9.0552 IACUC Rodent Euthanasia Policy
Institutional Animal Care and Use Committee

POLICY STATEMENT
Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function. In addition, the technique should minimize distress and anxiety experienced by the animal prior to loss of consciousness. This policy provides guidelines for the humane euthanasia of rodents using several techniques.

REASON FOR POLICY
The GRU IACUC is responsible for overseeing humane care and use of animals used in research and teaching and for ensuring adherence to regulatory expectations in the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act as well as standards contained in the Guide for the Care and Use of Laboratory Animals. The purpose of this policy is to educate personnel in proper technique to ensure humane euthanasia of rodents used in education and research.

AFFECTED STAKEHOLDER AND ORGANIZATION(S)
Any employee, student, contractor, or external consultant involved in animal use at GRU.

DEFINITIONS
- Applicable Species
  - All Rodent Species
- altricial - born in an undeveloped state and requiring care and feeding by the parents
- euthanasia – intentionally termination of life in a humane manner
- precocial - refers to species in which the young are relatively mature and mobile from the moment of birth

PROCESS & PROCEDURES

Euthanasia Equipment
- Euthanasia chamber with CO2 gas supply
- Barbiturates/euthanasia solution
- Decapitation device (i.e. guillotine) or dedicated scissors

Support Supplies
- Needles and syringes
- Animal restrainer (e.g. DecapiCone)
- Sharps container
- Bag or container for animal carcass disposal

General Requirements
1. Adhere to IACUC approved Animal Use Protocols (AUPs) and institutional policies.
2. Maintain equipment to ensure optimal performance.
3. All dead animals must be placed in plastic bags. The bags must be labeled with permanent marker including the following information: PI name, date, room #, Cage Card #, species and # of animals in the bag.

Non-physical Methods

CO2 Administration

Note: This method is not approved as a sole means of euthanasia.
1. Compressed CO2 gas in cylinders is the only acceptable source of CO2.
2. To minimize stress, animals should be kept in their existing home cage for euthanasia. When housed with other animals, those not being euthanized should be relocated to a different cage. The number of animals in a cage for euthanasia must not exceed the number of animals normally allowed to be housed in the same cage. Each animal must be able to stand on the floor of the chamber with all four feet and have sufficient space to turn around and perform normal postural adjustments.
3. The compressed CO2 gas cylinder is connected to the euthanasia chamber via a hose and dedicated chamber lid.
4. Flow rates are set according to AVMA Guidelines for the Euthanasia of Animals: 2013 Edition, p 20. Flow meters are set to displace 10-30% of the chamber volume per minute (standard size mouse cage 2 L/min or rat cage 5 L/min) to induce rapid unconsciousness with minimal distress to the animals. The regulator must be set between 10 and 20 PSI; only DLAS may make adjustments.
5. Gas flow should be maintained for at least 10 minutes of active exposure, including at least 1 minute after respiration has ceased. **Important:** Death must be verified before removing the animal from the chamber by making sure there is no respiratory movement for at least 3 minutes.

6. Secondary methods of euthanasia (e.g. bilateral thoracotomy, decapitation, exsanguination). Cervical Dislocation is not an appropriate secondary method.

**Overdose of Inhalant Anesthetic**

**Note:** This method is not approved as a sole means of euthanasia for neonates up to 7 days of age; see Euthanasia of Neonates below.

1. Animals are exposed to a high gas concentration using anesthetic vaporizer or soaked gauze in a closed container. If the latter method is used, animals should be exposed only to the vapor.
2. Animals should be exposed until respiration ceases and death ensues.
3. Secondary methods of euthanasia (e.g. bilateral thoracotomy, decapitation, and exsanguination). Cervical Dislocation is not an appropriate secondary method.

**Overdose of Injectable Barbiturates**

1. Approved veterinary or pharmaceutical grade euthanasia solutions are required (e.g. pentobarbital 120-150 mg/kg) intravenously (IV) or intraperitoneally (IP).
2. If the animal is not dead (or for additional security), follow with another method of euthanasia (e.g. bilateral thoracotomy).

**Physical Methods Considerations**

All staff utilizing physical methods of euthanasia must be trained, and have demonstrated proficiency to DLAS veterinary staff.

**Bilateral Thoracotomy**

This procedure opens both sides of the rodent’s chest, preventing the lungs from re-expanding.

1. Animals should be deeply anesthetized using a non-physical method.
2. Scissors and scalpels must be maintained to ensure sharpness and proper function.
   **Note:** Since a percentage of animals may have a complete mediastinum, the procedure chosen must invade both sides of the thorax, preventing any lung lobe from inflating!

**Cervical Dislocation**

This method cannot be used as a sole means of euthanasia in animals weighing more than 200 g. This method is not appropriate for weanlings younger than 1 month, since their tails can easily separate.

1. Apply prior anesthesia or sedation whenever possible.
2. Cervical dislocation requires use of secondary euthanasia method.

**Decapitation**

**Note:** This method requires IACUC approval.

1. Apply prior anesthesia or sedation whenever possible.
2. Appropriate sized guillotines must be used. Proper sharpness and function of guillotine, scissors, and scalpels should be verified prior to use.
3. Decapitation using scissors or a sharp blade is acceptable for altricial neonates less than 7 days of age. For precocial neonates (e.g., guinea pigs), follow guidelines for an adult.

**Exsanguination**

1. Animals should be deeply anesthetized using a non-physical method.
2. Animal death should be verified before disposal of the carcass by making sure there is no respiratory movement for at least 3 minutes. If the animal is not dead (or for additional security), follow the exsanguination by another method of euthanasia (e.g. bilateral thoracotomy).

**Euthanasia of Neonates**

Generally, neonatal rodents are more resistant to hypoxia than adults of the same species. Non-physical methods of euthanasia utilizing inhalant agents (e.g. CO₂, inhalant anesthetics) or hypothermia should be followed-up with a secondary technique (e.g. decapitation) in order to ensure death.
Acceptable procedures for euthanasia of neonatal mice, rats, and hamsters less than 7 days old:

1. Decapitation
2. Decapitation, preceded by loss of consciousness via CO₂, inhalant anesthetic administration, or hypothermia
3. Overdose of injectable barbiturate

For euthanasia of mice, rats, and hamsters greater than 7 days old follow guidelines for adults.
For euthanasia of precocial rodent neonates (e.g. Guinea pigs) follow guidelines for adults.

FORMS AND RELATED DOCUMENTS

- Public Health Service Policy on Humane Care and Use of Laboratory Animals [http://grants.nih.gov/grants/olaw/references/phspol.htm]
- USDA Care Policy (Euthanasia on page 6) [http://www.aphis.usda.gov/animal_welfare/policy.php?policy=3]

Approved by the Institutional Animal Care and Use Committee on 23 October 2014.

AUTHORIZING SIGNATURE

David W. Stepp, Ph.D. ____________________________ Date
Chairman Institutional Animal Care and Use Committee, Georgia Regents University

TO BE USED BY THE OFFICE OF COMPLIANCE & ENTERPRISE RISK MANAGEMENT

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