

# LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

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## MODULE 3 – Rodent Analgesia and Anesthesia

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# 1. ANALGESIA

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## 1.1. Definition of pain

Pain is defined by the International Association for the Study of Pain as an unpleasant sensory or emotional experience associated with actual or potential tissue damage.

## 1.2. Definition of analgesia

Analgesia is the inability to feel pain without loss of consciousness.

# 2. GENERAL CONSIDERATIONS

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- A procedure which would be expected to be painful if it was done on humans must be considered painful to the animal.
- When there is a question of whether or not a procedure is painful, the animal should receive the benefit of analgesia.
- General anesthetics used in rodents such as isoflurane and barbiturates do not provide significant analgesia and need to be complemented by appropriate analgesics.
- Analgesia should be provided at an appropriate dose and frequency to control pain.
- Any deviation from procedures described in your Animal Use Protocol (AUP) form must be justified by the investigator and approved by the appropriate Facility Animal Care Committee (FACC).

# 3. PAIN RECOGNITION AND ASSESSMENT

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- Because rodents are prey animals, they will tend to hide any signs of pain in presence of an observer. The most reliable signs of pain and distress are the changes in behavior, but those changes can be very subtle and this implies a sound knowledge of species-typical behavior by the observer and prolonged observation time. Because pain assessment is extremely difficult in rodents, we rely mainly on the basic principles listed in section 2. The absence of clinical signs does not rule out pain or distress, but the presence of clinical signs is indicative of pain or distress:
- Adapt the frequency of observation to the protocol (minimum once a day).
- Start by observing the animal from a distance so the animal's behavior is not altered by the presence of the observer. Then proceed to observe the animal more closely.
- Look for any changes in the behavior.
- Report animals which appear to be in pain to the veterinary care staff by filling out a Rodent Illness and Injury Report form.
- Common clinical signs indicative of pain or distress include:
  - Avoidance
  - Vocalization
  - Aggressiveness (mainly if the animal cannot escape)
  - Spontaneous activities are reduced. The animal is isolated from the social group
  - Altered gait
  - Hunched posture
  - Piloerection
  - Reduced grooming; dark-red stain around the eyes and nostrils (can be observed in rats only)
  - Reduced appetite and subsequent weight loss

### 3.1. The Mouse Grimace Scale (Langford et al. 2010)



National Centre  
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Refinement & Reduction  
of Animals in Research

## The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice.

The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

|   | Not present "0" | Moderately present "1" | Obviously present "2" |
|---|-----------------|------------------------|-----------------------|
| <b>Orbital tightening</b> <ul style="list-style-type: none"> <li>Closing of the eyelid (narrowing of orbital area)</li> <li>A wrinkle may be visible around the eye</li> </ul>  |                 |                        |                       |
| <b>Nose bulge</b> <ul style="list-style-type: none"> <li>Bulging on the bridge of the nose</li> <li>Vertical wrinkles on the side of the nose</li> </ul>  |                 |                        |                       |
| <b>Cheek bulge</b> <ul style="list-style-type: none"> <li>Bulging of the cheeks</li> </ul>  |                 |                        |                       |
| <b>Ear position</b> <ul style="list-style-type: none"> <li>Ears rotate outwards and/or backwards, away from the face</li> <li>Ears may fold to form a 'pointed' shape</li> <li>Space between the ears increases</li> </ul>                                |                 |                        |                       |
| <b>Whisker change</b> <ul style="list-style-type: none"> <li>Whiskers are either pulled back against the cheek, or pulled forward to 'stand on end'</li> <li>Whiskers may clump together</li> <li>Whiskers lose their natural 'downward' curve</li> </ul> |                 |                        |                       |

Read the original paper:  
Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, LaCroix-Fralich ML, Matsuniya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AMJM, Ferrari MD, Craig KD, Mogil JS. 2010. Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* 7(6): 447-449. doi:10.1038/nmeth.1455

For guidance on using the Mouse Grimace Scale, research papers that underpin this technique, and for grimace scales in other species, visit: [www.nc3rs.org.uk/grimacescales](http://www.nc3rs.org.uk/grimacescales). To request copies of this poster, please email: [enquiries@nc3rs.org.uk](mailto:enquiries@nc3rs.org.uk). The NC3Rs provides a range of 3Rs resources at: [www.nc3rs.org.uk/resources](http://www.nc3rs.org.uk/resources). Images kindly provided by Dr Jeffrey Mogil, McGill University

## 3.2. The Rat Grimace Scale (Sotocinal et al. 2011)



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of Animals in Research

# The Rat Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in rats.

The specific facial action units shown below have been used to generate the Rat Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

|   | Not present "0" | Moderately present "1" | Obviously present "2" |
|---|-----------------|------------------------|-----------------------|
| <p><b>Orbital tightening</b></p> <ul style="list-style-type: none"> <li>Closing of the eyelid (narrowing of orbital area)</li> <li>A wrinkle may be visible around the eye</li> </ul>                                 |                 |                        |                       |
| <p><b>Nose/cheek flattening</b></p> <ul style="list-style-type: none"> <li>Flattening and elongation of the bridge of the nose</li> <li>Flattening of the cheeks (potentially sunken look)</li> </ul>                 |                 |                        |                       |
| <p><b>Ear changes</b></p> <ul style="list-style-type: none"> <li>Ears curl inwards and are angled forward to form a 'pointed' shape</li> <li>Space between the ears increases</li> </ul>                              |                 |                        |                       |
| <p><b>Whisker change</b></p> <ul style="list-style-type: none"> <li>Whiskers stiffen and angle along the face</li> <li>Whiskers may 'clump' together</li> <li>Whiskers lose their natural 'downward' curve</li> </ul> |                 |                        |                       |

Read the original paper:  
Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JCS, Weil P, Zhan S, Zhang S, McDougall JJ, King OD, McGill JS. 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular Pain* 7: 55. doi:10.1186/1744-8059-7-55

For guidance on using the Rat Grimace Scale, research papers that underpin this technique, and for grimace scales in other species, visit: [www.nc3rs.org.uk/grimacescales](http://www.nc3rs.org.uk/grimacescales)  
To request copies of this poster, please email: [enquiries@nc3rs.org.uk](mailto:enquiries@nc3rs.org.uk)  
The NC3Rs provides a range of 3Rs resources at: [www.nc3rs.org.uk/resources](http://www.nc3rs.org.uk/resources)  
Images kindly provided by Dr Jeffrey Mogil, McGill University

## 4. ANALGESIA PLAN

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- If possible, provide analgesia before the painful stimulus, as it is more effective in preventing pain, e.g., administer analgesic before surgery
- Try to use a combination of analgesics, which is often more effective than using a single agent. For example, administer a combination of buprenorphine, carprofen, and local infiltration of lidocaine/bupivacaine.
- For surgical procedures, extend analgesia from pre-op to 72 hours post-op, unless specified otherwise in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).

## 5. CLASSES OF ANALGESIC DRUGS

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### 5.1. General Analgesics

#### 5.1.1. Opioids

Opioid agents will mostly have an action on the pain receptors of the brain and the spinal cord, e.g., buprenorphine. Opioids are controlled drugs.

#### 5.1.2. Non-Steroidal Anti-Inflammatory Drugs

Non-steroidal anti-inflammatory drugs, also known as NSAIDs, are potent analgesics acting on the central nervous system. They will also help to reduce inflammation which contributes to pain.

When administering NSAIDs ensure good water intake and monitor hydration status. Suspend water restriction prior to administration.

Examples of NSAIDs include ketoprofen (Anafen®), carprofen (Rimadyl®), meloxicam (Metacam®).

### 5.2. General Analgesics

#### Mouse / Hamster / Gerbil

| Analgesic                              | Dose      | Route  | Frequency  | Note   |
|--|-----------|--------|------------|--|
| *Buprenorphine<br>Slow Release<br>(SR) | 1 mg/kg   | SC     | once       | Buprenorphine SR (0.6mg/ml) is a sustained release buprenorphine product that has been developed to provide 72 hours of analgesia and is specifically designed for use in rodents.<br>See administration instructions in section 7.1.              |
| *Buprenorphine                         | 0.1 mg/kg | SC, IP | 4–8 hrs.   | Mild to moderate pain.<br>Controlled drug.   |
| *Carprofen                             | 20 mg/kg  | SC, PO | 12–24 hrs. | Mild to moderate pain.<br>Use carprofen 50mg/ml injectable solution.<br>To prepare a 4mg/ml dilution: add 0.8ml of carprofen 50mg/ml to 9.2 ml of sterile water for injection.<br>Administer 5µL/g of bodyweight. Discard dilution after 3 months. |

| <b>Analgesic</b> | <b>Dose</b> | <b>Route</b> | <b>Frequency</b> | <b>Note</b>   |
|------------------|-------------|--------------|------------------|---|
| *Meloxicam       | 20 mg/kg    | SC, PO       | 24 hrs.          | Mild to moderate pain.<br>Ensure good water intake and monitor hydration status.<br>Suspend water restriction prior to administration.  |
| Ketoprofen       | 15 mg/kg    | SC           | 12-24 hrs.       | Mild to moderate pain.<br>Do not administer after an adrenalectomy.<br>Ensure good water intake and monitor hydration status.<br>Suspend water restriction prior to administration. |
| Acetaminophen    | 300 mg/kg   | PO           | 4 hrs.           | Mild pain; to combine with another analgesic.<br>Ensure good water intake and monitor hydration status.<br>Suspend water restriction prior to administration.                       |
| Fentanyl         | 0.1 mg/kg   | SC, IP       | 30 min.          | Moderate to severe pain.<br>Controlled drug.  |
| Morphine         | 20 mg/kg    | SC, IP       | 2 hrs.           | Moderate to severe pain.<br>Controlled drug.  |

\*most commonly used

#### Rat

| <b>Analgesic</b>                       | <b>Dose</b> | <b>Route</b> | <b>Frequency</b> | <b>Note</b>   |
|--|-------------|--------------|------------------|---|
| *Buprenorphine<br>Slow Release<br>(SR) | 1 mg/kg     | SC           | once             | Buprenorphine SR (0.6mg/ml) is a sustained release buprenorphine product that has been developed to provide 72 hours of analgesia and is specifically designed for use in rodents.<br>See administration instructions in section 7.1. |
| *Buprenorphine                         | 0.05 mg/kg  | SC, IP, IV   | 8-12 hrs.        | Mild to moderate pain.<br>Controlled drug.<br>Give every 12 hours when combined with meloxicam or carprofen.  |

**Rat**

| <b>Analgesic</b> | <b>Dose</b>   | <b>Route</b> | <b>Frequency</b>   | <b>Note</b>  |
|------------------|---|--------------|--|--|
| *Carprofen       | 5-10 mg/kg  | SC, PO       | 12–24 hrs.   | Mild to moderate pain.<br>Use carprofen 50mg/ml injectable solution.<br>To prepare a 5mg/ml dilution: add 1ml of carprofen 50mg/ml to 9 ml of sterile water for injection. Administer 0.1-0.2ml/100g of bodyweight. Discard dilution after 3 months.<br>Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose.  |
| *Meloxicam       | 1 mg/kg   | SC, PO       | 24 hrs.  | Mild to moderate pain.<br>Ensure good water intake and monitor hydration status.<br>Suspend water restriction prior to administration.   |
| Ketoprofen       | 2–5 mg/kg   | SC, PO       | 12–24 hrs.   | Mild to moderate pain.<br>Do not administer after an adrenalectomy.<br>Ensure good water intake and monitor hydration status.<br>Suspend water restriction prior to administration.  |
| Acetaminophen    | 100–300 mg/kg<br><br>5 mg/ml solution in drinking water | PO           | 4 hr.<br><br>Give medicated water 24 hours before painful procedure. | Mild pain; to combine with another analgesic.<br>Provide acetaminophen-treated drinking water at least 24 hours in advance of painful procedure. Used in conjunction with buprenorphine enhances pain relief. Prepare solution by mixing Children’s Tylenol Elixir to drinking water. Mix bottle thoroughly and use. Fresh solution should be prepared and added to clean bottles every 3-4 days for maximum efficacy.<br>Ensure good water intake and monitor hydration status.<br>Suspend water restriction prior to administration. |
| Fentanyl         | 0.1 mg/kg   | SC, IP       | 30 min.  | Moderate to severe pain.<br>Controlled drug.   |
| Morphine         | 20 mg/kg  | SC, IP       | 2 hrs.   | Moderate to severe pain.<br>Controlled drug.   |

\*most commonly used

## Guinea pig / Chinchilla

| Analgesic      | Dose            | Route  | Frequency  | Note  |
|----------------|-----------------|--------|------------|---|
| *Buprenorphine | 0.01–0.05 mg/kg | SC, IP | 8–12 hrs.  | Mild to moderate pain.<br>Controlled drug.  |
| *Carprofen     | 5-10 mg/kg      | SC, PO | 12–24 hrs. | Mild to moderate pain.<br>Use carprofen 50mg/ml injectable solution.<br>To prepare a 5mg/ml dilution: add 1ml of carprofen 50mg/ml to 9 ml of sterile water for injection. Administer 0.1-0.2ml/100g of bodyweight. Discard dilution after 3 months.<br>Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose. |
| *Meloxicam     | 1 mg/kg         | SC, PO | 24 hrs.    | Mild to moderate pain.<br>Ensure good water intake and monitor hydration status.<br>Suspend water restriction prior to administration.  |
| Ketoprofen     | 2 mg/kg         | SC     | 12–24 hrs. | Mild pain.<br>Do not administer after an adrenalectomy.<br>Ensure good water intake and monitor hydration status.<br>Suspend water restriction prior to administration.   |
| Acetaminophen  | 100–300 mg/kg   | PO     | 4 hrs.     | Mild pain; to combine with another analgesic.<br>Ensure good water intake and monitor hydration status.<br>Suspend water restriction prior to administration.   |

\*most commonly used

### 5.2.1. Administration instructions for buprenorphine slow release (SR):

- Avoid contact with the skin to prevent the development of injection site reactions.
- For use in mice: draw up the buprenorphine SR with a 25g or 27g needle attached to a 1cc low waste syringe (alternatively, use a 28g insulin syringe) and wipe needle off with an alcohol swab to remove any drug residue from the needle prior to administration.
- For use in rats: draw up the buprenorphine SR LAB with an 18g needle and change to a 23g needle prior to administration. Consider using a luer-lock syringe.
- Administer subcutaneously on back of neck between the shoulder blades while the animal is anesthetized.
- Administer slowly and finish injecting before the needle is pulled out through skin.
- Pinch the injection site for approximately 10 seconds after removing the needle
- Do not combine the buprenorphine SR with any other drugs in the same syringe and do not attempt to dilute the formulation.



### 5.3. Local Analgesics

- Are applied topically to prevent pain at the skin level. Examples include lidocaine, bupivacaine, EMLA® cream
- Infiltrate or apply local analgesic to areas where a painful stimulus may be induced. Apply lidocaine/bupivacaine mixture prior to closing an incision.

| Analgesic                       | Dose         | Duration         | Note   |
|---------------------------------|--------------|------------------|--|
| Lidocaine                       | < 2 mg/kg    | 30–60 min.       | Use lidocaine HCl 2% (20mg/ml) injectable solution.<br>Because this drug is acidic, it is recommended to dilute it 3:1 with sodium bicarbonate injectable solution (at 5 or 8.4%).<br>Dilution with sodium bicarbonate is not necessary if lidocaine is to be administered to an anesthetized animal.<br>Dilution must be prepared immediately before use and should not be stored. Diluted solution is as effective but induction of analgesia is slightly prolonged. |
| Bupivacaine                     | < 2 mg/kg    | 3–4 hrs.         | Use bupivacaine HCl 0.50% (5mg/ml) injectable solution.<br>Same comment as for lidocaine.  |
| * Lidocaine-bupivacaine mixture | -            | 30min. to 4 hrs. | Same comment as for lidocaine.<br>Combining both drugs allows for rapid induction and prolonged effect.<br>Discard mixture after 3 months.   |
| EMLA cream                      | Thick spread | 30–60 min.       | Shave or pluck the fur and apply a thick layer of cream ideally 10 minutes before the painful procedure.   |

## 6. ANESTHESIA

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### 6.1. General considerations

#### 6.1.1. Choice of anesthetic method

Rodents can be anesthetized with either inhalant gas or injectable drugs. The use of inhalant gases is the preferred method of anesthesia whenever possible.

#### 6.1.2. Animal Observation

Animal should be observed prior to anesthesia to prevent any complications such as:

- Excess respiratory secretions that can cause a blockage of the airways, e.g., respiratory infections.
- Gastrointestinal problems that can lead to dehydration, e.g., diarrhea.

#### 6.1.3. Fasting

Rodents are not routinely fasted prior to anesthesia due to their inability to vomit. Withholding the food from a small rodent for a long period of time can also lead to hypoglycemia.

#### 6.1.4. Analgesia

If analgesia is required, it should be administered before the anesthesia. Please refer to the Analgesia section above.

### 6.1.5. Dehydration

Rodents dehydrate much faster than other larger species.

Administer from 0.2 to 0.5mL/10g body weight of isotonic fluids (a solution having the same osmotic pressure as blood) such as 0.9% saline, subcutaneously, prior to or during anesthesia for survival procedures. Isotonic fluids can be warmed prior to administration.

### 6.1.6. Eye Lubrication

Since the rodent's eyes remain open during anesthesia, it is important to lubricate them with a sterile ophthalmic ointment as it can cause irreversible damage to their corneas and alter their sight. Ophthalmic ointment should be reapplied as needed.

### 6.1.7. Hypothermia

Heat loss is rapid in anesthetized rodents due to their small size, the absence of muscular activity and the cooling that can result from the inhalation of anesthetic gases. If hypothermia is not prevented it can lead to death.

It is important to keep the animal warm during the procedure and until it recovers from the anesthesia. Heat loss can be prevented by using one of the following:

- Gauze pad: place gauze on the animal's tail.
- Towel: wrapped around the animal.
- Heat lamp with a red light bulb: at a reasonable distance away from the animal. (Approximately 2-3 feet for a 250W bulb).
- Warm water circulating pad: under the animal cage or in indirect contact with the animal.
- Bubble wrap: wrapped around the animal.
- Warm bottles: placed against the animal.
- Microwavable heating disc: under the animal cage or in indirect contact with the animal.

Note: The use of electrical heating pads is strongly discouraged. These types of pads heat unevenly and may cause skin burns.

Do not overheat the animal as it can cause an irreversible hyperthermia.

## 7. MONITORING DURING ANESTHESIA

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### 7.1. Reflexes

- Palpebral reflex is stimulated by gently touching the edge of the eyelids with the tip of a finger or the corner of a clean gauze for smaller animals.  
Note: not reliable with the use of ketamine as the animal will lose this reflex before losing consciousness.
- Pedal withdrawal reflex is stimulated by pinching the skin between the toes and/or toe pads using blunt forceps. The pinch needs to be strong enough to create a painful stimulus in order to evaluate the depth of anesthesia. Under general anesthesia, this reflex becomes weaker as the depth of anesthesia increases. It disappears completely at the 3<sup>rd</sup> stage of anesthesia (animal is anesthetized deeply enough to undergo surgery).

### 7.2. Respiration

- Assessed by observing the animal unless specific monitoring equipment is present.
- Should be regular, thoracic and abdominal.

### 7.3. Heart Rate

- Cannot be evaluated unless specific monitoring equipment is present since the heart rate is too fast to be visually assessed.

### 7.4. Oxygen Saturation

- Assessed by observing the color of the mucous membranes of the nose and the mouth. Their color should remain pink. Pink color of the mucous membranes indicates adequate blood oxygen levels. Specialized monitoring equipment can also be used to monitor the oxygen saturation.

### 7.5. Temperature

- Can be determined using an infrared thermometer or other specific monitoring equipment designed for small rodents.

## 8. GAS ANESTHESIA

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### 8.1. Advantages

- Gas anesthetics are not controlled agents.
- The depth of anesthesia can easily be adjusted by the anesthetist.
- The elimination of the anesthetic gas is mainly through the lungs.
- Allows a constant and high concentration of oxygen to be delivered to the animal (close to 100%).
- Induction and recovery are rapid.

### 8.2. Disadvantages

- Waste anesthetic gases need to be eliminated as they can be harmful to the user.

### 8.3. Procedure for Isoflurane Anesthesia

Prior to anesthesia:

- Open the oxygen tank and verify the level on the tank pressure gauge.
- Verify the level of isoflurane left in the vaporizer through the sight glass.

Induction:

- Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.
- Place the animal(s) in the induction chamber.

Note: Do not overload the induction chamber.

Mice can be placed with their cage mates in the chamber.

Rats must be placed in the induction chamber individually.

Keep the induction chamber clean to minimize odors that might distress animals subsequently anesthetized.

- Set the isoflurane vaporizer between 3% and 5%.
- Wait until the animal loses consciousness and then follow the steps in the maintenance section below.

Maintenance:

- Use the mask connected to the Bain circuit.
- Adjust the oxygen flowmeter to 0.4 to 0.8 L/min.
- Set the isoflurane vaporizer between 2 and 2.5%.

- Apply lubricating ophthalmic ointment to both eyes. Reapply as needed.
- Asses the depth of anesthesia by testing the pedal reflexes of the animal by pinching the toe pads with a pair of forceps.
- Adjust the isoflurane vaporizer according to the desired depth of anesthesia.

Recovery:

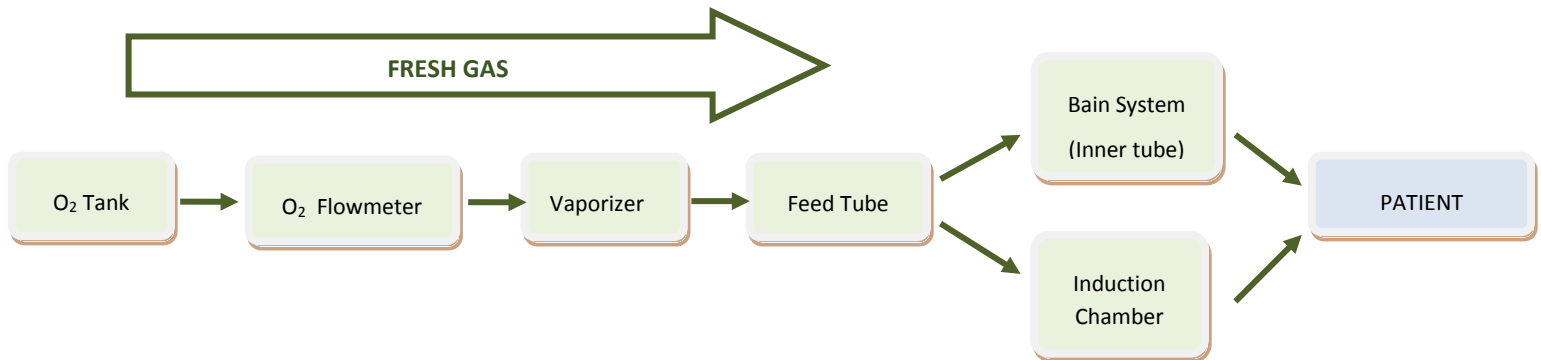
- Turn off the isoflurane vaporizer but keep the animal on oxygen. Provide heat source.
- Transfer the animal to its cage once it begins to move and allow to recover fully (sternal position).

Note: Never leave an anesthetized animal unattended.

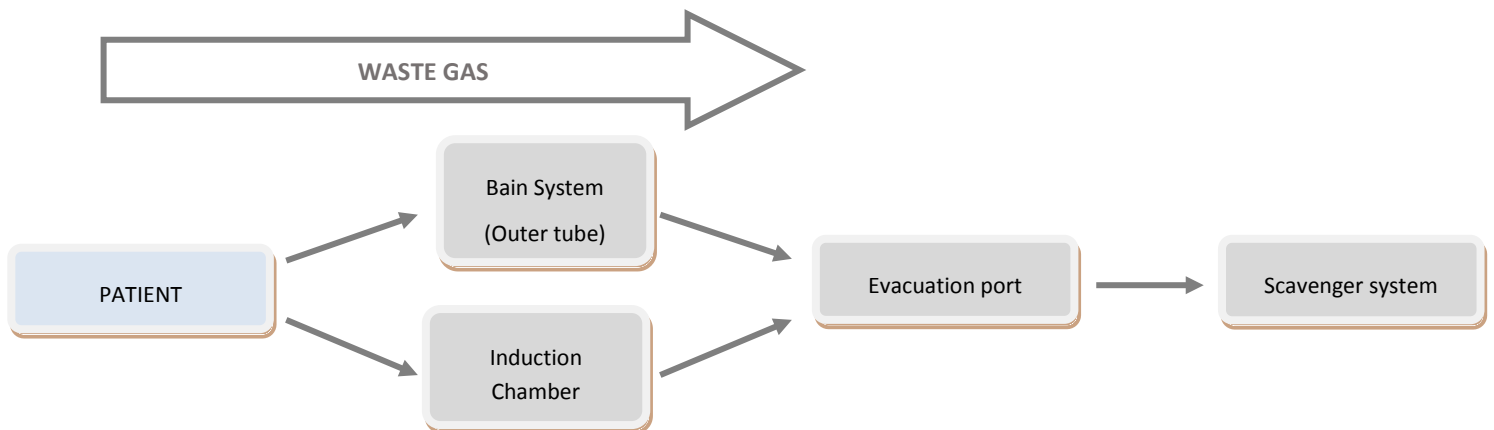
#### 8.4. In Case of Respiratory or Cardiac Arrest:

- Decrease or stop isoflurane administration.
- Keep the animal under 100% oxygen.
- Stimulate the animal by pinching a toe or inserting a 25G needle in between the two nostrils.

#### 8.5. Flow of Anesthetic Gases



The oxygen contained under pressure in the tank will pass through the oxygen flowmeter which delivers oxygen at a controlled rate measured in liters per minute. Once the vaporizer is opened, the volatile liquid anesthetic is converted into gas. As the oxygen passes through the vaporizer, a controlled amount of anesthetic gas mixes with the oxygen and exits through the feed tube. The flow of fresh gas (oxygen mixed with anesthetic gas) will pass through the induction chamber or the inner tube of the Bain system and will be delivered to the patient.



The patient will exhale the waste anesthetic gases in the induction chamber or the outer tube of the Bain system. The waste gas will then pass through the evacuation port to finally be released through the scavenger system.

## 8.6. Anesthetic Machine Components

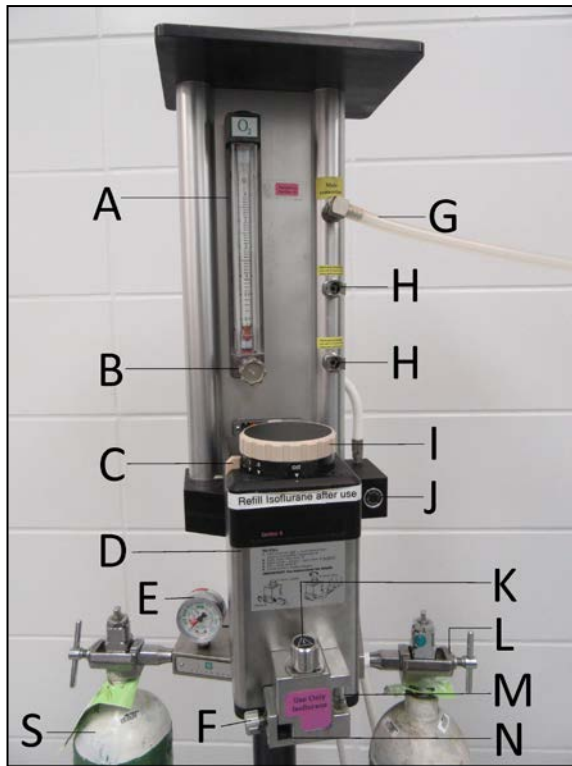


Figure 1 (mobile anesthetic machine)

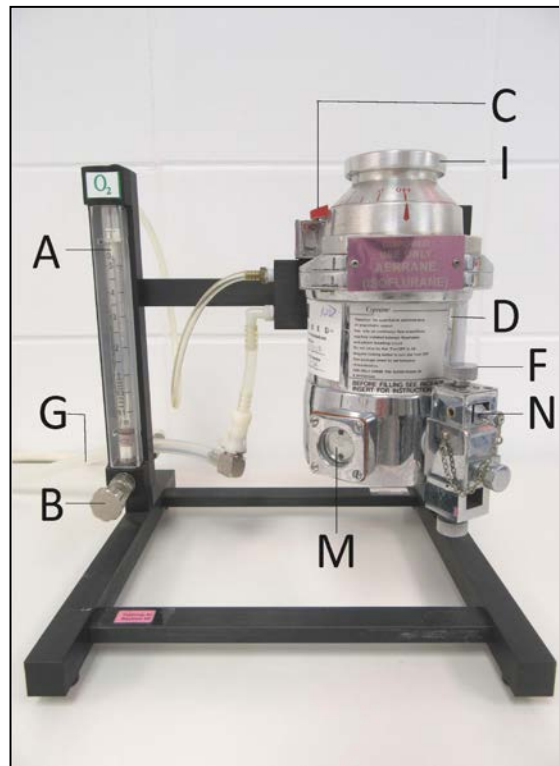


Figure 2 (table-top anesthetic machine)

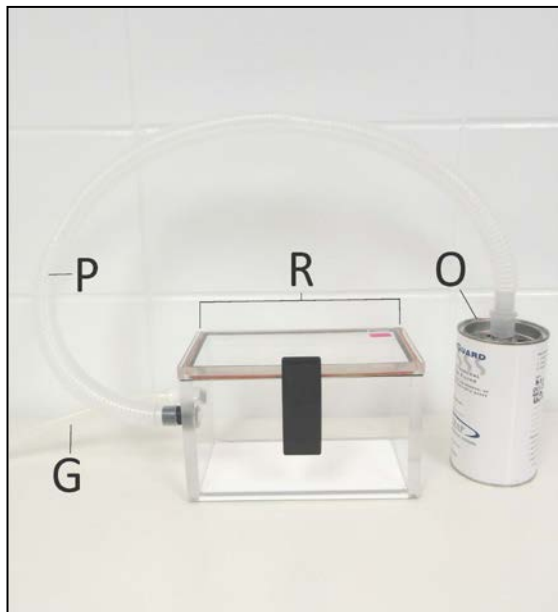


Figure 3

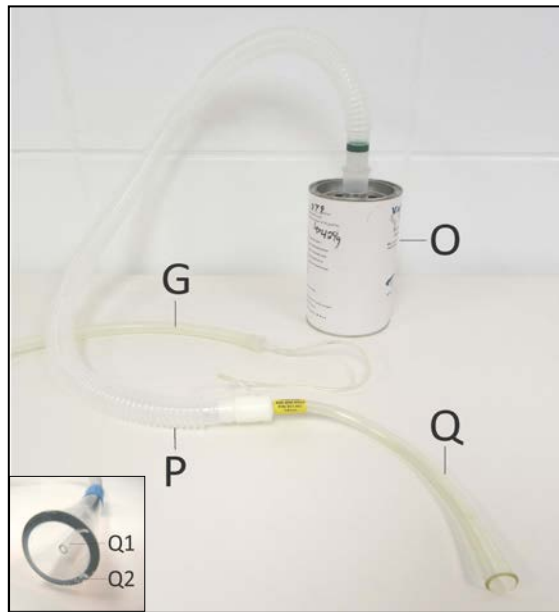
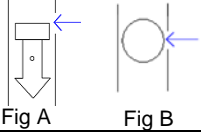


Figure 4

| Location | Figure  | Component                                | Description  |
|----------|---------|--|--|
| <b>A</b> | 1,2     | Oxygen Flowmeter                         | Determines the amount of oxygen delivered to the patient in liters per minute.   |
| <b>B</b> | 1,2     | Oxygen Flowmeter valve                   | Allows precise adjustment of the gas flow delivered to the patient. The vane rotameter should be read at the top of the rotor (Fig A). The ball indicator should be read at the center of the ball. (Fig B). Do not tighten valve. <div style="text-align: right;">  </div> |
| <b>C</b> | 1,2     | Release Button                           | Unlocks the control dial on the vaporizer.   |
| <b>D</b> | 1,2     | Vaporizer                                | Converts liquid anesthetic to gas and adds controlled amounts of vaporized anesthetic to oxygen.   |
| <b>E</b> | 1       | Tank Pressure Gauge                      | Indicates the pressure of gas remaining in the tank.   |
| <b>F</b> | 1       | Optional Attachment Site                 | Allows the use of up to 3 components (feed tubes or induction chambers) on the same anesthetic machine.  |
| <b>G</b> | 1,2,3,4 | Feed-tube                                | Delivers the anesthetic gas to the animal.   |
| <b>H</b> | 1,2     | Tank Holder or Yoke                      | Holds the oxygen tank in place   |
| <b>I</b> | 1,2     | Control Dial                             | Controls the amount of isoflurane gas delivered to the patient. Graduated from 1% to 5%.   |
| <b>J</b> | 1,2     | Oxygen Flush Valve                       | If pressed, it will deliver pure oxygen by bypassing the oxygen flowmeter and vaporizer. Note: Cannot be used with a Bain system with rodents as the high flow rate of oxygen would severely damage the animal's lungs   |
| <b>K</b> | 1       | Valve Knob                               | When opened, allows filling of the vaporizer   |
| <b>L</b> | 1,2     | Sight Glass                              | Indicates the level of liquid anesthetic in the vaporizer (isoflurane)   |
| <b>M</b> | 1       | Filling Tunnel                           | Point of insertion of the keyed block of the liquid anesthetic bottle adaptor  |
| <b>N</b> | 1       | Retaining Screw                          | Locks the keyed block in place for the filling of the vaporizer  |
| <b>O</b> | 3,4     | Charcoal Filter Canister                 | Remove waste anesthetic gases from the room environment<br>Note: refer to the instructions on the filter canister  |
| <b>P</b> | 3,4     | Evacuation Port                          | Transports the waste anesthetic gases  |
| <b>Q</b> | 3,5,6   | Bain system<br>(Non-Rebreathing Circuit) | Supplies fresh gas (isoflurane mixed with oxygen) to the patient through the inner tube (Q1)<br>Directs exhaled gas from the patient to the evacuation system (filter) by the outer tube (Q2)  |
| <b>R</b> | 4       | Induction Chamber                        | Used for anesthetic induction of the patient   |
| <b>S</b> | 1       | Oxygen Tank                              | Contains oxygen under pressure   |

## 8.7. Filling Of Anesthetic Vaporizer

When filling vaporizer always wear gloves and work in a well ventilated area.

### 8.7.1. Funnel Filler

- Ensure the vaporizer control dial is set in the OFF position.
  - Remove the filler cap by turning it counter-clockwise. (Figure 1)
  - Pour anesthetic gas slowly into opening. (Figure 2)
  - Observe the level of anesthetic gas through sight glass and stop pouring when it reaches the upper line.
  - Replace the cap filler by turning it clockwise. (Figure 3)
- Note: Cap should be tight to prevent any leaks.



Figure 1



Figure 2



Figure 3

### 8.7.2. Keyed Filler

- Always refer to your vaporizer's manual for specific filling instructions.
- Ensure the vaporizer control dial is set in the OFF position.
- Turn the retaining screw on vaporizer filler unit counter-clockwise.
- Withdraw filler plug (if applicable).
- Insert the keyed block of the bottle adapter completely in the filling tunnel. There is only one way to correctly insert the keyed block (Figure 1).  
Note: Maintain the bottle below the level of the vaporizer to prevent spillage.
- Tighten the retaining screw on vaporizer filler unit clockwise to seal the adapter.
- Open the valve knob (Figure 2).
- Slowly raise the bottle above the level of the filling tunnel (Figure 3).  
Note: Do not kink the tube.
- Observe the level of anesthetic gas through the sight glass.
- When the level of anesthetic reaches the "FULL" line, turn the valve knob on vaporizer filler unit counter-clockwise. (Figure 4).
- Lower the bottle and remove the keyed block of the bottle adapter from the filling tunnel (Figure 5).  
Note: Excess liquid anesthetic may drain from the filling tunnel.
- Insert the filler plug in the filling tunnel and tighten (if applicable).



Figure 1



Figure 2

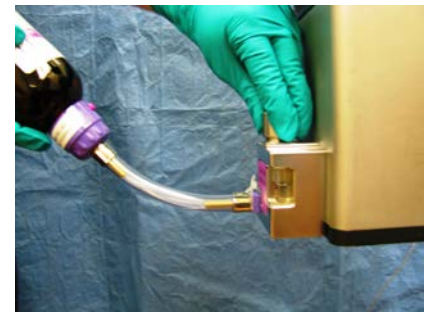


Figure 3

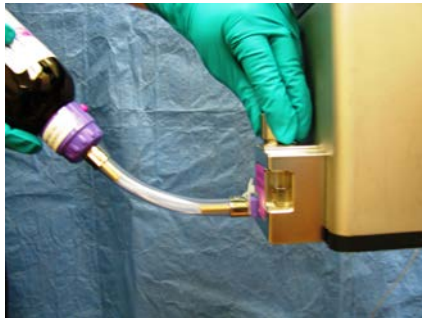


Figure 4



Figure 5

## 8.8. Waste Anesthetic Gas Scavenging

Waste anesthetic gases can be potentially hazardous to personnel. Adequate waste anesthetic gas scavenging systems must be used to minimize exposure.

To eliminate waste anesthetic gases from the work area and minimize exposure of personnel, follow these guidelines:

- Work in a well-ventilated area, ideally under a fume hood or a hard-ducted biosafety cabinet (which vents directly to the outside without recirculation within the building).
- Maintain a reasonable distance from the source of the gas.
- Use an adequately-sized anesthetic mask to ensure a tight seal around the animal's face and prevent leaks.
- Clean the induction chambers with disinfecting solution immediately after each use to avoid residual anesthetic waste release into the environment. Waste anesthetic gases can continue to be released for up to 3 hours.
- Use an appropriate scavenging option:
  - a. Direct the exhaust from the anesthetic circuit to a fume hood, a hard-ducted biosafety cabinet or to the room exhaust if it is evacuated directly outside the building without recirculation. Ensure a minimum room ventilation air exchange is maintained (10–15 air changes per hour).
  - b. Perform procedures under a certified fume hood when using an induction chamber to capture the gas escaping the chamber when the lid is opened.
  - c. Activated charcoal canisters:
    - Do not to occlude the vent holes.
    - Weigh the canister before and after each use to evaluate the remaining absorption capacity, and record the weight on the canister in the space provided
    - Shake canister briefly before replacing to evenly redistribute contents.
    - Discard the canisters in the trash inside a sealed plastic bag after the maximum recommended weight increase.



## 9. INJECTABLE ANESTHESIA

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### 9.1. Advantages

No specialized equipment is required.

### 9.2. Disadvantages

- Some anesthetic drugs are controlled; an exemption from Health Canada must be obtained for their use. Refer to SOP 401 for guidelines on the use of controlled drugs. E.g.: ketamine
- The depth of anesthesia cannot be easily controlled.
- Injectable drugs are usually metabolised by the liver and excreted by the kidneys which may be problematic in some cases.
- The animal only receives the oxygen breathed in room air (approximately 20%).
- The induction and recovery periods are longer than with gas anesthesia.

### 9.3. Injectable anesthetic administration

- Injectable anesthetic dose can vary with the sex, the age, the strain, and the body condition of the animal.
- Contact your veterinarian for advice on the appropriate dose prior to use.
- Weigh the animal prior to drug administration.
- If you are unsure of the animal's sensitivity to anesthetic drugs, first administer 75% of the calculated dose and assess the depth of anesthesia by testing the pedal reflex 5 minutes after the anesthetic administration. If the reflex is still present, administer the remaining 25% of the calculated dose
- It is not recommended to administer more than 125% of the calculated dose.
- Recovery:
  - Provide a source of heat to the animals such as a heating disc, warming pad or warm-water circulating pad (do not use electric heating pads) until full recovery.
  - Observe the animal until it regains righting reflexes. Observe respiration and coloration of the eyes (for albinos), mucous membranes and skin. Once the procedure is completed, transfer the animal to its cage. Do not return animals that have not completely recovered to an animal room.  
Note: Never leave an anesthetized animal unattended.

### 9.4. Ketamine/Xylazine/Acepromazine Anesthesia (Rodent Anesthetic Cocktail)

#### 9.4.1. Mice

- Anesthetic dose in mice: ketamine 100mg/kg, xylazine 10mg/kg, acepromazine 3mg/kg.
- Prepare the solution the day before or shake it thoroughly before use.
- Preparation of anesthetic cocktail for mice:
  - In a sterile vial or bottle with a rubber stopper, mix:
    - 1 mL of ketamine (100mg/mL)
    - 0.5 mL xylazine (20mg/mL)
    - 0.3 mL acepromazine (10mg/mL)
    - 8.2 mL of sterile isotonic saline or sterile water for injection.
  - Label as "Mouse Cocktail" and indicate expiration date on vial or bottle (the cocktail must be discarded after 6 months). Mixed cocktail should be protected from light and stored in a cool place.
  - Administer 0.05-0.1mL/10g body weight intraperitoneally.

- Duration of anesthesia is approximately 20 minutes.
- After 20 minutes, a half dose may be re-administered as needed.
- Consider administering atipamezole 1-2 mg/kg SC or IP to improve respiration or speed up the recovery if needed. Atipamezole is the antidote for xylazine.

#### 9.4.2. Rats

- Anesthetic dose in rats: ketamine 50mg/kg, xylazine 5mg/kg, acepromazine 1mg/kg.
- Prepare the solution the day before or shake it thoroughly before use.
- Preparation of anesthetic cocktail for rats:
  - In a sterile vial or bottle with a rubber stopper, mix:
    - 5mL of ketamine (100mg/mL)
    - 2.5mL xylazine (20mg/mL)
    - 1mL acepromazine (10mg/mL)
    - 1.5mL of sterile isotonic saline or sterile water for injection.
  - Label as “Rat Cocktail” and indicate expiration date on vial or bottle (the cocktail must be discarded after 6 months). Mixed cocktail should be protected from light and stored in a cool place.
  - Administer 0.1mL/100g body weight intraperitoneally.
- Duration of anesthesia is approximately 30 minutes.
- After 30 minutes, a half dose may be re-administered as needed.
- Consider administering atipamezole 1-2 mg/kg SC or IP to improve respiration or speed up the recovery if needed. Atipamezole is the antidote for xylazine.

#### 9.5. In Case of Respiratory or Cardiac Arrest:

- Provide 100% oxygen to the animal if the equipment is available.
- Stimulate the animal by pinching a toe or inserting a 25G needle between the two nostrils.

## 10. REFERENCES

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### 10.1. Comparative Medicine & Animal Resources Centre

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| CMARC website  | <a href="http://www.mcgill.ca/cmarc">www.mcgill.ca/cmarc</a>                               |
| Veterinary Care  | <a href="mailto:aht.arc@mcgill.ca">aht.arc@mcgill.ca</a>                                   |
| Technical Services, Equipment rental (Anesthetic machines) | <a href="mailto:rts.arc@mcgill.ca">rts.arc@mcgill.ca</a>                                   |
| Imports, Transfers and Quarantine                          | <a href="mailto:import.cmarc@mcgill.ca">import.cmarc@mcgill.ca</a>                         |
| Imaging Services   | <a href="mailto:imaging.cmarc@mcgill.ca">imaging.cmarc@mcgill.ca</a>                       |
| Irradiator Services  | <a href="mailto:irradiator.cmarc@mcgill.ca">irradiator.cmarc@mcgill.ca</a>                 |
| Workshop and Training                                      | <a href="mailto:workshop.cmarc@mcgill.ca">workshop.cmarc@mcgill.ca</a>                     |
| Polyclonal Antibody Production                             | <a href="mailto:antibodyproduction.cmarc@mcgill.ca">antibodyproduction.cmarc@mcgill.ca</a> |
| Materials and drug sales                                   | <a href="mailto:drss@mcgill.ca">drss@mcgill.ca</a>   |
| Comparative Pathology                                      | <a href="mailto:comparative.pathology@mcgill.ca">comparative.pathology@mcgill.ca</a>       |

### 10.2. McGill Standard Operating Procedures (SOP)

<http://www.mcgill.ca/research/researchers/compliance/animal/sop>

## Before you begin

### Analgesia

- Check expiry date on drugs.
- Find the concentration and the dose to administer to the animal(s).
- Weigh the animal(s).
- Calculate the amount to administer.
- Analgesia can be given approximately 20-30 min. prior to the procedure.

### Preparation of Anesthetic Machine

- Open the oxygen tank and verify the level of gas left on the tank pressure gauge.
- Verify the level of isoflurane left in the vaporizer by looking through the sight glass.

## Induction

- Check for proper connections.
- Adjust the oxygen flowmeter between 0.8 to 1.5 L/min.
- Place the animal in the induction chamber.
- Adjust the isoflurane vaporizer between 3% to 5% and wait until the animal loses consciousness.

## Maintenance

- Once the animal is unconscious, decrease the oxygen flow between 0.4 to 0.8L/min.
- Change the connection to the bain system.
- Remove the animal from the induction chamber and use the mask connected to the bain system.
- Adjust the isoflurane vaporizer between 2% to 2.5%.
- Provide a source of heat to the animal (microwavable disc, heat lamp with a red bulb, etc)
- Apply ophthalmic ointment.
- Assess the depth of anesthesia by testing the pedal reflex of the animal by pinching the toe pads and the paws with a pair of forceps.
- If the animal still has reflexes, adjust the isoflurane vaporizer accordingly.

## Monitoring During Anesthesia (approximately every 3-5 minutes)

- Reflexes: pedal withdrawal reflex (toe pads + paws).
- Respiration: should be regular, thoracic and abdominal.
- Mucous Membranes: color surrounding the nose and mouth should remain pink.

## Recovery

- Administer from 0.2 to 0.5mL/10g body weight of isotonic fluids subcutaneously.
- Turn off the isoflurane vaporizer.
- Keep the animal on 100% oxygen.
- Transfer the animal to its cage once it begins to move.
- Provide a source of heat to the animals until full recovery (keep animals in sternal position).

## Post Procedure

- Close the oxygen flowmeter and close the oxygen tank.
- Press on the oxygen flush valve to empty the lines. If properly closed, the tank pressure gauge should indicate "0".
- Disconnect tubings.
- Clean equipment and work surface.

## INJECTABLE ANESTHESIA CHECKLIST

### Prior to Procedure

#### Analgesia

- Check expiry date on drugs.
- Find the concentration and the dose to administer to the animal(s).
- Weigh the animal(s).
- Calculate the amount to administer.
- Analgesia can be given approximately 20-30 min. prior to the procedure.

#### Anesthetic Drug

- Check expiry date on drugs.
- Find the concentration of the anesthetic drug and the dose to administer.
- Weigh the animal(s).
- Calculate the amount to administer.

### Administration

- Administer the calculated dose via intraperitoneal injection.
- Provide a source of heat to the animal (microwavable heating disc, heat lamp, etc.)
- Apply ophthalmic ointment.
- Assess the depth of anesthesia after approximately 7-10 minutes by testing the pedal reflex of the animal by pinching the toe pads and the paws with a pair of forceps.
- If reflexes still present after 10 to 12 minutes, give an extra 25% of the calculated dose.

Note: It is not recommended to administer more than 125% of the calculated dose.

### Monitoring During Anesthesia (Approximately every 3-5 minutes)

- Reflexes: pedal withdrawal reflex (toe pads + paws).
- Respiration: should be regular, thoracic and abdominal.
- Mucous Membranes: color surrounding the nose and mouth should remain pink.

Note: If a procedure is prolonged, an extra dose can be readministered.

### Recovery

- Administer from 0.2 to 0.5mL/10g body weight of isotonic fluids subcutaneously.
- Transfer the animal to its cage.
- Provide a source of heat to the animals until full recovery (keep animals in sternal position).
- Observe the animal until it regains reflexes.

### Post Procedure

- Complete the controlled drug log with the exact amount used.
- Clean material and work surface.

## ANALGESICS

The Comparative Medicine & Animal Resources Centre can provide the following analgesics at no cost to McGill investigators. We provide solutions already diluted to the adequate concentration for use in mice or rats. Please contact [aht.arc@mcgill.ca](mailto:aht.arc@mcgill.ca) to request free analgesics.

### **Carprofen**

- Dose for mice: 20mg/kg every 24 hours.
- Dose for rats: 5-10mg/kg every 24 hours.
- The volume to administer is indicated on the vial.
- Administer first dose 20 to 30 minutes before the procedure. Second and third doses are administered every 24 hours.
- Administer carprofen subcutaneously.
- Carprofen can be administered in conjunction with buprenorphine and local anesthetics with no adverse effects.
- Do not use past expiry date indicated on bottle.
- Protect product from light and store in refrigerator.

### **Lidocaine/bupivacaine mixture (local analgesic)**

- Apply a few of drops to surgical incision just prior to closing the wound.
- Should be used in conjunction to carprofen and/or buprenorphine.
- Do not use past expiry date indicated on bottle.
- Protect product from light.

## ANESTHETIC MACHINE RENTAL

- Anesthetic machines and accessories are available to use in CMARC animal facilities at no cost.
- Laboratory personnel must provide their own isoflurane. Isoflurane can be purchased through the CMARC pharmacy (McIntyre room 1440, [drss@mcgill.ca](mailto:drss@mcgill.ca)).
- To rent the anesthetic machine, a Request for Anesthetic Machine Use form needs to be completed. The form is available on the CMARC website [www.mcgill.ca/cmARC](http://www.mcgill.ca/cmARC)
- Submit all requests by e-mail to [rts.arc@mcgill.ca](mailto:rts.arc@mcgill.ca) or in person to McIntyre room 1440 at least 5 working days prior to date required. An e-mail confirmation will be sent.
- The anesthetic machine must be returned with a full isoflurane reservoir. If the reservoir is not full at the time the machine is returned, the cost of isoflurane will be charged directly to the account provided on the request form.
- The anesthetic machine and its accessories should be returned in their original state. If material is found to be missing or damaged, it will be charged directly to the account provided on the request form.
- If the machine is to be used in a BSL2 room, charges for the disinfection of the anesthetic machine and gas sterilization of its components will be assumed by the user.