University Animal Care Committee Standard Operating Procedure		
Document No: 7.6	**Subject: Anesthesia in Mice**	
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Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe approved methods commonly used to anesthetize mice.

1. Introduction and Definitions:

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI,** subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP,** intramuscular **IM**, per os **PO,** per rectum **PR**

The anaesthetic regimen must be compatible with the needs and restrictions of the procedure to be performed, taking into account the length of the procedure, whether the procedure is terminal, the degree of invasiveness and the scientific goals.

Fasting prior to anaesthesia is generally not required in mice as vomition is extremely rare during induction and anaesthesia. If scientifically justified, fasting in mice should be as short as possible due to the high metabolic rate of these small mammals.

Either inhalant or injectable anaesthetics can be used to anaesthetise mice. Inhalant anaesthetics with the use of a vaporiser are the agents of choice as they provide a safe, reliable, reversible, and reproducible method.

Once anaesthetized, ophthalmic ointment must be applied using a sterile cotton tip applicator to prevent drying of the eyes (e.g. Hypotears, Lubrithal Eye Gel).

Monitor the mouse continuously as an anaesthetised animal should never be left unattended.

As rodents have a high surface area to body mass ratio, it is extremely important to provide thermal support starting immediately after induction of anaesthesia and continuing until full recovery.

Fluid therapy must be provided after each and every anesthetic event (regardless of the procedure performed). The only exception is if fluid may confound results; this deviation must then be described within the approved Animal Use Protocol.

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2. Materials:

- Warm water recirculating blanket, heating disks, etc.
- Gas anaesthesia machine (calibrated within the last 12 months, and with scavenging capabilities or a passive filter)
- Transparent induction chamber

- Nosecone
- Isoflurane
- Ketamine 100mg/kg (controlled substance – exemption permit required)
- Xylazine (20mg/mL)
- Acepromazine (10mg/mL)
- Medetomidine
- Atipamezole
- Sterile sodium chloride 0.9% or
- Sterile water (diluent)
- Lactated Ringers solution (fluid therapy)
- Sterile needles (various sizes)
- Alcohol swabs
- Gauze
- Eye Lubricant
- 10mL red top vacutainers

3. Procedures:

- ***Isoflurane Anaesthesia***:

i. Induction
   1. Place the animal in the induction chamber.
   2. Adjust the oxygen flow to 1 – 1.5 L/min.
   3. Adjust the vaporiser to 3% - 5%.
   4. Once the mouse is in lateral recumbency and cannot right itself, apply eye lubricant using a sterile cotton tip applicator, and transfer the animal to a nosecone for maintenance.

ii. Maintenance
   1. Ensure the mouse's snout is firmly seated in the nosecone with a good seal.
   2. Adjust the oxygen flow to 800-1000 ml/min.
   3. Adjust the vaporiser from 1 – 2.5% (this is variable depending on the depth of anesthesia required for the particular procedure or for the strain of the mouse).
   4. Maintain the mouse on a heating device the duration of the procedure.

iii. Recovery
   1. Turn off the isoflurane and leave the animal on 100% oxygen (short term).
   2. Administer fluid therapy.
   3. Transfer the animal to a clean cage and place it on a piece of paper towel until it is mobile.
   4. Provide heat during this recovery phase to half of the cage, to ensure that the mouse has the ability to escape the heat as necessary.
   5. Return the animal to the colony room once it has fully recovered from anaesthesia (blinking and righting reflex have returned).

- ***Ketamine/Xylazine Anaesthesia:***

i. Anaesthetic dose: ketamine 150 mg/kg, xylazine 10 mg/kg
ii. To prepare the cocktail, in a sterile vial mix:
    1. 0.75 ml (75mg) ketamine (100 mg/ml)
    2. 0.25 ml (5mg) xylazine (20 mg/ml)
    3. 4 ml diluent
    4. Label with the drug name(s), expiration date of the drugs, date mixed and initials.
iii. Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.1 ml (100 ul) per 10g of body weight IP.
iv. Duration of anaesthesia is 20 – 40 minutes depending on the strain, sex, and body weight of the animal.
v. If a top up is required, a half dose of only ketamine (75 mg/kg) should be administered. To prepare the half dose:
    1. 0.38 ml (37.5 mg) ketamine (100 mg/ml)
    2. 4.62 ml diluent
    3. Write on the vial the name of the drug, expiration date, date mixed and initials
vi. Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.1ml (100ul) per 10g body weight IP.
vii. Atipamezole (5 mg/ml) can be administered to reverse xylazine and facilitate recovery at a dose of 0.1 to 5.0 mg/kg SC or IP.
viii. Mixed cocktail should be protected from light and stored in a cool place with the controlled substances.

- ***Ketamine/Xylazine/Acepromazine Anaesthesia:***

i. Anaesthetic dose: ketamine 50 mg/kg, xylazine 5mg/kg, acepromazine 1mg/kg.
ii. To prepare cocktail, in a sterile vial or bottle with a rubber stopper, mix:
    1. 0.5 ml (50 mg) of ketamine (100 mg/ml)
    2. 0.25 ml (5 mg) xylazine (20 mg/ml)
    3. 0.1 ml (1 mg) acepromazine (10 mg/ml)
    4. 9.15 ml diluent
    5. Label with the drug name(s), expiration date of each of the drugs, date mixed and initials.
iii. Mixed cocktail should be protected from light and stored in a cool place with the controlled substances.
iv. Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.1ml

(100ul) per 10g body weight IP.

v. Duration of anaesthesia is approximately 30 minutes depending on the strain, sex, and body weight of the animal.
vi. After 30 minutes, a half dose (0.05 ml per 10g) may be administered as needed.
vii. Atipamezole (5 mg/ml) can be administered to reverse xylazine and facilitate recovery at a dose of 0.1 to 5.0 mg/kg SC or IP.

- ***Ketamine/Medetomidine Anaesthesia:***

i. Anaesthetic dose: ketamine 75 mg/kg, medetomidine 1 mg/kg
1. 0.3 ml (30 mg) ketamine (100 mg/ml)
2. 0.4 ml (0.4 mg) medetomidine (1 mg/ml)
3. 7.3 ml diluent
4. Label with the drug name(s), expiration date of the drugs, date mixed and initials.

ii. Mixed cocktail should be protected from light and stored in a cool place.
iii. Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.2 ml per 10g body weight IP.
iv. Duration of anaesthesia is approximately 30 minutes depending on the strain, sex, and body weight of the animal.
v. If a top up dose is required, only ketamine should be administered (75mg/kg). To prepare the ketamine dose:
1. 0.38ml (37.5 mg) ketamine (100 mg/ml)
2. 4.62 ml diluent
3. Write on the vial the name of the drug, expiration date, date mixed and initials

vi. Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.1 ml per 10g body weight IP.
vii. Atipamezole (5 mg/ml) can be administered to reverse medetomidine and facilitate recovery at a dose of 0.1 to 5.0 mg/kg SC or IP.

SOP Revision History:

Date	New Version
March 14, 2012	Created
December 15, 2016	Review and update
February 28, 2019	Triennial Review
June 20, 2019	Update
August 8, 2022	Triennial Review