

UH IACUC Policy on Euthanasia
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Policy

The University of Hawaii (UH) Institutional Animal Care and Use Committee (IACUC) adheres to the *AVMA Guideline for the Euthanasia of Animals*¹ (AVMA 2020 or later editions. Guidance documents such as those from the NIH Office of Laboratory Animal Welfare, as well as State and institutional requirements are also applied when necessary. In evaluating the appropriateness of methods, consideration is given to: ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety; reliability; irreversibility; time required to induce unconsciousness; appropriateness for the species and age of the animal; compatibility with research objectives; and the safety of and emotional effect on personnel².

Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the *AVMA Guidelines*. All methods of euthanasia will be reviewed by the veterinarian and IACUC².

All persons performing euthanasia must be skilled in the methods for the species and must be approved for the specific procedure by the UH IACUC. All persons must have written documentation of demonstrating proficiency to the University Veterinarians or their designee prior to performing the procedures independently. Exemptions for demonstration of proficiency (e.g. field studies) will be addressed by the veterinarian during IACUC protocol reviews.

The operator will adhere to the guidance found in the AVMA Guidelines. In addition, the following are required by the UH IACUC:

Carbon Dioxide (CO₂) Euthanasia (Acceptable with most birds and mammals excluding companion animals)¹

- A displacement rate from 30-70% of the chamber volume/min is recommended for rodents. Consideration should be given to the benefits of using a darkened home cage, while also keeping in mind the need to observe the animals. Whenever gradual displacement methods are used, CO₂ flow should be maintained for a t least 1 minute after respiratory arrest. The practice of immersion, where conscious rodents are placed directly into a container prefilled with 100% CO₂ is unacceptable.
- There is no flow rate requirement at this time for the use of carbon dioxide in poultry. Because neonatal birds may be more accustomed to high concentration of CO₂, concentrations necessary to achieve rapid euthanasia of pipped eggs or newly hatched

¹ AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, AVMA, Schaumburg, IL https://www.avma.org/sites/default/files/2020-01/2020_Euthanasia_Final_1-15-20.pdf

² The Guide for the Care and Use of Laboratory Animals, 8th edition, NRC, Washington DC

chicks may be substantially greater (as high as 80-90%) than for adults of the same species.

- For safety, all CO₂ tanks must be properly stored. Use of fabric straps, are not used as a sole means to secure tanks, in the event of fire destroying its integrity.
- For humane euthanasia, all CO₂ tanks must be outfitted with an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size container being utilized.
- Neonatal altricial rodents (e.g. mice and rats) (less than 10 days old) are more resistant to the hypoxic effects of CO₂. Neonatal mice may take up to 50 minutes to die from CO₂ exposure, and neonatal rats may take as long as 35 minutes. Adequate exposure time, or an adjunctive method (e.g. cervical dislocation or decapitation) should be performed after a neonate is nonresponsive to painful stimuli. Instruments for decapitation should be kept in good condition (rust-free, easily cuts through tissues, sharpness maintained).
- Secondary methods to confirm death after CO₂, such as cervical dislocation or decapitation or pneumothorax do not require documentation of demonstrate of proficiency by the veterinarian.
- Logs must be maintained for all CO₂ euthanasia performed, to include date, operator, species, number of animals euthanized.
- Death must be confirmed prior to disposal of bodies.
- Facility-specific SOPs for utilizing CO₂ euthanasia equipment should be reviewed and approved by the UH IACUC prior to use by the operators.

Inhalation Anesthetic Overdose (e.g. Isoflurane)

- Inhaled anesthetics can be useful as the sole euthanasia agent or as part of a 2-step process, where animals are first rendered unconscious through exposure to inhaled anesthetic agent and subsequently killed via an adjunctive (secondary) method. Decapitation instruments should be kept in good condition (rust-free, easily cuts through tissues, sharpness maintained).
- Secondary methods to confirm death after anesthetic overdose, such as cervical dislocation or decapitation or pneumothorax do not require documentation of demonstrate of proficiency by the veterinarian.
- Inhaled anesthetics are aversive to rabbits and laboratory rodents and may be true for other species. Onset of unconsciousness may be delayed due to breath holding.
- An adjunctive method (e.g. cervical dislocation or decapitation) must be performed when halogenated anesthetics are used on neonatal rodents to avoid the possibility of recovery.
- When used as a sole euthanasia agent, animals may need to be exposed for prolonged period of time to ensure death.
- When use of open-drop technique care must be taken to ensure that the rodent does not come in direct contact with the anesthetic.
- Confirmation of death should be based not on a single sign, such as cessation of breathing, but on multiple signs, such as physical examination, exposure to room air (under observation).

Cervical Dislocation (Acceptable with conditions for small rodents and small birds)

UH IACUC Policy on Euthanasia

- If cervical dislocation is used as a primary method of euthanasia, the operator must have documentation of demonstration of proficiency by the veterinarian and must meet a performance standard of luxation of the cervical vertebrae without primary crushing of the vertebrae and spinal cord.
- Justification for its use must be approved by the UH IACUC
- Size limits for cervical dislocation on animals – see AVMA Guidelines, p. 60-61 acceptable for mice and rats <200 g. Avian (p. 81) Cervical dislocation has generally been used for small birds (<200 g) when no other method is available, but the procedure has been performed on birds as large as 2.3 kg (5.1 lb).

Decapitation

- The equipment must be maintained in good working order and serviced on a regular basis to ensure sharpness of the blades. A written description of maintaining blade sharpness must be included in the IACUC protocol. For example, test the sharpness of scissors by regularly verifying that the scissors can easily cut scissors testing paper or equivalent, prior to use on animals. If unable to cut these cleanly, sharpen blades or replace instrument.
- A log of maintaining sharpness of blade should be available for review during IACUC, Post-Approval Monitoring (PAM), or veterinary reviews.
- Justification for its use should be based on experimental design use must be approved by the UH IACUC.
- If decapitation is used as the primary method of euthanasia, documentation of demonstration of proficiency by the veterinarian is required.
- If decapitation is used as an adjunctive (secondary method) to ensure euthanasia, instruments must be kept in good condition (rust-free, easily cuts through tissues, sharpness maintained). However, demonstration of proficiency is not required when using decapitation as an adjunctive method of euthanasia.
- Justification for its use must be approved by the UH IACUC
- Decapitation is acceptable with conditions for mice and rats. Based on information currently available, decapitation is considered to be acceptable with conditions for euthanasia of small (<200 g) birds.

Captive Bolt

- Any protocol that involves the use of captive bolt for euthanasia shall submit a maintenance Standard Operating Procedure (SOP) to ensure that the bolt extends properly with the required force to cause rapid brain death. The SOP must be consistently applied to ensure humane euthanasia.

Fire Arms

- PIs proposing the use, transport, or possession of firearms on their IACUC protocols
 - Individuals listed shall have completed an approved gun safety course prior to submitting the protocol
 - Be aware of the following policies
 - UH Executive Policy E9.210: “Weapons: The possession or use of lethal weapons on University premises is strictly prohibited unless specifically

authorized by the Senior Vice President/Chancellor. Lethal weapons include but are not limited to firearms, ammunition, spear guns, explosives, and dangerous substances. Any person found in violation may be subject to the provisions of state law, University policy, and the Student Conduct Code.”

- RCUH Policy 3.40 Firearms “The policy covers the training in, use of, and conditions of use of firearms for authorized project-related activities. The policy ensures RCUH’s compliance with all legal requirements associated with the use of firearms, ammunition, and related matters in support of projects and programs. It is aligned with applicable State and federal firearms regulations as provided under Hawai‘i Revised Statutes (HRS) Chapter 134 – Firearms, Ammunition and Dangerous Weapons, Title 18, USC; Chapter 44 -- Federal Gun Control Act of 1968; and relevant amendments (e.g., September 30, 1996, Lautenberg Amendment). In addition, this policy ensures compliance with required criminal history/background checks as required by federal and State laws.”

Kill Traps (for collection and killing of small, free-ranging mammals)

- Must be used in accordance with the *Guidelines for the American Society of Mammologists for the Use of Wild Mammals in Research (2011)*³, especially that traps are checked at least once daily.
- Kill traps should only be used when other acceptable methods are not practical or have failed.
- Use of kill traps requires UH IACUC review and approval

Finfish

Tricaine methanesulfonate (MS 222): A concentration of 250-500 mg/L is effective for most species. Immersion of fish in solutions of buffered MS 222 for 30 minutes following loss of rhythmic opercular movement is sufficient for euthanasia of most fish. Due to species differences in response to MS 222, a secondary method of euthanasia is recommended in many fish to ensure death (e.g. Goldfish). In the USA, there is a 21-day withdrawal time for MS 222; therefore, it is not appropriate for euthanasia of animals intended for consumption.

Vermin Control

- Refer to the *UH IACUC Guidelines on Non-Grant Funded Rodent Control at UH Campus Facilities and by Private Contractors Providing Rodent Control* for details.
- If a UH facility wishes to do their own non-grant funded rodent control or utilize private contractors for rodent control, please consider the following recommendations based on the *Guidelines for the American Society of Mammologists for the Use of Wild Mammals in Research (2011)*³.

³ Sikes, R. S., W. L. Gannon, and the Animal Care and Use Committee of the American Society of Mammologists. 2011. Guidelines of the American Society of Mammologists for the use of wild mammals in research. *Journal of Mammalogy* 92(1):235-253.

UH IACUC Policy on Euthanasia

- Operators must wear appropriate personal protective equipment and follow procedures to minimize risk of zoonotic disease or injury before engaging in activities involving rodent control. Operators should be trained by the Biosafety office before engaging in rodent control activities to mitigate the risks of zoonotic diseases.