

Adult Rodent Anesthesia SOP

1. Purpose

This Standard Operating Procedure (SOP) describes methods for anesthetizing adult rats and mice. This SOP follows the UBC and CCAC guidelines for the care of anesthetized rodents.

2. References:

Associated Guidelines and SOP's can be found on the ACC SOP/Guidelines website:
<https://animalcare.ubc.ca/planning-your-research/sops-guidelines>

TECH 10 - IP injection in mice and rats

TECH 11 – Subcutaneous injection in mice and rats

UBC Animal Care Centre Rodent Anesthesia and Analgesia

Policy # 16 Survival Surgery of Rodents

Policy # 17 Monitoring of Animals Used for Research, Teaching and testing

Surgical Class and Analgesia Guidelines

Rodent Anesthesia and Analgesia Formulary and General Drug Information

Physiological normal values

(Depends on age, strain, health status, type of anesthesia, etc.)

Rats		Mice	
Normal core body temperature	37.5 – 38.5°C	Normal core body temperature	37.0 – 38.0°C
Normal heart rate range	260 – 600 bpm	Normal heart rate range	350 - 800 bpm
<i>Under anesthesia</i>	<i>250 - 400 bpm</i>	<i>Under anesthesia</i>	<i>400 - 600 bpm</i>
Normal respiratory rate range	75 - 120 bpm	Normal respiratory rate range	90 - 250 bpm
<i>Under anesthesia</i>	<i>60 – 90 bpm</i>	<i>Under anesthesia</i>	<i>80 - 120 bpm</i>
Pulse oximetry (blood oxygen saturation)	>95%	Pulse oximetry (blood oxygen saturation)	>95%

Heart rates under anesthesia can be lower than above values if Xylazine or Dexmedetomidine are used.

3. Responsibility

- 3.1. Principal Investigators (PIs) and their research staff and animal and veterinary care staff.
- 3.2. All animal users anesthetizing rodents must have successfully completed the UBC (or equivalent) Introduction to Working with Rodents in Research, Restraint, SQ and IP injection courses and the Rodent Anesthesia course.

4. Key Components of Anesthesia

- 4.1. Rodents **should not** be fasted (food deprived) prior to anesthesia. In rodents, fasting prior to anesthesia leads to dehydration.
- 4.2. Rodents can be anesthetized with inhalant gas, injectable drugs or a combination of both.
- 4.3. Heat loss is rapid in anesthetized rodents. Providing a safe supplemental heat source is critical to maintaining normal body temperature during anesthesia and until animal has recovered from anesthesia. In addition, heat loss can be minimized by providing heat support during induction of anesthesia, covering anesthetized rodents and protecting them from contact with cool surfaces.
- 4.4. Never leave an anesthetized animal unattended. Monitor animal until it has recovered from anesthesia and is able to move around the cage normally.

5. Materials

- 5.1. Safe heat sources: homeothermic feedback blanket, warm-water circulating pad, or infrared heater. Electrical heating pads can be used but the surface temperature must be measured before and during use to prevent burns. Some form of barrier (e.g. cloth) should be placed between the heating pad and patient.
- 5.2. Corneal protectant (e.g.: lacrilube, isoptotears, TearGel, etc.)
- 5.3. Accurate animal weigh scale
- 5.4. Calculator
- 5.5. 70% Isopropyl alcohol
- 5.6. Warmed sterile SQ fluids (i.e. 0.9% saline or lactated ringer's solution) if anesthesia is expected to last more than 10 minutes.
- 5.7. Appropriately sized rectal thermometer if anesthesia is expected to last more than 10 minutes (typically, if the probe diameter is smaller than the diameter of the animal's normal fecal pellet diameter, it can be safely used)
- 5.8. Appropriately sized needles and syringes for size of animals (See TECH 11 SQ injection in mice and rats and TECH 10- IP injection in mice and rats).
- 5.9. Additional monitoring equipment if available – pulse oximeter, capnograph (CO₂ monitor)
- 5.10. Warmed recovery cage with paper towel covering bottom. No loose bedding should be present until animals are fully recovered from the anesthetic.
- 5.11. Easily accessible food (rodent pellets) or gel food/treats (e.g. Nutragel, Recovery gel, etc.) and water or gel water replacement (e.g. Transgel, Napanectar, Hydrogel, etc.).
- 5.12. Appropriate analgesics when surgery is planned (see Policy # 16 Survival Surgery of Rodents, Surgical Class and Analgesia Guidelines and Rodent Anesthesia and Analgesia Formulary and General Drug Information).
- 5.13. Cage flags, procedure log and/or monitoring sheets (see Policy # 17 Monitoring of Animals Used for Research, Teaching and Testing).

5.14. For Gas (Isoflurane) Anesthesia – 5.1-5.13 plus:

- 5.14.1. Isoflurane
- 5.14.2. Calibrated Isoflurane machine (precision vaporizer, flow meter, oxygen)
- 5.14.3. Anesthetic delivery tubing – a non-rebreathing circuit such as a Bain's or Mapleson circuit is typically used
- 5.14.4. Adequate scavenging for waste gases (active scavenging or charcoal filter (weighed prior to use to determine if using a charcoal scavenge canister – follow manufacturers' directions for when to discard).
- 5.14.5. Appropriately sized warmed induction chamber (transparent so rodent can be viewed). The chamber should allow the animal some room to move and turn around so that the anesthetist can determine when the righting reflex is lost.

Example: Mice → 1 litre chamber

Rats ≤ 400 gm. → 2 litre chamber

Rats > 400gm → 2.5-3 litre chamber.

- 5.14.6. Appropriately sized nose cone that fits snugly over just the nose without covering eyes.

5.15. For injectable anesthesia - 5.1 - 5.13 plus:

- 5.15.1. Injectable anesthetic(s) of choice in sterile multiuse vials with an expiry date (see referenced UBC Animal Care Centre Rodent Anesthesia and Analgesia)
- 5.15.2. Appropriately sized warmed cage with a paper towel covering the cage floor
- 5.15.3. Oxygen support if available.

6. Isoflurane Anesthesia Procedure

6.1. Induction:

- 6.1.1. Ensure Isoflurane vaporizer is full of Isoflurane (to “fill line”) and has oxygen supply connected and open.
- 6.1.2. Perform leak test of Isoflurane vaporizer to ensure all tubing is connected properly and there are no cracks/leaks in any component.
- 6.1.3. Gently remove rodent from home cage and weigh using accurate weigh scale. Record weight on anesthesia log.
- 6.1.4. Place induction chamber on or under safe heat source to pre-warm and provide heat support while animal is being induced.
- 6.1.5. Connect fresh gas flow tubing to induction chamber and ensure that scavenge tubing is also properly connected to the scavenge device to remove waste gases.
- 6.1.6. Place animal in warmed induction chamber which is lined with paper towel (clean the induction chamber between animals).
- 6.1.7. Turn oxygen flow meter to 1-2 L/min flow rate. Typically the O₂ flow rate for induction is the same as the volume of the chamber. I.e.: for a 1 Litre chamber, the O₂ flow rate would be 1 L/min. For chambers larger than 3 Litres, the O₂ flow rate can be 2L/min so that the oxygen flow into the chamber does not cool the animal down too quickly or create a “wind tunnel” effect in the chamber.
- 6.1.8. Begin the flow of isoflurane. Isoflurane exposure can be aversive so begin the flow rate at 0.5% to begin with and slowly increase up to 5% over approximately 5 minutes.
- 6.1.9. 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 O₂ flow rate up to 4 L/min for 2-3 minutes).
- 6.1.10. Open induction chamber and gently remove rodent while supporting the entire body and keeping it level. Do not pick up and hang by tail.

6.2. Maintenance:

- 6.2.1. Immediately transfer animal to non-rebreathing circuit with appropriately sized nose cone. Turn oxygen flow meter to 0.5-1 L/min (depending on what the vaporizer has been calibrated to) and turn Isoflurane vaporizer dial to 2-3%. Ensure scavenging is adequate. **Neonatal rodents typically need much higher Isoflurane % to maintain a surgical plane of anesthesia (4-5%). It is vital to keep neonates warm and hydrated or they will stop breathing under anesthesia.
- 6.2.2. Place animal on/under heat source. Lubricate and gently place rectal probe into rectum of animal (approximately 1 cm in adult mice up to 2 cm in adult rats). Tape probe to tail if necessary to keep in place. Ensure animal cannot overheat or burn.
- 6.2.3. Place corneal protectant into both eyes (be generous).
- 6.2.4. Administer any required analgesics if performing surgery or a painful procedure.
- 6.2.5. Record all drugs administered on anesthesia log.
- 6.2.6. Administer warmed fluids SQ. Give up to 20 ml/kg SQ initially if animal is having surgery or anesthesia is expected to last >10 minutes.
- 6.2.7. 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 anesthesia.
- 6.2.8. Routinely throughout anesthetic procedure (i.e. at least every 10 minutes), check animal's breathing pattern/rate, temperature, surgical plane (toe pinch, blink reflex) and colors of extremities. Adjust anesthetic level and heat support to ensure animal remains at surgical plane of anesthesia if surgery is being performed and at physiologically normal levels.

6.3. Recovery:

- 6.3.1. Turn the Isoflurane to 0% but keep oxygen flow running.
- 6.3.2. Administer additional SQ fluids (10 ml/kg) if procedure lasted more than 1 hour, there was blood loss or if surgery may prevent animal from eating and drinking normally after procedure.
- 6.3.3. Remove rectal probe from animal but keep on heat source.
- 6.3.4. Place more corneal protectant in eyes.

- 6.3.5. Monitor animal continuously until it begins to move on its own.
- 6.3.6. Once animal begins to move, remove from nose cone and place (by itself) in pre-warmed recovery cage (lined with paper towels – no bedding) and continue to provide heat support. Monitor frequently (at least every 10 minutes) until animal is moving about cage and grooming.
- 6.3.7. Place animal back into normal home cage (with normal bedding).
- 6.3.8. Provide food pellets or gel food/treats and supplemental water source (i.e. Transgel, Hydrogel) on floor of cage, especially if prolonged or painful procedure performed).
- 6.3.9. Place cage flag card on cage to indicate what procedure animal had performed and all drugs administered.
- 6.3.10. Ensure all monitoring sheets appropriate to procedure are completed according to approved animal care protocol.

7. Injectable anesthesia

7.1. Induction:

- 7.1.1. Weigh animal using accurate weigh scale and record weight on anesthesia record
- 7.1.2. Calculate drug dosage volume based on concentration of drug, animal's weight and drug dosages.

Volume of drug to inject (ml) = (animal's weight (in kg) x drug dosage (mg/kg)) / drug concentration mg/ml

Remember: 1 gm = 0.001kg

- 7.1.3. Disinfect the top of the drug vial with 70% isopropyl alcohol wipe and draw up the calculated dose in an appropriately sized syringe/needle.
- 7.1.4. Record all drug doses administered on anesthesia record.
- 7.1.5. Place clean cage lined with paper towel (no loose bedding) onto/under a safe heat source to pre-warm.
- 7.1.6. Gently restrain animal and inject drug(s) via proper route (typically either SQ or IP).
- 7.1.7. Place animal back into warmed cage and monitor closely until it has lost its righting reflex.

7.2. Maintenance and Monitoring:

- 7.2.1. Gently remove rodent while supporting the entire body and keeping it level. Do not pick up and hang by tail. Place animal on/under safe heat source.
- 7.2.2. Lubricate and gently place rectal probe into rectum of animal (approximately 1 cm in adult mice up to 2 cm in adult rats). Tape probe to tail if necessary to keep in place. Ensure animal cannot overheat or burn.
- 7.2.3. Place corneal protectant into both eyes (be generous).
- 7.2.4. Administer any required analgesics if performing surgery or a painful procedure.
- 7.2.5. Record all drugs administered on anesthesia log.
- 7.2.6. Administer warmed fluids SQ. Give up to 20 ml/kg SQ initially if animal is having surgery or anesthesia is expected to last >10 minutes.
- 7.2.7. Check breathing pattern, color of extremities, temperature and toe pinch response.
 - 7.2.7.1. If extremities are bluish, administer supplemental oxygen via nose cone
- 7.2.8. Routinely throughout anesthetic procedure (i.e. at least every 10 minutes), check animal's breathing pattern/rate, temperature, surgical plane (toe pinch, blink reflex) and colors of extremities. Adjust anesthetic level and heat support to ensure animal remains at surgical plane of anesthesia if surgery is being performed and at physiologically normal levels.
- 7.2.9. If animal is not reaching surgical plane of anesthesia or begins to "lighten" the plane of anesthesia before the procedure is complete, either inject additional analgesics (keeping in mind maximum doses), inject additional anesthetic drugs (start at 1/3 original calculated dose keeping in mind maximum safe dose), and/or begin administering Isoflurane via nose cone and precision vaporizer.

7.3. Recovery:

- 7.3.1. Continue supplemental oxygen, if available.

- 7.3.2. Administer additional SQ (10 ml/kg) fluids if procedure lasted more than 1 hour, there was blood loss, Xylazine or Dexmedetomidine was used, or if procedure may prevent animal from eating and drinking normally after procedure.
- 7.3.3. Remove rectal probe from animal but keep animal on/under heat source.
- 7.3.4. Place more corneal protectant in eyes.
- 7.3.5. Monitor animal continuously until it begins to move on its own.
- 7.3.6. Once animal begins to move, place in pre-warmed recovery cage by itself (lined with paper towels, no loose bedding) and continue to provide heat support. Monitor frequently (at least every 10 minutes) until animal is moving about cage and grooming.
- 7.3.7. Place animal back into normal home cage (normal bedding; provide food pellets or gel food/treats and supplemental water source (i.e. Transgel, hydrogel) on floor of cage, especially if prolonged or painful procedure performed).
- 7.3.8. Place cage flag card on cage to indicate what procedure animal had performed and all drugs administered.
- 7.3.9. Ensure all monitoring sheets appropriate to procedure are completed according to approved animal care protocol.