

The Forsyth Institute

Rodent Animal Facilities

CATEGORY: Procedures

TITLE: Euthanasia of animals in carbon dioxide chamber

CREATION/ REVIEW DATE: Nov. 2013/Revised May 2017/Revised July 2020

RESPONSIBILITY: S.Yoganathan, Animal Care and Research Staff

In compliance with PHS Policy on adoption of 2020 AVMA Guidelines on Euthanasia, the following procedure must be followed when sacrificing small groups of rodents.

- Mice and rats are euthanized in the euthanasia chamber in Procedure Room 2 or BL2 room.
- A regulated flow valve restricts the flow of the gas to this chamber to 30-70% of chamber volume per minute. It is installed in the supply side and preset based on the volume of the euthanasia chamber. Opening the main valve will supply carbon dioxide to the correct level and no additional steps are needed. It will take a few minutes for the CO₂ to reach an anesthetic concentration and death may take several minutes longer.
- **Prefilling of the euthanasia chamber with carbon dioxide is not allowed.**
- When possible mice should be left in their home cage, and the entire cage, without lid, placed into the euthanasia chamber. Alternatively, if mice are to be euthanized one by one, place a clean cage in the euthanasia chamber and place mice inside the cage. This saves having to clean the chamber every time.
- Rats should be euthanized one by one in a clean mouse cage without lid.
- A maximum of five to eight mice per cage may be euthanized at one time. No overcrowded cages allowed.
- Procedure:
 1. Place the mice (in their home cage if possible) into the chamber.
 2. Open the main valve and allow gas to flow (do not prefill the chamber). Observe the animals for complete cessation of breathing.
 3. **Gas flow MUST continue for at least one minute post apparent death.**
 4. The staff must be physically present during the entire time of euthanasia.
 5. Neonates (up to 10 days of age) and fetal mice >15 days pc are resistant to carbon dioxide asphyxiation and must be humanely euthanized after deep narcosis, either by decapitation or opening of the chest.
 6. Euthanized animals are taken to the freezer by the staff for collection and disposal.

AGENTS AND METHODS OF EUTHANASIA AT THE FORSYTH INSTITUTE

Acceptable

Agent	Classification	Mode of Action	Rapidity	Ease of Performance	Safety of Personnel	Species Suitability	Efficacy and Comments
Barbiturates	Hypoxia attributable to depression of vital centers	Direct depression of cerebral cortex, subcortical structures, and vital centers; direct depression of heart muscle	Rapid onset of anesthesia	Animal must be restrained; personnel must be skilled to perform IV injection	Safe except human abuse potential; DEA-controlled substances	Most species	Highly effective when appropriately administered; acceptable IP in small animals and IV
Carbon dioxide (bottled gas only)	Hypoxia attributable to depression of vital centers	Direct depression of cerebral cortex, subcortical structures, and vital centers; direct depression of heart muscle	Moderately rapid	Used in closed container	Minimal hazard	Small laboratory animals, birds, cats, small dogs, rabbits, mink (high concentrations required), zoo animals, amphibians, fish, some reptiles, swine	Effective, but time required may be prolonged in immature and neonatal animals
Inhalant anesthetics	Hypoxia attributable to depression of vital centers	Direct depression of cerebral cortex, subcortical structures and vital centers	Moderately rapid onset of anesthesia, excitation may develop during induction	Easily performed with closed container; can be administered to large animals by means of a mask.	Must be properly scavenged or vented to minimize exposure to personnel	Some amphibians, birds, cats, dogs, furbearing animals, rabbits, some reptiles, rodents and other small mammals, zoo animals, fish free-ranging wildlife	Highly effective provided that subject is sufficiently exposed, ether is conditionally acceptable
Microwave irradiation	Brain enzyme inactivation	Direct inactivation of brain enzymes by rapid heating of brain	Very rapid	Requires training and highly specialized equipment	Safe	Mice, rats	Highly effective for special needs

Conditionally acceptable

Agent	Classification	Mode of Action	Rapidity	Ease of Performance	Safety of Personnel	Species Suitability	Efficacy and Comments
Cervical dislocation	Hypoxia due to disruption of vital centers	Direct depression of brain	Moderately rapid	Requires training and skill	Safe	Poultry, birds, laboratory mice, rats (<200 g), rabbits (<1 kg)	Irreversible; violent muscle contractions can occur after cervical dislocation
Decapitation	Hypoxia due to disruption of vital centers	Direct depression of brain	Rapid	Requires training and skill	Guillotine poses potential employee injury hazard	Laboratory rodents, small rabbits, birds, some fish, amphibians and reptiles (latter 3 with pithing)	Irreversible, violent muscle contractions can occur after decapitation
Nitrogen, argon	Hypoxia	Reduces partial pressure of oxygen available to blood	Rapid	Used in closed chamber with rapid filling	Safe if used with ventilation	Cats, small dogs, birds, rodents, rabbits, other small species, mink, zoo animals, nonhuman primates, free-ranging wildlife	Effective except in young and neonates; an effective agent, but other methods are preferable

Generally unacceptable

Agent or Method	Comments
Exsanguination	Because of the anxiety associated with extreme hypovolemia, exsanguination should be done only in sedated, stunned, or anesthetized animals.