Euthanasia

IACUP Policy
Effective Date: January 2024

UCSF Office of Research
Institutional Animal
Care and Use Program

I. Purpose

This policy establishes the standards for euthanasia of laboratory animals at UCSF. This policy has been created to ensure that euthanasia complies with the requirements of the Animal Welfare Act and Regulations, the Guide for the Care and Use of Laboratory Animals, 8th Edition (the Guide), and the AVMA Guidelines on Euthanasia of Animals (AVMA Guidelines).

II. Regulatory or Accreditation Authority

9 C.F.R. §2.31 – Institutional Animal Care and Use Committee
(xi) Methods of euthanasia used must be in accordance with the definition of the term set forth in 9 CFR part 1, §1.1 of this subchapter, unless a deviation is justified for scientific reasons, in writing, by the investigator.

Guide for the Care and Use of Laboratory Animals, 8th Edition, November 2013. *Euthanasia. pp. 123-124*

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, ISBN 978-1-882691-54-8

III. Scope

This policy applies to all animals euthanized at UCSF.

IV. Definitions

Euthanasia: the humane disposition of an animal accomplished by a method that minimizes or eliminates pain and distress. This is routinely accomplished by rapid unconsciousness and subsequent death.

V. Policy

A. General

In order to minimize animal suffering, laboratory animals must be euthanized either as described in the protocol at established endpoints, or expeditiously if criteria for humane endpoints have been reached.

Animals must be continually observed and never be left unattended during the euthanasia procedure. All methods used must result in the confirmed death of the animal; for several methods this requires a secondary physical method after the primary chemical method to ensure death. Animal carcasses and tissues must be properly disposed of after euthanasia.

B. LARC Authority

In the event that LARC facility staff discover animals suffering from unrelieved pain or distress, LARC facility staff will attempt to contact the PI or designated alternate. If the PI or alternate cannot be reached, euthanasia may be performed at the discretion of LARC veterinary staff.

C. Protocol Requirements

The method of euthanasia must be consistent with the AVMA Guidelines, appropriate for the species and age of animal and performed by trained personnel. Protocols must include a description of methods used for euthanasia, including method(s) to confirm death.

D. Rodent Euthanasia

- Use of an anesthetic agent for euthanasia must be administered at an overdose, not an anesthetic dose.
- To confirm death, any chemical method used for euthanasia must be followed by a physical method from which the animal cannot recover (e.g., decapitation, exsanguination, cervical dislocation, bilateral thoracotomy, tissue perfusion, or dissecting of a **vital** organ). The animal must be completely non-responsive to noxious stimuli (confirmed by lack of response to hind foot pad pinch on each foot) before any physical method is performed. All agents used must be pharmaceutical grade, unless non-pharmaceutical grade agents are scientifically justified and approved in the IACUC protocol.
- The techniques listed below are methods commonly approved in UCSF IACUC protocols for the euthanasia of rodents. Other methods outlined in the AVMA Guidelines on Euthanasia are acceptable when approved in the IACUC protocol.

E. Chemical Methods

Carbon Dioxide Inhalation/administration (≥ 7 days of age): CO_2 is delivered from a pressurized tank into an un-crowded cage to ensure precise regulation of gas inflow. The flow rate must be set to displace 30-70% of the chamber or cage volume/minute, allowing CO_2 to enter the chamber and induce unconsciousness prior to death. Prefilled chambers are unacceptable. CO_2 flow should be maintained for at least one minute after respiratory arrest; animals must be left in the chamber for a sufficient time so that death has occurred prior to performing a physical method. When euthanizing mice, see 'Temporary Holding Cages' below.

To ensure compliance with the *AVMA Guidelines*, a precision CO_2 gauge/regulator with a pressure reducing valve or flow meter must be used. Units in centralized care (LARC) are equipped with precision preset flow restriction valves. Units in decentralized care must be similarly equipped or a flow meter may be used that is set to the proper flow rate for the size of cage utilized. Inspection and verification that flow rate complies with the AVMA Guidelines should be confirmed on an approximately triennial basis by the lab.

When possible, euthanize rodents in their home cage to minimize the stress of being placed into an unfamiliar enclosure and to prevent social aggression. Cages/containers used for euthanasia must allow clear visibility from all sides, be a size that permits full posture to be expressed, and be disinfected between uses to remove the potential distress that may be

caused by exposure to remaining pheromones. CO_2 is denser than room air and will remain at the bottom of the chamber. Do not place animals in a pre-charged (containing CO2 from the previous group) chamber.

Injectable Anesthetic Overdose: Intraperitoneal injection of at least 200 mg/kg sodium pentobarbital is recommended; other injectable anesthetics may be approved and delivered at an **overdose**. Sodium pentobarbital containing solutions can be viscous and are best diluted to a concentration of no more than 60 mg/ml. Intracardiac injections are suitable only if the animal is adequately anesthetized. For rats, it is recommended to refine the method by combining sodium pentobarbital with Lidocaine in the same syringe to reduce the irritant effects of sodium pentobarbital; consult LARC veterinarians for guidance.

Inhalant Anesthetic Overdose: Isoflurane inhalation at an overdose may be utilized as a method of euthanasia, either by precision vaporizer or open-drop method. If open-drop isoflurane is utilized, it must be approved in the IACUC protocol and adequately scavenged to prevent personnel exposure. Animals may need to be exposed for prolonged time periods to ensure death.

Euthanasia while under Anesthesia: When animals are fully anesthetized as at the end of a non-survival surgery, methods such as bilateral thoracotomy, exsanguination, removal of a vital organ or perfusion are acceptable.

Temporary Holding Cages: On occasion, it may be useful for investigators to temporarily hold more than 5 mice per cage. For example: mice being collected for immediate euthanasia. This is acceptable as long as the following conditions are met:

- Up to 10 compatible adult (> P21) mice may be placed in a temporary holding cage for up to 30 minutes and holding cages are never left unattended.
- If fighting is observed, mice must be immediately separated.
- Adult males ≥ 6 weeks old from different cages should never be combined.
- For pups >12 days:
  - An established breeding cage can be euthanized as a single cage regardless of the number of animals (e.g., approved trio breeding cage with 12 pups >12 days of age)
  - When combining cages, no more than 10 animals (including adults and pups >12 days of age) can be combined.
- For pups ≤ 12 days of age):
  - Cages with any pups ≤ 12 days of age cannot be combined with pups > 12 days of age or non-parental adult mice.
  - No more than 2-4 litters of pups ≤ 12 days of age can be placed in single layer within the cage without adult mice, and ALL pups must be ≤ 12 days of age.

F. Physical Methods

As secondary methods: Chemical methods must be followed by a confirmatory physical method. Decapitation, exsanguination, cervical dislocation, bilateral thoracotomy, tissue perfusion, or dissecting of a **vital** organ must occur after the animal has been determined to be non-responsive to noxious stimuli.

As primary methods: Physical-only methods of euthanasia such as decapitation or cervical dislocation of un-anesthetized animals must be approved by the IACUC with appropriate scientific justification in the IACUC protocol. The PI must ensure that personnel are experienced or properly trained, and demonstration and documentation of competence is required. To schedule an appointment to demonstrate competency please contact *trainerIACUC@ucsf.edu*.

- **Species-specific requirements:**
    1. **Mice and Gerbils:** Decapitation, cervical dislocation
    2. **Rats and Hamsters:** Decapitation, cervical dislocation (animals must be less than 21 days and/or weighing less than 200 grams).
    3. **Guinea pigs:** Decapitation only. Cervical dislocation may not be performed on guinea pigs.

G. Fetuses and Neonates

- It is not necessary to remove fetuses for euthanasia after the dam is euthanized as they are unconscious in utero and hypoxia does not evoke a response. Precocial (able to independently feed and move almost immediately at birth) young should be euthanized as adults (for example: guinea pigs). Altricial neonates are defined as less than postnatal day 12.

- Anesthetic overdose, as listed in the chemical methods above, can be used.

- Inhalation anesthetics:

    1. Exposure time for inhaled anesthetics may take up to 50 minutes for euthanasia to be successful. Adequate exposure must be provided and followed by cervical dislocation, decapitation, or bilateral thoracotomy

    2. CO_2 (Prior to Physical Method): Neonates should not be combined into cages with non-parental adult mice. Place no more than 2 - 4 litters of neonates in a single layer in a mouse or rat cage. Place the cage within the euthanasia chamber, and fill with CO_2 for at least 4 or 5 minutes with CO_2 tank regulator set to displace 30-70% of the cage volume per minute. Neonates can then be removed and physical method performed.

- Decapitation using scissors or sharp blades is acceptable as a sole means of euthanasia.

- Hypothermia followed by secondary physical method can be used for animals ≤7 days of age. Fetuses and altricial neonates can be gradually cooled until anesthetized, careful to prevent direct contact with ice or precooled surfaces, followed by decapitation, bilateral thoracotomy, or removal of major organ(s).

- Rapid freezing in liquid N_2 is acceptable for less than 5 days of age

H. Non-Rodent Mammal Euthanasia

- Use of an anesthetic agent for euthanasia must be at an **overdose**, not an anesthetic dose.
- To confirm death, the administration of any chemical agent used for euthanasia must be followed by a physical method from which the animal cannot recover, such as bilateral thoracotomy or fixative perfusion. The animal must be completely non-responsive to noxious stimuli before any physical method is performed. All agents used must be pharmaceutical grade.
- The techniques listed below are methods commonly approved by the UCSF IACUC for the euthanasia of non-rodent mammals. Appropriate restraint for the species must always be applied. Sedation, anesthesia, or tranquilization may be necessary for some species or individual animals prior to the administration of the euthanasia agent(s).

I. Techniques

Injectable anesthetic overdose: Intravenous injection of an anesthetic agent may be an acceptable method; however, intracardiac injections are acceptable only when the animal is adequately anesthetized. Intraperitoneal injection may be approved for smaller species (mice, rats, birds). Sodium pentobarbital containing agents are recommended, though other injectable anesthetics may be acceptable.

Euthanasia while under anesthesia: When animals are fully anesthetized (e.g., at the end of non-survival surgery), methods such as bilateral thoracotomy, exsanguination, perfusion, or intravenous or intracardiac injection of potassium chloride are acceptable.

Maintenance and Use of Decapitation Equipment:

Equipment used for euthanasia of unanesthetized animals such as commercial guillotines, scissors or shears must be kept clean and serviced on a regular basis to ensure sharpness of blades. Clean and disinfect after each use and rinse with 70% ethanol to promote drying. Blades that are in use should be sharpened at least annually and blades that have been out of service must be sharpened before the first procedure. The guillotine apparatus should be lubricated periodically with silicone.

The blades should be checked prior to each use for rust, cleanliness and ability to move freely without resistance. Dull blades should be replaced or sharpened by a professional sharpening service. **Sharpening and maintenance records need to be available during IACUC inspections and upon request.**

Lab staff must be appropriately experienced or trained (see Physical Methods above). When using decapitation equipment always make sure hands and fingers are clear of blade path. The use of plastic restraint cones (i.e. Decapicones®) is recommended to restrain adult animals as it may reduce distress from handling, minimize the chance of injury to personnel and improves positioning of the animal in the guillotine.

J. Zebrafish Euthanasia

Approved methods for zebrafish euthanasia by age and agent:

Adults 7 days post fertilization (dpf) and older include:

- Tricaine (MS-222): Immerse fish in a solution of tricaine methanesulfonate (Finquel or Tricaine-S). The solution must be buffered with sodium bicarbonate to a pH of 7.0-7.5. Stock preparation is 4g/L buffered to pH7 in sodium bicarbonate (at 2:1 bicarb to MS-222). Euthanasia dosage 300ug/ml or 7.5ml stock solution to a total of 100ml. Fish must remain in the solution for 10 minutes following cessation of opercular (gill) movement.

- Rapid chilling: Submerge fish in 2 -4^0 C chilled system water (5 parts ice to 1 part water). Confirm with a thermometer that the water is ≤ 4°C before placing the fish. They should never be in direct contact with ice. Adult fish should be exposed for 10 minutes following cessation of opercular movement.

Fry 4-7 days post fertilization:

Tricaine or rapid chilling may be used as above, but fry should remain submerged in solution for 20 minutes following cessation of opercular movement.

Embryos 0-3 days post fertilization:

- Tricaine or rapid chilling may be used as above, but embryos < 3 dpf should be followed with an adjunctive method such as dilute bleach. Add dilute bleach solution (1 part sodium hypochlorite 6.15% to 5 parts deionized water) to the water for 5 minutes to ensure embryonic lethality.

After one of the above methods has been performed, acceptable adjunctive physical methods for all stages of development will be done including (for non-transgenic animals) maceration (use of a specially designed mechanical apparatus having rotating blades or projections, causing immediate fragmentation) or placement of the animal carcasses in the freezer. Carcasses will be transferred to biohazard bags at -20ºC for a minimum of 24 hours and subsequently disposed of as potentially hazardous tissue by LARC.

K. Disposal

UCSF policy is to treat ALL animal carcasses as infected biohazardous waste and to discard them in red biohazard bags. The bags must be sealed and stored in waterproof containers with tight-fitting lids in designated cold rooms or freezers until removed by the animal waste management contractors; these containers should not weigh more than 50 pounds when filled. Carcasses weighing more than 50 pounds should be disposed of one per container; contact the EH&S at (415) 476-1300 or ehs@ucsf.edu to arrange disposal of carcasses totaling more than 50 pounds or for further information regarding disposal.

L. Training

Only trained individuals may perform euthanasia. Training is provided in individual or group workshops through IACUC Training and Compliance, *trainerIACUC@ucsf.edu*

References:

- American Veterinary Medical Association. 2020. AVMA Guidelines for the Euthanasia of Animals, 2020 edition.
- Koerber AS and Kalishman J. 2009. Preparing for a Semiannual IACUC Inspection of a Satellite Zebrafish (*Danio rerio*) Facility. Journal of the American Association for Laboratory Animal Science. **48**: 65-75.
- NIH. 2020. Guidelines for Use of Zebrafish in the NIH Intramural Research Program.