University Animal Care Committee Standard Operating Procedure		
Document No: **7.6**	**Subject: Anaesthesia in Mice**	
Date Issued: March 14, 2012	**Revision:** 5	**Page No:** 1

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,** Waste Anesthetic Gas **WAG,** Litres per Minute **(LPM)**

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, seedling mat, or other approved warming device
- Gas anaesthesia machine calibrated within the last 12 months, and with scavenging capabilities (Moduflex) or a passive filter (carbon filter, weigh scale)
    - (Note: active scavenge systems utilize a vacuum to draw waste gases away, while passive systems rely on pressure differences and the physical properties of the gases)
- Transparent induction chamber
- Nosecone
- Isoflurane
- Oxygen supply
- Ketamine 100mg/kg (controlled substance – exemption permit required)
- Xylazine (20mg/mL)
- Acepromazine (10mg/mL)
- Medetomidine (1 mg/mL)
- Atipamezole (5 mg/mL)
- Sterile sodium chloride 0.9% (diluent and/or fluid therapy)
- Sterile water (diluent)
- Lactated Ringers solution (fluid therapy)
- Sterile needles (various sizes)
- Alcohol swabs
- Gauze
- Sterile eye Lubricant
- 10mL red top vacutainers
- Accelerated hydrogen peroxide (0.5%)
- Heat support/monitoring and analgesics as outlined in animal use protocol

3. Procedures:

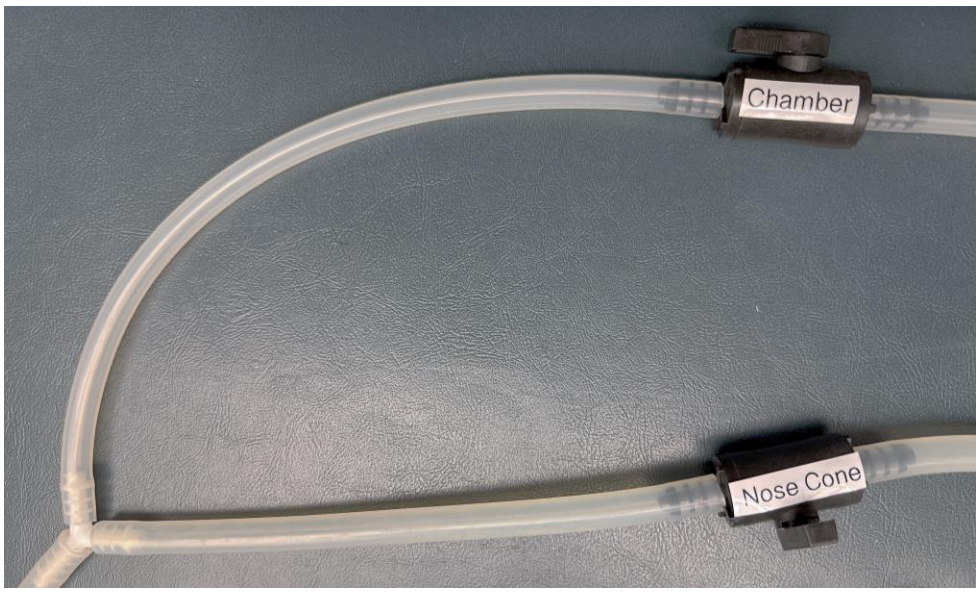
- ***Isoflurane Anaesthesia***:
    - Clean all surfaces, including inside of the induction chamber, with 0.5% accelerated hydrogen peroxide; allow to dry
    - Ensure Isoflurane vaporizer is full of Isoflurane. Fill to "fill line" using key filler bottle adaptor provided
    - Ensure oxygen supply is connected and shut off valve is open
    - Ensure the scavenge line is connected to the exhaust port on the induction chamber as well as the nose cone and that the lines are connected to the wall mounted Moduflex active scavenge interface
    - Ensure wall mounted Moduflex active scavenge interface is on, turn flow meter to 15 LPM (this scavenges the waste anaesthetic gases (WAG) from the patient)

Image of isoflurane key filler bottle adaptor (left) and Moduflex set to 15 LPM (right)

    - If using a passive system (carbon filter), follow manufacturer's instructions on use
    - Ensure the dual anaesthetic gas supply lines from the machine to the patient breathing circuit (chamber or nose cone) are attached

- Dual anaesthetic supply lines have valves that allows for the anaesthetic gas to flow to either the induction chamber, nose cone, or both. These lines are labelled “Chamber” or “Nose Cone”

- Ensure the oxygen flow meter is functioning correctly; turn on flow meter to 1L to 1.5L
- Gently remove rodent from home cage and place in the clean induction chamber. Ensure that the valve labelled “chamber” is open (valve aligned with supply line) and the “Nose Cone” valve remains closed (valve perpendicular to the supply line); see photo



Dual delivery line with “Y” adaptor. The valve is “open” to the chamber and “closed” to the nose cone in this image.

- Increase isoflurane to 4–5% on the vaporiser
- Once the animal has lost its righting reflex and the breathing pattern has become deeper and slower, turn Isoflurane vaporizer to 0% and flush oxygen through induction chamber to remove waste gases (use flush valve or turn O_2 flow rate up to 4 L/min for 30 seconds)
- Open induction chamber and gently remove rodent while supporting the entire body and keeping it level. Do not pick up and hang by tail

- Transfer the rodent to the nose cone, open the "Nose Cone" valve and close the valve labelled "Chamber". Decrease the oxygen flow to 1L/min and turn vaporiser dial to 1.5 - 2%
- Place eye lubricant in both eyes, administer any required analgesics per approved Animal Use Protocol
- Administer SQ fluids per SOP 7.4 (Rodent Post-operative Care (Mice))
- When the mouse is in an adequate plane of anaesthesia, perform the procedure
- Check breathing pattern, color of extremities, temperature and toe pinch response. Adjust Isoflurane % to level required to maintain stable surgical plane (no response to toe pinch) of anaesthesia (1.5 - 2%)
- Routinely throughout anesthetic procedure (i.e., at least every 5 minutes), check animal's breathing pattern/rate, temperature, surgical plane (toe pinch, blink reflex) and colors of extremities. Adjust anaesthetic level and heat support to ensure animal remains at surgical plane of anaesthesia if surgery is being performed and at physiologically normal levels
- At completion of the procedure, turn the Isoflurane to 0% but keep oxygen flow running
- Continuously monitor the animal, once it starts moving, place the animal back in a home cage that has been lined with paper towel. Continuously monitor the animal until it has made a full recovery. Heat should be provided under half of the cage during the recovery period
- Turn off oxygen
- Turn off active scavenge
- Refill the vaporiser back to the fill line level so that it is ready for the next user
- Clean the chamber, nose cone and procedure tables with accelerated hydrogen peroxide

NOTES:

1. In addition to the procedure room space that you are using, the anaesthetic machines should also be reserved using the Outlook calendar system
2. The chamber and nose cone valves must not be closed at the same time while the vaporizer is on as it will blow the line and affect the calibration of the vaporizer

3. Anaesthetic machines are not to be moved or relocated between rooms without approval from ACS, moving them can affect the calibration of the vaporizer

- ***Ketamine/Xylazine Anaesthesia:***
  - Anaesthetic dose: ketamine 150 mg/kg, xylazine 10 mg/kg
  - To prepare the cocktail, in a sterile vial mix:
    - 0.75 ml (75mg) ketamine (100 mg/ml)
    - 0.25 ml (5mg) xylazine (20 mg/ml)
    - 4 ml diluent
  - Label with the drug name(s), expiration date of the drugs, date mixed and initials
  - Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.1ml (100 ul) per 10g of body weight IP
  - Duration of anaesthesia is 20 – 40 minutes depending on the strain, sex, and body weight of the animal
  - If a top up is required, a half dose of only ketamine (75 mg/kg) should be administered
  - To prepare the half dose:
    - 0.38 ml (37.5 mg) ketamine (100 mg/ml)
    - 4.62 ml diluent
    - Write on the vial the name of the drug, expiration date, date mixed and initials
      - Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.1ml (100ul) per 10g body weight IP
      - 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
      - Mixed cocktail should be protected from light and stored in a cool place with the controlled substances

- ***Ketamine/Xylazine/Acepromazine Anaesthesia:***
  - Anaesthetic dose: ketamine 50 mg/kg, xylazine 5mg/kg, acepromazine 1mg/kg

- To prepare cocktail, in a sterile vial or bottle with a rubber stopper, mix:
  - 0.5 ml (50 mg) of ketamine (100 mg/ml)
  - 0.25 ml (5 mg) xylazine (20 mg/ml)
  - 0.1ml (1 mg) acepromazine (10 mg/ml)
  - 9.15 ml diluent
- Label with the drug name(s), expiration date of each of the drugs, date mixed and initials
- Mixed cocktail should be protected from light and stored in a cool place with the controlled substances
- Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.1ml (100ul) per 10g body weight IP
- Duration of anaesthesia is approximately 30 minutes depending on the strain, sex, and body weight of the animal
- After 30 minutes, a half dose (0.05 ml per 10g) may be administered as needed
- 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:***
  - Anaesthetic dose: ketamine 75 mg/kg, medetomidine 1 mg/kg
    - 0.3 ml (30 mg) ketamine (100 mg/ml)
    - 0.4 ml (0.4 mg) medetomidine (1 mg/ml)
    - 7.3 ml diluent
  - Label with the drug name(s), expiration date of the drugs, date mixed and initials
  - Mixed cocktail should be protected from light and stored in a cool place.
  - Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.2 ml per 10g body weight IP
  - Duration of anaesthesia is approximately 30 minutes depending on the strain, sex, and body weight of the animal
  - If a top up dose is required, only ketamine should be administered (75mg/kg). To prepare the ketamine dose:
    - 0.38ml (37.5 mg) ketamine (100 mg/ml)
    - 4.62 ml diluent
    - Write on the vial the name of the drug, expiration date, date mixed and initials
    - Gently restrain the animal, swab the injection site with 70% alcohol and administer 0.1 ml per 10g body weight IP

- 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
August 13, 2025	Triennial Review, updated anesthetic machine set up and delivery