Cornell University
Cornell Center for Animal Resources and Education

# CARE101.01 Rodent Anesthesia

The intent of this standard operating procedure (SOP) is to describe commonly used methods to anesthetize rodents. This SOP is intended for use by researchers and CARE staff that anesthetize rodents. This procedure is approved by the Cornell Institutional Animal Care and Use Committee (IACUC) and the Cornell Center for Animal Resources and Education (CARE). Any exemption must be submitted for approval to the IACUC prior to its application

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## 1. Introduction

Rodents can be anesthetized with either inhalant gas or injectable drugs. The use of inhalant gas is the preferred method of anesthesia whenever possible. In cases where the use of inhalants is not possible, recipes for anesthetic cocktails for rats and mice have been given.

## 2. Materials

- Induction chamber – clear
- Gas anesthesia machine
- Isoflurane
- Material or equipment to provide or conserve body heat (e.g., gauze pads or heating pad)
- 100 mg/mL ketamine
- 100 mg/mL xylazine
- Sterile water
- Needles and syringes
- Permanent marker
- 2,2,2-Tribromoethanol
- Tertiary amyl alcohol
- Sterile saline
- Crushed ice

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## 3. Procedures:

**Note:** Heat loss is rapid in anesthetized rodents. Keep animals warm by covering them (e.g. gauze pad or towel) &/or providing a heat source until they have recovered from anesthesia.

a. Mouse anesthesia
   i. Isoflurane

- Place the animal in the induction chamber.
- Adjust the oxygen flowmeter to 0.8–1.5 L/min.
- Adjust the isoflurane vaporizer to 2%–3%.
- For maintenance, use a mask connected to a Bain circuit, and adjust the flowmeter to 400–800 mL/min.
- Please see Appendix, Part I for adverse effects and precautions for use of isoflurane.

ii. Xylazine–Ketamine

- In a sterile 10 mL bottle with a rubber stopper, mix 1 mL of ketamine (100 mg/mL) + 0.1 mL of xylazine (100 mg/mL) + 8.9 mL of sterile water for injection. SHAKE WELL BEFORE USE.
- Write on the bottle:
  - "XYLAZINE–KETAMINE FOR MOUSE: 0.1 mL/10 g"
  - Your initials
  - Expiration date (selecting the earliest expiration date between ketamine, xylazine, or water, for a maximum of 3 months)
- Store away from light, in a cool place.
- Inject 0.1 mL/10 g intraperitoneally (IP).
- Repeat if necessary, half a dose at a time (approximately every 30 minutes).
- Please see Appendix, Part II for adverse effects and precautions for use of xylazine.

iii. 2,2,2-Tribromoethanol (a.k.a. TBE or Avertin)

**Note:** TBE administration can result in sensitization of the animal; thus, it is recommended to be given only on a single occasion. If multiple usage is required, this usage and monitoring for adverse reactions must be explicitly detailed in the IACUC approved protocol.

- Prepare concentrated solution by mixing 25 g Tribromomethanol in 15.5 mL amyl alcohol in a sterile container.
- Write on the bottle:
  - "50X Tribromomethanol"
  - Your initials
  - Expiration date (1 year)
- Store in freezer, away from light.
- Prepare solution for injection:
  - Mix 1 mL of the concentrated solution in 50 mL of 0.9% sterile saline for injection, in a sterile container.
  - Heat the solution to 40 °C until dissolved, maintaining sterility.
  - Shake well.
  - Label the container (date, contents, concentration-32 mg/ml, and initials) and store in a dark environment at 4 - 6 °C. Shelf life of properly stored TBE solution is 4 months.
  - Filter with a 0.2 micron filter prior to use.
  - Inject 0.15 mL/10 g IP. Dosage may vary depending on the strain/genotype of mice.
- Please see Appendix, Part III for adverse effects and precautions for use of TBE.

b. Rat anesthesia
   i. Isoflurane
      - Place the animal in the induction chamber.
      - Adjust the flowmeter to 0.8–1.5 L/min.
      - Adjust the isoflurane vaporizer to 2%–3%.
      - For maintenance, use a mask connected to a Bain circuit, and adjust the flowmeter to 400–800 mL/min.
   ii. Xylazine–Ketamine
      - Recipe A
        - Mix 8.75 mL of Ketamine (100 mg/mL), and 1.25 mL of Xylazine (100 mg/mL) in a sterile 10 ml bottle with a rubber stopper. SHAKE WELL BEFORE USE.
        - Write on the bottle:
          - "XYLAZINE–KETAMINE FOR RAT: 0.05–0.10 mL/100 g IP"
          - Your initials
          - Expiration date (selecting the earliest expiration date between ketamine, xylazine, or water, for a maximum of 3 months)
        - Store away from light, in a cool place.
        - Administer 0.05–0.10 mL/100 g IP.
        - Repeat as required with 1/3 to 1/2 dose at a time (approximately every 30 minutes).
      - Recipe B
        - Mix 3.75 mL of Ketamine (100 mg/mL), 0.5 mL of Xylazine (100 mg/mL), and 5.75 mL of sterile water for injection in a sterile 10 ml bottle with a rubber stopper. SHAKE WELL BEFORE USE.
        - Write on the bottle:
          - "XYLAZINE–KETAMINE FOR RAT: 0.2 mL/100 g IP"
          - your initials
          - expiration date (selecting the earliest expiration date between ketamine, xylazine, or water, for a maximum of 3 months.)
        - Store away from light, in a cool place.
        - Administer 0.2 mL/100 g IP.
        - Repeat as needed with 1/3 to 1/2 dose at a time (approx. every 30 minutes).

c. Rodent neonates

   **Note:** This technique may only be used in neonatal altricial (hairless) rodents, generally <10 days old. Time of onset of lethargy may be considerably slower than in other methods.

   i. Place the **neonate** on a latex covered bed of crushed ice, or place in the cut off finger of latex glove and immerse in ice water.
   ii. Alternatively, place the neonate inside a glass test tube lined with paper and immerse in ice water.

iii. Monitor the readiness of the animal for a procedure by noting lethargy and immobility. Expect the neonate to remain immobile for up to 10 minutes. If additional time is needed for the procedure, maintain immobility by keeping the neonate on a latex covered cold pack.

iv. Illuminate the surgical field by use of a fiber optic light source, as incandescent bulbs may cause inadvertent and uncontrollable warming.

v. Recover pups and slowly re-warm them in an incubator at 33 °C or in a warm nest. Complete recovery typically requires 30–60 minutes.

## 4. Safety

- Assure that all anesthetic waste gases are properly scavenged- see CARE SOP 712: Waste Anesthetic Gas Scavenging Systems.
- Assure that all heat sources used for recovery are either thermostatically controlled or are carefully monitored to prevent burns.

## 5. Contingencies

- Contact Cornell Environmental Health and Safety at www.ehs.cornell.edu or 255-8200 for concerns regarding the use of chemical agents and monitoring of waste anesthetics gas.
- Contact CARE at 1-800-349-2456 or care@cornell.edu for concerns regarding the use of particular anesthetic regimes or additional training.

## 6. References


- Flecknell, P., Laboratory Animal Anesthesia; Academic Press, New York; 1996.
- Kohn, D.F., Benson, G.J., Wixson, S.K., White, W.J., Anesthesia and Analgesia in Laboratory Animals; Academic Press, New York, 1997; Chapter 15.
- Danneman, P.J., Mandrell, T.D. Evaluation of five agents/methods for anesthesia of neonatal rats. .Lab. Anim. Sci. 1997 Aug;47(4):386-95.
- CARE SOP 712: Waste Anesthetic Gas Scavenging Systems http://www.research.cornell.edu/care/documents/SOPs/CARE712.pdf
- NIH Animal Care & Use Committee Subcommittee on Training; recommendations for perioperative medications in laboratory animals: http://oacu.od.nih.gov/ARAC/tablebyspecies.pdf

Appendix

## Part I

Isoflurane :
Isoflurane is a vasodilator and can result in fatal hypotension. Use isoflurane with caution (e.g. close hemodynamics monitoring) in animals that may be dehydrated or otherwise at risk for hypotension. Maintaining animals with isoflurance concentrations in excess of the recommended levels may result death of healthy and compromized animals.

## Part II

Xylazine :
Xylazine can result in poor tissue perfusion and bradycardia. This drug should be avoided with organ dysfunction (e.g. renal or heart failure) and very young or old animals.

## Part III

Tribromomethanol (TBE or Avertin):

Proper storage of TBE is critical to prevent formation of irritating degradation products which may cause severe peritonitis. TBE administration can result in sensitization of the animal; thus, it is recommended to be given only on a single occasion. If multiple usage is required, this usage and monitoring for adverse reactions must be explicitly detailed in the IACUC approved protocol.

Tribromoethanol is a non-pharmaceutical grade compound. Use of non-pharmaceutical grade compounds is regulated by the federal USDA Animal Welfare Act. Prior to TBE use, scientific justification must be reviewed and approved by IACUC.

USDA Policy 3:
Investigators are expected to use pharmaceutical-grade medications whenever available, even in acute procedures. Non-pharmaceutical-grade chemical compounds should only be used after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings alone are not an adequate justification for using non-pharmaceutical grade compounds in animals. For further details, see
http://www.aphis.usda.gov/animal_welfare/downloads/policy/policy3.pdf