i. A "flow rate for CO2 euthanasia systems should displace 30% to 70% of the chamber or cage volume/min."
        ii. A "commercially supplied cylinder or tank; an appropriate pressure-reducing regulator and flow meter or equivalent equipment must be used."

    c. The chamber may not be pre-filled with carbon dioxide.
    d. The chambers must not be overcrowded. Incompatible animals must not be mixed together in the same chamber/box.
    e. Dry ice may not be used to produce CO2 for euthanasia.

9. Potassium Chloride, IV or IC, must be administered to animals under general anesthesia.

10. By federal regulation, Attending Veterinarians may euthanize animals that, in their professional judgment, cannot be adequately treated.

11. Specialized rodent guillotines and scissors used for decapitation must be kept clean, in good condition and have sharp blades. A record of maintenance/sharpening for guillotines must be kept near the equipment and be available for inspectors to review.

V. Related Documents

Euthanasia Equipment Maintenance	http://iacuc.ucsd.edu/PDF_References/EuthanasiaEquipmentMaintenance.pdf
Guillotine Use and Maintenance log	http://iacuc.ucsd.edu/PDF_References/GuillotineUseandMaintenanceLog.doc

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition	https://www.avma.org/KB/Policies/Documents/euthanasia.pdf

VI. Additional information

EUTHANASIA OF RODENTS

1. Fetuses and neonates: 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.
   - 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.
   - Inhaled agents: Non-flammable volatile anesthetics (e.g. isoflurane) are effective for both in utero fetuses and neonatal rodents but exposure times may be prolonged. Neonatal mice may take up to 50 minutes to die from exposure to CO2. Adequate exposure times need to be provided, especially up to 10 days of age, or an adjunctive method (e.g. cervical dislocation or decapitation) should be performed after a neonate is nonresponsive to painful stimuli.
   - Injectable barbiturates can be used for euthanasia of fetuses and neonates.
   - Decapitation is acceptable for altricial rodents <7 days of age.
   - Cervical dislocation can be used for both fetal and neonatal mice.

2. Carbon Dioxide Gas

   CO2 may cause distress to animals due to:
   a. pain secondary to the formation of carbonic acid on respiratory and ocular membranes,
   b. production of air hunger, and
   c. direct stimulation of ion channels within the amygdala associated with the fear response.
   d. An air displacement rate from 30% to 70% of the chamber volume/min will minimize the above effects. The flow of CO2 should be maintained for at least 1 minute after respiratory arrest.

- ACP has pre-approved certain types of equipment for use by investigators who conduct CO2 euthanasia in their laboratory. Investigators may be able to obtain this equipment, or information on approval of alternative equipment and methods by contacting ACP-vetservices@ucsd.edu. Provide your name, PI, IACUC protocol number, and phone number.

- Rodents are sensitive to their environment and to handling. Aversion and distress should be minimized. Whenever possible they should be euthanized in their home cages with animals that they are familiar with.

- Prior to euthanasia, rodents may not be contained (even temporarily) at a greater density than what is acceptable for housing.

- Euthanasia chambers should be large enough to permit each animal to stand on the floor of the chamber with all 4 feet and have sufficient space to turn around and perform normal postural adjustments.
- Euthanasia chambers should be kept clean and free of debris and excreta.
- Euthanasia chambers should be clear so that the animals are easily visualized.

3. Cervical Dislocation as a primary method (not anesthetized)

- Cervical dislocation in conscious mice and rats requires scientific justification and prior approval by the IACUC. Personnel must demonstrate a high degree of technical proficiency.
- Cervical dislocation as a primary method may only be done in rats weighing less than 200 grams.
- Personnel must be properly trained and able to consistently perform the technique humanely and effectively.

4. Decapitation as a primary method (not anesthetized)

- Decapitation in conscious mice and rats requires scientific justification and prior approval by the IACUC. Personnel must demonstrate a high degree of technical proficiency.
- Guillotines must be maintained in good working order and serviced on a regular basis to ensure sharpness of the blades.

EUTHANASIA OF FINFISH

1. Immersion and Injection
   - Tricaine methanesulfonate (MS 222)
     For comments regarding handling and preparation please see IACUC Policy 34.01, Anesthesia.
     - Finfish: 200 to 1000 mg/L; Buffer with NaHCO3 to pH 7-7.5. The exact dose will be determined by variables such as species of fish to be euthanized, size of fish, life stage, and water chemistry parameters.
     - Fish should be left in the anesthetic solution for a minimum of 30 minutes after cessation of opercular movement.
     - If the fish are too large for practical or cost effective immersion with lethal doses of MS 222, the concentrated buffered solution can be applied directly to the gills.
     - Embryonic Zebrafish (<3dpf {days post fertilization}): Immersion into buffered MS 222 has been shown to be unreliable, and should be followed by an adjunctive method such as immersion into dilute sodium or calcium hypochlorite solution at 500mg/L.
   - Pentobarbital
     - Sodium pentobarbital at 60 to 100 mg/kg can be administered by IV, or intracoelomic routes
     - Pentobarbital can also be administered intracardiac in anesthetized animals.
     - Death usually occurs within 30 minutes depending on the route used

2. Physical methods
   - Decapitation/cervical transection
     - Rapid severance of the head and brain from the spinal cord or the spinal cord from the cervical vertebrae.
     - Must be followed by pithing of the brain
   - Rapid chilling/hypothermic shock
     - Immersion into ice chilled water (2 to 4 degrees C). Chilling must occur rapidly and the fish should not come into direct contact with the ice.
     - Acceptable for use in zebra fish and other small bodied (3.8 cm long or smaller) tropical and subtropical stenothermic finfish for which the lower lethal temperature range is above 4 degrees C.
     - Adults: Immerse in chilled water for a minimum of 10 minutes following loss of opercular movement and orientation.
     - Fry (4 to 7 dpf): Immerse in chilled water for a minimum of 20 minutes following the loss of opercular movement and orientation.
     - Embryos (< 3dpf): Rapid chilling by itself has been shown to be unreliable and should be followed by an adjunctive method such as immersion into dilute sodium or calcium hypochlorite solution at 500 mg/L.

EUTHANSIA OF AMPHIBIANS AND REPTILES

Amphibian and reptilian hearts can continue to beat even after brain death so death should always be confirmed by physical intervention.

1. Immersion and Injection
   - MS 222
     - Buffered tricaine can be administered via water bath (amphibians), or injected directly into the lymph sacs (amphibians) or the coelomic cavity (amphibians and reptiles).
     - Dose for use in amphibians is 5 to 10 gm/L buffered with Na HCO3 to pH 7-7.5. Prolonged immersion for up to 1 hour may be necessary.
     - This agent does not create histopathologic artifacts.
   - Pentobarbital
     - As venous access can be challenging, acceptable routes of administration are intracoelomic, subcutaneous, lymph spaces and lymph sacs.
     - Dose range: 60 to 1,100 mg/kg. Higher doses may be required for intracoelomic injection of Xenopus.
     - Time to death may vary from instantaneously to up to 30 minutes.
2. Physical methods
   - Rapid Freezing
     - Must result in immediate death.
     - Animals must be < 4 g and placed into liquid N2.
     - Should not be used in animals who have adapted freeze tolerance strategies, as it may not result in instant death.
   - Decapitation
     - Animal must be under anesthesia.
     - Heavy shears or a guillotine are effective for most species.
     - Must be followed by pithing or another method of destroying brain tissue.

EUTHANASIA OF BIRDS

Birds lack a diaphragm so they have a single coelomic cavity. They also have hollow pneumatic bones that communicate directly with their respiratory system. When giving intracoelomic or intraosseous injections, care must be taken to ensure that the material is not injected into the air sacs which could potentially drown the bird or expose the respiratory tract to irritating substances.

1. Injectable and Inhalation
   - Pentobarbital
     - Optimal route is IV in the properly restrained or anesthetized bird
     - If the IV route is not possible then the intracoelomic, intracardiac, or intraosseous routes can be used, but only in the anesthetized bird.
   - Isoflurane, Sevoflurane
     - Can be used as the sole method of euthanasia or can precede (loss of consciousness, anesthesia) the use of another method of euthanasia
     - Induces minimal tissue damage and results in the least amount of tissue artifact for necropsy.
2. Physical Methods
   - Cervical dislocation
     - Generally used in small birds < 200 grams
     - Should only be performed by well trained personnel who are regularly monitored to ensure proficiency
   - Decapitation
     - Considered to be acceptable for euthanasia of birds < 200 grams

EUTHANASIA OF RABBITS

1. Injectable agents
   - Pentobarbital
     - Can be given IV thru an ear vein in awake juvenile and adult rabbits.
     - If venous access is not possible then it can be given IP in neonatal animals. However this route may be associated with pain.
     - Can be given intra-cardiac if the rabbit is under anesthesia.

2. Inhalant agents
   Rabbits will struggle and breath hold when confronted with any unpleasant or unfamiliar odors. This makes most inhaled methods difficult to use in rabbits without premedication.
   - Rabbits can be euthanized with an overdose of an inhalant if they are already under anesthesia.
   - CO2 when used as the sole agent for euthanasia in rabbits results in apparent distress therefore it is not recommended.

3. Exsanguination
   - Can only be used in an anesthetized rabbit.

EUTHANSIA FOR OTHER SPECIES

Methods and techniques of euthanasia for other species are described in the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Consultation and training in these techniques is available from the Animal Care Program.