UNIVERSITY STANDARD

Title

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
STANDARD ON INHALATIONAL ANESTHESIA/EUTHANASIA
(DROP METHOD)

Introduction

PURPOSE
The standards and procedures described below provide guidance to all researchers and animal handlers in the proper use of a sealed container for rapid anesthetization of mice or rats and safety of the user.

SCOPE OF APPLICABILITY
All personnel engaged in the use of isoflurane for short-term anesthesia or euthanasia of rodents, without the use of a vaporizer.

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard. Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

Standard

Inhalational anesthesia/euthanasia, also known as the ‘drop method’, is a rapid method to anesthetize rodents for very short procedures or to perform euthanasia via overdose. For a sealed container, laboratories can purchase desiccators; either glass or plastic, from various vendors or the lab may construct a custom version for use. Regardless of version, it is essential that the container be the appropriate size required to prevent overcrowding of animals and the need for large volumes of the isoflurane, which slows induction time. The container lid must be tight fitting to prevent potential leaks of isoflurane and easily sanitizable. A protective layer (such as a perforated platform in a desiccator) is required inside the container to prevent direct contact of the animals with isoflurane soaked materials. Contact with the anesthetic can cause skin irritation and potential overdose since isoflurane is absorbed through the skin.

Due to potential risk of human health, the use of the ‘drop method’ must occur in a fume hood, a ducted biosafety cabinet, or with a properly functioning active scavenging system. If this cannot occur, EHS approval is required to use the ‘drop method’ on a
bench top. Measurement of air quality and approval from EHS is required. Once approved, a posted copy of the approval in the area is required.

1. Pre-charge the sealed container by placing two to three pieces of gauze/absorbent material on the bottom of the chamber, below the protective layer (i.e. perforated platform). Add approximately 5 ml (1 to 2 capfuls) of isoflurane liquid to the gauze. Close the lid and wait 5 minutes for the liquid to form a volatile gas within the chamber. (Note: the amount of isoflurane to add depends on the size of the chamber, so the actual amount needed may vary.)

2. All isoflurane usage for anesthesia requires documentation; labs may use the Isoflurane Drop Method Log Template or create a similar log for the lab to use.

3. Remove the lid of the container, quickly place the animals onto the protective layer within the chamber, and immediately close the lid. (For perforated platforms, ensure that the perforations are not large enough for the animal to crawl through and risk contact with the isoflurane soaked material.)

4. The animals will first exhibit an excitatory phase, after which they become anesthetized typically within 2-5 minutes. Neonates must remain in the chamber for at least five (5) minutes.

5. When animals are recumbent and breathing is slow and steady, remove them from the container and replace the lid.

6. For painful procedures, a noxious stimulus (i.e. toe pinch) on all four paws is required and documentation recorded. An absent withdrawal reaction in all four paws indicates appropriate anesthetic depth and the procedure can be performed.
   - **Note:** Isofluráne is highly volatile and animals will quickly regain consciousness once removed from the chamber; thus, it is imperative to perform the procedure immediately.

7. Once the procedure is completed, recover the animal in a clean cage, bedding covered if present, until the mouse/rat is fully ambulatory.

8. If utilizing the drop method for euthanasia, the animal should be left inside the container until cessation of breathing is observed (neonates may require longer exposure). Remove the animal quickly and perform a secondary physical method.
   - **Note:** All gas euthanasia must be followed with a secondary physical method.
EXCEPTIONS
Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

Definitions

IACUC: Institutional Animal Care and Use Committee
DCM: Division of Comparative Medicine
EHS: Environment, Health and Safety
University Standard: The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC
Desiccator: A sealable enclosure
Volatile Gas: The tendency of a substance to vaporize
Anesthesia: The loss of sensation with or without loss of consciousness
Recumbent: The state of lying down
Excitatory Phase: The period marked by excited and delirious activity during anesthesia
Ambulatory: Able to walk

Related Requirements

EXTERNAL REGULATIONS AND CONSEQUENCES

UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES

For more detailed guidance, please refer to the University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes.

Contact Information

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Important Dates

- Effective Date and title of Approver: September 2007; UNC IACUC
- Revision and Review Dates, Change notes, title of Reviewer or Approver: Revised July 2018; Revised contact information and added documentation template; UNC IACUC

Approved by: UNC IACUC

Dr. Roland Tisch
UNC IACUC Chair