LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 3

THE LABORATORY RODENT

Anesthesia and Analgesia

Analgesia:

1. Definition of Analgesia and Pain
2. General Considerations
3. Pain Recognition and Assessment
4. Classes of Analgesic Drugs
5. Analgesia Plan

Anesthesia:

1. Pre-anesthetic Considerations
2. Animal Monitoring
3. Gas Anesthesia
4. Injectable anesthesia

ANALGESIA

1. DEFINITION OF ANALGESIA AND PAIN

1.1. What is 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].

1.2. What is Analgesia?

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

2. GENERAL CONSIDERATIONS

- 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 form must be justified by the investigator and approved by the appropriate Facility Animal Care Committee (FACC).

3. PAIN RECOGNITION AND ASSESSMENT

- 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 do not rule out pain or distress, but the presence of clinical signs is indicative of pain or distress:
- 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

[1] This often quoted definition was first published in 1979 by IASP in *Pain* journal, number 6, page 250. It is derived from a definition of pain given earlier by Harold Merskey: "An unpleasant experience that we primarily associate with tissue damage or describe in terms of tissue damage or both." Merskey, H. (1964), *An Investigation of Pain in Psychological Illness*, DM Thesis, Oxford.

- 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

4. CLASSES OF ANALGESIC DRUGS

4.1. General Analgesics

· Opioids

Opioid agents will mostly have an action on the pain receptors of the brain and the spinal cord.
Ex: Buprenorphine

· Non-Steroidal Anti-Inflammatory Drugs

Non-steroidal anti-inflammatory drugs are also known as NSAIDs are potent analgesics acting on the central nervous system. They will also help to reduce inflammation which contributes to pain.
Ex: ketoprofen (Anafen®), carprofen (Rimadyl®), meloxicam (Metacam®)

Mouse/Hamster

Analgesic	Dose	Route	Frequency	Note
*Buprenorphine	0.05–0.1 mg/kg	SC, IP	4–8 hr.	Controlled drug.
*Carprofen	5-10 mg/kg	SC, PO	12–24 hr.	Should not be used if there is impaired liver and/or kidney function
Ketoprofen	2–5 mg/kg	SC	12-24 hr.	Should not be used if there is impaired liver and/or kidney function, or coagulation disorders [2].

Rat

Analgesic	Dose	Route	Frequency	Note
*Buprenorphine	0.01–0.05 mg/kg	SC, IP, IV	4–8 hr.	Controlled drug.
*Carprofen	5-10 mg/kg	SC, PO	12–24 hr.	Should not be used if there is impaired liver and/or kidney function
Ketoprofen	2–5 mg/kg	SC, PO	12–24 hr.	Should not be used if there is impaired liver and/or kidney function, or coagulation disorders[2].

*most commonly used

[2] Compendium of Veterinary Products, 6th Ed. 1999. Canadian Animal Health Institute. North American Compendiums, Hensall, Ontario Pain Management in Animals. 2000 Flecknell P and Waterman-Pearson A (eds). WB Saunders, London 184pp. Laboratory Animal Anesthesia. 1996. Flecknell P. Academic Press, London. 274 pp.

4.2. Local Analgesics

- Are applied topically to prevent pain at the skin level.
  Ex : Lidocaine, bupivacaine, EMLA® cream

- Infiltrate or apply local analgesic to areas where a painful stimulus may be induced.
  Ex: Apply lidocaine/bupivacaine mixture prior to closing an incision.

- Repeat application of local agent at specified intervals to maintain analgesia. In some cases, a sedative is recommended when using local analgesia alone without the use of anesthesia.

Mouse/Rat/Hamster

Analgesic	Dose	Duration	Note
Lidocaine	< 2 mg/kg	30–60 min.	Because these drugs are acidic, it is recommended to dilute it 3:1 with sodium bicarbonate solution. · Dilution must be prepared immediately before use and should not be stored. · Diluted solution is as effective but induction of analgesia is slightly prolonged. · Dilution with sodium bicarbonate is not necessary if lidocaine is to be administered to an anesthetized animal.
Bupivacaine	< 2 mg/kg	3–4 hr.	Same precautions as for lidocaine.
* Lidocaine-bupivacaine mixture	-	30–60 min.	Same precautions as for lidocaine and bupivacaine. Combining both drugs allows for rapid induction and prolonged effect.
EMLA® cream	Thick spread	30–60 min.	Should be applied on the skin at least 45 minutes before procedure. Site should be covered with an occlusive dressing. Ex: plastic wrap

*most commonly used

5. ANALGESIA PLAN

- If possible, provide analgesia before the painful stimulus, as it is more effective in preventing pain.
  Ex: give 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).

ANESTHESIA

1. PREANESTHETIC CONSIDERATIONS

1.1. 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. Ex: respiratory infections.
- Gastrointestinal problems that can lead to dehydration. Ex: diarrhea.

1.2. 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. However, if a surgery needs to be performed on the stomach, the animal could be fasted 3 to 4 hours prior to the procedure.

1.3. 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 disk: under the animal cage or in indirect contact with the animal.

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

1.4. 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 anesthesia for survival procedures. Isotonic fluids can be warmed prior to administration.

1.5. Eye Lubrication

Since the rodent's eyes remain open during anesthesia, it is important to lubricate them with a sterile ophthalmic ointment.

1.6. Analgesia

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

2. MONITORING DURING ANESTHESIA

2.1. Reflexes

- Palpebral reflex: 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: stimulated by pinching the skin between the toes and/or toe pads using, for example, mosquito hemostats. The pinch needs to be strong enough to create a painful stimulus in order to evaluate the depth of anaesthesia. Under general anaesthesia, this reflex becomes weaker as the depth of anesthesia increases. It disappears completely at the 3rd stage of anesthesia (animal is anesthetised deeply enough to undergo surgery).

2.2. Respiration

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

2.3. Heart Rate

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

2.4. Mucous Membrane

- Assessed by observing the colour of the mucous membrane of the nose and the mouth. Their colour should remain pink.

2.5. Temperature

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

3. GAS ANESTHESIA

3.1. 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.

3.2. Disadvantages

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

3.3. 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:
  - Place the animal in the induction chamber.

    Note: Do not overload the induction chamber.

    Mice: can be placed with their cagemates in the chamber.

    Rats: place individually in the chamber.

    Keep the induction chamber clean to minimize odors that might distress animals subsequently anesthetized.
  - Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.
  - Adjust the isoflurane vaporizer between 3% to 5%.
  - Once the animal looses consciousness, decrease the isoflurane concentration to the maintenance level.

- Maintenance:
  - Use the mask connected to the Bain circuit.
  - Adjust the flowmeter to 0.4 to 0.8mL/min.
  - Adjust the isoflurane vaporizer between 2 to 2.5%.
  - Asses the depth of anesthesia by testing the pedal reflexes of the animal by pinching the toe pads with a pair or forceps.
  - Adjust the isoflurane vaporizer according to the desired depth of anesthesia.

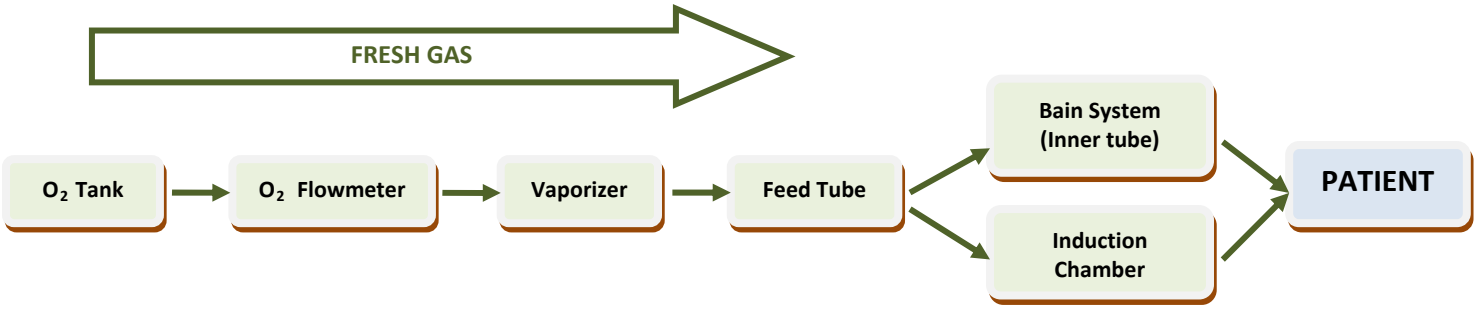
- Recovery:
  - Turn off the isoflurane vaporizer but keep the animal on oxygen.
  - Transfer the animal to its cage once it begins to move and allow to recover fully (sternal position).

    Note: Never leave an anesthetized animal unattended.

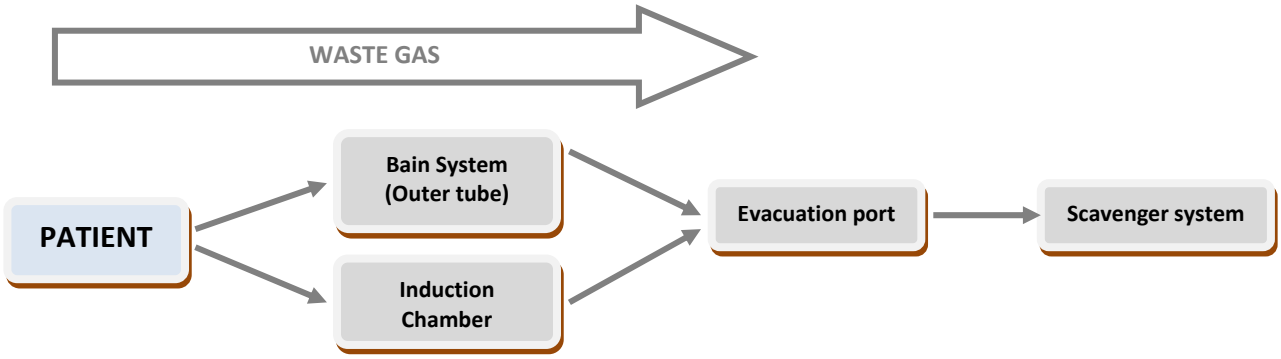
3.4. In Case of Emergency (respiratory/cardiac arrest):

- Stop the isoflurane administration (set vaporizer to 0).
- Keep the animal under 100% oxygen and compress the chest (gently) rapidly
- Stimulate the animal by pinching a toe or inserting a 25G needle in between the two nostrils.

3.5. Flow of Gases



The oxygen contained under pressure in the tank will be brought in the oxygen flowmeter and delivered accordingly in litres per minute. Then, once the vaporizer is open, the liquid anesthetic is converted into gas. The flow of oxygen will then pass inside in order to bring a controlled amount of anesthetic gas in the feed tube. The flow of fresh gas (oxygen mixed with anesthetic gas) will pass trough the induction chamber or the inner tube of the Bain system to finally reach the patient.



The patient will exhale the waste gas 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 trough the scavenger system.

3.6. Anesthetic Machine Components

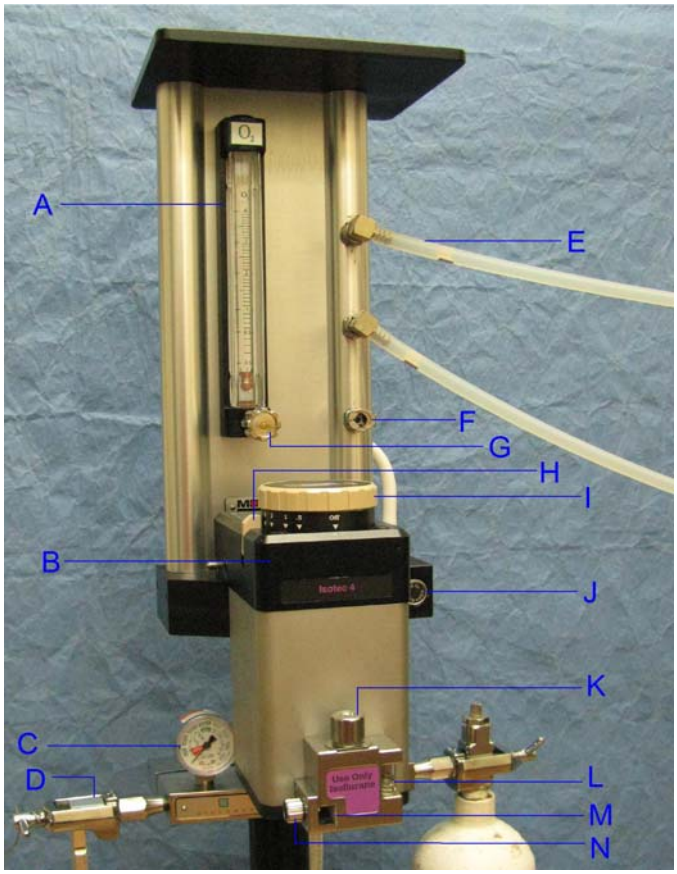


Figure 1 (mobile anesthetic machine)

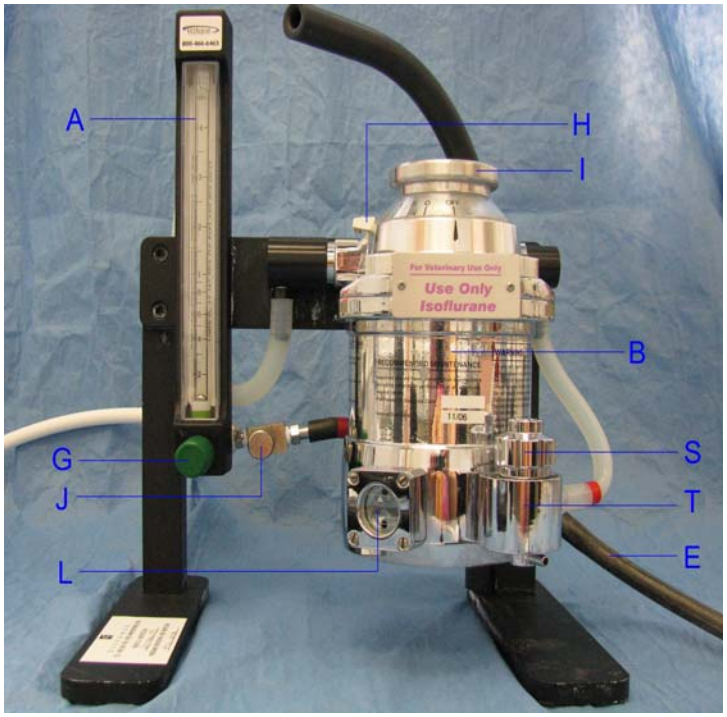


Figure 2(table-top anesthetic machine)

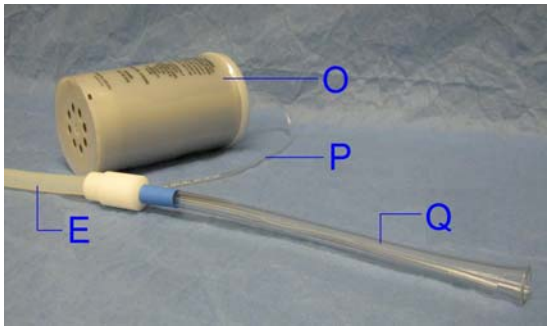


Figure 3

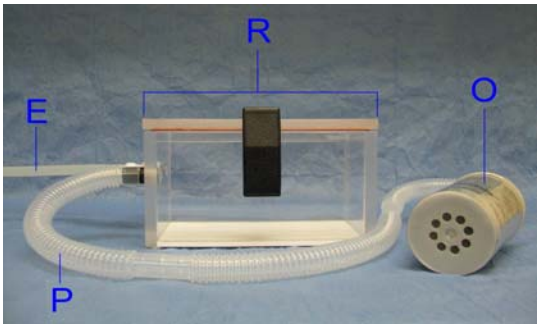


Figure 4

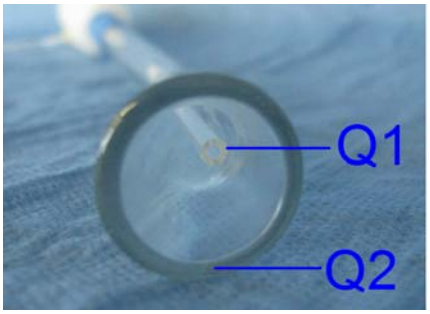
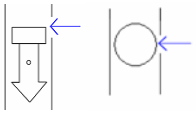


Figure 5

Figure 6

Location	Figure	Component	Description
A	1,2	Oxygen Flowmeter	Determines the amount of oxygen delivered to the patient in litres per minute.
B	1,2	Vaporizer	Converts liquid anesthetic to a gas and adds controlled amounts of vaporized anesthetic to oxygen .
C	1	Tank Pressure Gauge	Indicates the pressure of gas remaining in the tank.
D	1	Tank Holder or Yoke	Holds the oxygen tank in place.
E	1,2,3,4	Feed-tube	Delivers the anesthetic gas to the animal
F	1	Optional Attachment Site	Allows the use of up to 3 components (feed tubes or induction chambers) on the same anesthetic machine..
G	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).  Fig A Fig B
H	1,2	Release Button	Unlocks the control dial on the vaporizer
I	1,2	Control Dial	Graduated in percent concentration from 1% to 5%. Controls the amount of isoflurane gas delivered to the patient.
J	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.
K	1	Valve Knob	When opened, allows filling of the vaporizer.
L	1,2	Sight Glass	Indicates the level of liquid anesthetic in the vaporizer. (isoflurane)
M	1	Filling Tunnel	Point of insertion of the keyed block of the liquid anesthetic bottle adaptor.
N	1	Retaining Screw	Locks the keyed block in place for the filling of the vaporizer.
O	3,4	Charcoal Filter Canister	Remove waste anesthetic gases from the room environment. Note: refer to the instructions on the filter canister
P	3,4	Evacuation Port	Transports the waste anesthetic gases
Q	3,5,6	Bain system (Non-Rebreathing Circuit)	Supplies fresh gas (isoflurane mixed with oxygen) to the patient through the inner tube (Q1). Directs exhaled gas from the patient to the evacuation system (filter) by the outer tube (Q2).
R	4	Induction Chamber	Used for anesthetic induction of the patient.
S	2	Filler Cap	Provides access to the funnel filler.
T	2	Funnel Filler	Allows the liquid anesthetic to be poured into the vaporizer for filling.
U	1	Oxygen Tank	Contains oxygen under pressure

3.7. Filling of Anesthetic Vaporizer

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

3.7.1. 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 trough 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

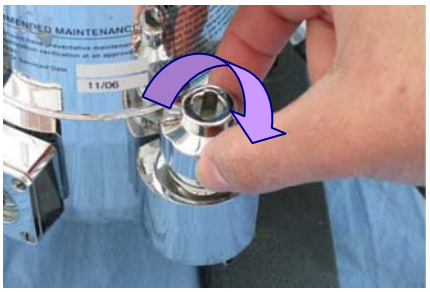


Figure 3

3.7.2. 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 at a lower level 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

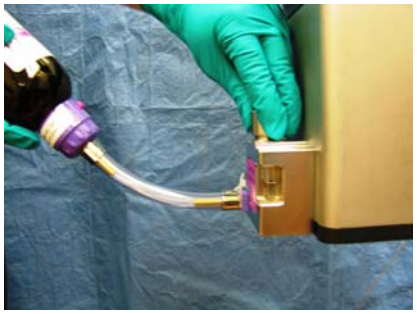


Figure 3

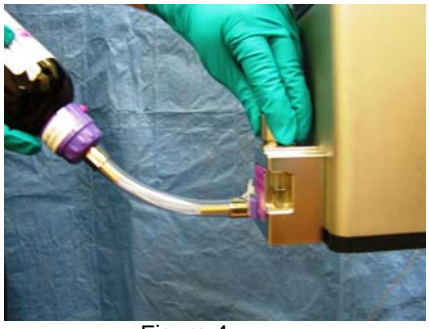


Figure 4

Figure 5

3.8. 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 appropriately sized anesthetic mask to ensure a tight seal around the animal's face and prevent leaks.
  - Clean the induction chambers with soap and water 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 a 50 gram increase.

4. INJECTABLE ANESTHESIA

4.1. Advantages

- No equipment is required.

4.2. 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.Ex.: 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.

4.3. Procedure for Ketamine/Xylazine/Acepromazine Anesthesia (Rodent Cocktail)

- Anesthetic dose: ketamine 50mg/kg, xylazine 5mg/kg, acepromazine 1mg/kg.
- 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 “Rodent 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.

  - For mice:
    - In a sterile vial or bottle with a rubber stopper, mix:

      0.5mL of ketamine (100mg/mL)
      0.25mL xylazine (20mg/mL)
      0.1mL acepromazine (10mg/mL)
      9.15mL 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 0.1mL/10g body weight intraperitoneally.

- Cocktail administration:
  - Weigh the animal prior to drug administration.
  - An animal's response to injectable anesthetics can vary by strain. If you are unsure of the animal's sensitivity to anesthetic drugs, first administer 75% of the calculated dose and asses 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.
  - Duration of anesthesia is approximately 30 minutes.
  - After 30 minutes, a half dose may be re-administered as needed.

- Recovery:
  - Provide a source of heat to the animals until full recovery such as a heating disc, warming pad or warm-water circulating pad (do not use electric heating pads).
  - 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.

4.4. In Case of Emergency (respiratory/cardiac arrest):

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