Standard Operating Procedures	
UNIVERSITY OF GUELPH **Animal Care Committee (ACC)**	Title: **Rodent Survival Anesthesia - Inhalant**
	SOP.ACC. 817. Rodent Survival Anesthesia-Inhalant **Approval Date: November 10, 2023** **Revision Date: October 9, 2024**

1. ***Purpose:*** To provide instructions for performing inhalant anesthesia during survival procedures on rodents and to meet or exceed CCAC standards.

2. ***Responsibilities:*** Animal care staff, veterinarians, and trained individuals listed on an approved Animal Utilization Protocols (AUPs). All animal users performing procedures in animals must have successfully completed Mouse/Rat A/B training courses and any relevant surgery/anesthesia training. Anyone performing anesthesia independently must have been certified by an Animal Care Services veterinarian.

3. ***Introduction:*** Anesthesia is defined as "loss of sensation". Anesthesia can be produced either by blocking sensation to a particular region of the body (local or regional anesthesia), or by administration of drugs that produce loss of consciousness (general anesthesia). General anesthesia is used to prevent, pain, or movement during experimental procedures such as imaging and surgery. The depth of anesthesia and the choice and dose of anesthetic agent may vary depending upon which of these aims is to be achieved; this document describes the use of the inhalant anesthetic isoflurane.

   Any anesthesia in rodents must be outlined in an approved Animal Utilization Protocol (AUP). Any deviations from this policy must be detailed in the protocol and approved by the Animal Care Committee.

   For detailed instructions on set-up of the inhalant anesthetic machine and preparing for surgical procedures, see CAF.342 Isoflurane Anesthetic Machine Set up and Operation and **SOP.ACS.818.** Asepsis for Rodent Survival Surgery, respectively. A list of potential sources for described equipment is available on request from ACS Veterinarians or the Central Animal Facility.

4. Procedures

For a checklist of major steps associated with preparing, maintaining, and recovering an animal from anesthesia, see Appendix 1.

ANESTHETIC ENVIRONMENT:

- Anesthesia should be conducted in dedicated facilities or spaces (dedicated at the time of the procedure).
- Traffic and activities should be minimized in the room or area at the time of the procedure
- Conduct anesthesia on a table that is impervious to liquids.
- If conscious animals will be transported to the anesthetic area, the cages should be kept in a quiet area of the room and covered with a drape.

PREPARING FOR ANESTHESIA

Before an animal is anesthetized (or even transported to the anesthetic environment), both the animal and the equipment must be examined.

Steps related to the **animal** before anesthesia:

1. Acclimatization: where possible and applicable, animals should be acclimatized to syringe feeding to allow for voluntary oral analgesia, and positively habituated (i.e., with a food reward) to any restraint required for drug administration. This process needs to begin days – weeks before surgery.

2. Weight: a healthy, baseline weight should be taken 2-3 times during the week leading up to surgery to allow for accurate post-operative monitoring. Multiple weight measurements are preferable to a single measurement, as a rodent's weight can fluctuate based on timing of the last meal, urination, defecation etc.

3. Health check: rats and mice must be in good health before undergoing anesthesia. Externally, they should be bright, alert, and responsive with a smooth hair coat. Amongst other signs, a hunched posture, ruffled hair coat, poor body condition, decreased activity, or agitation are indicators that an animal is not healthy enough to be anesthetized.

4. Analgesia: depending on the analgesic of choice, the type of procedure, and the length of time between induction and any aversive procedures, it may be optimal to provide analgesia to an awake animal (vs. right after induction). Speak to an ACS veterinarian to determine an optimal analgesia protocol.

Equipment checks before anesthesia:

1. Set-up your anesthetic machine (see CAF.342 Isoflurane Anesthetic Machine Set up and Operation) next to a downdraft table so that the induction chamber and procedure area are on the surface of the table. In the case of surgery, plan for separate shaving and surgery areas (see SOP.ACS.818 Asepsis for Rodent Survival Surgery).
2. Check that all tubing connections between the anesthetic machine and (1) the induction chamber and (2) the procedure area are secure and sending gas to the correct place.
3. Open the oxygen (O_2 cylinder and check that it has sufficient oxygen: a full cylinder has about 137 barr of pressure (just under 2000 psi), which falls steadily as oxygen is used.
4. Check that the flowmeter can be opened and closed, and that oxygen is flowing.
5. Check that the vaporizer dial can be moved freely, and that the vaporizer window shows sufficient liquid anesthetic. Refill if needed and replenish when finished.
6. Check the weight of the F/AIR filters (one for each of the induction chamber and procedure area) and confirm they have not reached the discard weight. Record the new weight on the side of the filter.
7. If the machine has one, check that the oxygen flush valve is working correctly.
8. Prepare a recovery cage (and/or incubator): place a soft towel/fleece in a clean cage and place half of the cage on a supplemental heating device.
9. Check the temperature of the supplemental heating devices for the induction chamber, procedure area (+/- shaving area), and recovery cages. To be effective for rats and mice, the surface temperature (that will touch the animal) should be between 39 and 41˚C.
10. Draw up any fluid/analgesia injections +/- prepare soaps and solutions for the skin and place them on a heating pad.
11. If performing surgery, prepare the sterile field and instrument area and cover equipment touch surfaces as described in SOP.ACS.818 – Asepsis forRodent Survival Surgery.

INDUCTION AND INITIAL MAINTENANCE OF ANESTHESIA

1. Place the induction chamber on a heat pad/puck and allow to warm. Place something soft (e.g., towel/fleece) on the floor of the chamber. Paper towel offers no padding and is not preferred.
2. Place the animal in the induction chamber and adjust the oxygen flow to 1.5 – 2 L/min. Pre-oxygenate the animal for a minimum of 2 minutes.
3. Adjust the vaporiser to 4-5%.
4. Once the animal is recumbent, gently tip the induction chamber to confirm the righting reflex is lost. Wait until the animal's breathing has slowed further, which usually takes an additional 10-30 seconds. Open the flow of oxygen and isoflurane to the nose cone to allow the tubing to pre-fill (especially important for long tubing).

5. Transfer the animal to a nose cone/face mask on a homeothermic heat pad and turn off the flow of oxygen and isoflurane to the induction chamber.
6. Ensure the animal's snout is firmly seated in the nose cone with a good seal. Mice and rats are obligate nose breathers – only the nose needs to be seated in the cone. Ensure the nose cone is not contacting the ocular surface.
7. Adjust the vaporiser initially to 1-2.5% (based on the depth of anesthesia required for the procedure, analgesic protocol, and variation between individuals/strains).
8. Adjust oxygen flow to 0.8 – 1 mL/min.
9. Apply ophthalmic ointment to eyes; non-ophthalmic lubricants such as mineral oil, Vaseline, etc. are not appropriate.
10. Ensure the animal's head, limbs, and spine are in a neutral position (elevate the heat pad/nose cone as needed). The head should be level with the spine and the limbs should not be trapped under the body or in hyperextension or flexion.
11. Administer analgesics (local and systemic) & antibiotics if indicated in the protocol.
12. Administer WARMED SQ or IP fluids (saline or lactated Ringer's solution, syringe or bag placed on a heating pad) at 10-20 mL/kg. Fluids can also be given post-operatively or split between a pre-operative and post-operative dose.
    - Adult rat: generally 5 -10 mL
    - Adult mouse: generally 0.5 -1 mL
13. Begin monitoring body temperature with a rectal thermometer. If possible, use a second temperature probe to monitor the heat pad temperature (can be signed out from the Central Animal Facility).
14. Begin monitoring oxygen saturation (SpO_2) with a pulse oximeter (can be signed out from the Central Animal Facility).

For a detailed description of aseptic preparation of the animal, the surgeon, and the surgical area for survival surgery, see SOP.ACS.818 – Asepsis for Rodent Survival Surgery. However, guiding principles of preparation of the animal for surgery to maximize anesthetic success include:

- Providing supplemental heat at every step: induction, clipping, prepping, surgery, recovery.
- Warming soaps and solutions on a heating pad before applying them to the animal.
- Clipping the minimum area of hair and applying the minimum amount of soap/solution necessary to prevent contamination of the surgical site (using Q-tips prevents soaking)
- Using a transparent, impermeable drape (such as GLAD Press'n Seal) to trap heat.
- Confirming a surgical plane of anesthesia with a withdrawal (toe pinch) reflex prior to starting surgery.

MAINTAINING ANESTHESIA

1. Observe core temperature, respiratory rate (RR), SpO_2, heart rate (HR), and colour (mucous membranes, feet, ears, nose, tail) every 3-5 minutes and record these parameters on an anesthetic monitoring sheet every 10 minutes (see Appendix 2).
    a. Check pedal withdrawal reflex if these parameters suggest a change in depth
    b. For RR, the number of breaths is counted over 15 sec and then multiplied by 4
2. Continue to adjust the vaporizer in 0.25-0.5 % increments based on monitoring parameters
    a. A higher % may be required at the beginning of surgery before analgesia has peaked in the bloodstream and during highly stimulating activities such as the initial incision(s) and changing animal positioning (e.g., from sternal to lateral).
    b. The use of pre-operative opioids generally decreases respiration rate and the % of isoflurane required to maintain anesthesia

	Temperature (rectal)	Surface temperature of heat pad	Respiratory rate (per min)	SpO_2	Heart rate (per min)	Mucous membrane colour
Mouse	37.5˚C	39 – 41˚C	60 – 80	>95%	300 – 600	Pink
Rat	37.5˚C	39 – 41˚C	60 – 80	>95%	300 – 350	Pink

Table 1. Normal vital parameters for mice and rats while under general anesthesia. Respiratory rate and heart rate can vary widely between strains and individuals; all parameters should be interpreted together to inform decision-making. Heart rate data is approximate in rodents and the trend is more important than the absolute number.

PROBLEM-SOLVING

If you observe decreasing body temperature, depressed respiration (consistently slow breathing at <50 BPM or gasping), SpO_2 consistently less than <95%, sudden jumps up or down in heart rate, or blood loss, then you need to act. Note that the use of drugs to restore stable cardiac rhythm and output requires considerable care and expertise. If such problems are anticipated, obtain advice from a veterinarian.

If body temperature is steadily decreasing:

1. Check the positioning of the rectal probe and ensure the reading is accurate.
2. Check the temperature of the heat pad and rewarm if necessary.
3. If not already in place, apply a layer of Press'n Seal to trap heat.
4. If temperature cannot be maintained above 35.8˚C, consider shortening the procedure.

If you observe respiratory depression, low SpO_2, or cyanotic (blue or grey) colouring:

1. Check the animal's airway is not obstructed: extend its head and neck, and if you suspect obstruction, swab the mouth with a cotton wool bud or use suction to clear the oropharynx.
2. Check that the breathing system, which will be delivering oxygen, is still connected to the anesthetic machine and to the animal and that the flowmeter is at 0.8 L/min or above.
3. Reduce the depth of anesthesia in small (0.25-0.5%) increments - but remember that if surgery is underway, you need to avoid the animal regaining consciousness.
4. Consider other possible causes of depressed respiration: is the surgeon restricting movement of the animal's chest (e.g., leaning on the thorax) or is the animal's spine not fully supported (e.g., animals that are "hanging" from the nose cone or ear bars with their front limbs dangling)?
5. If respiration stops, compress the chest gently with thumb and forefinger at a rate of about 1 compression per second. Stop if the animal inhales but be prepared to assist them again if needed.

If you detect signs of depressed or deteriorating cardiovascular function (e.g., progressive decline in heart rate, pale colouring) or anticipate deterioration secondary to blood loss:

1. Check whether the depth of anesthesia has increased or if the animal is developing hypothermia and correct as needed by reducing the % of isoflurane and/or rewarming the animal with a secondary heat source and a covering to trap heat (such as Press'n Seal).
2. Proactively address blood loss of more than 10% of circulating volume (conservatively 5 mL/kg for rats and mice)
    a. The degree of blood loss can be assessed easily by weighing the gauze used to absorb blood during the procedure and comparing it to the weight of a dry swab. The increase of weight in grams approximates the volume of blood lost in mL and indicates what volume should be replaced. IV or IP injection is preferred to SQ in that order. Whole blood is preferred but is usually not available.
    b. This volume is in addition to the volume of fluid administered to account for normal water and electrolyte loss (20-50 mL/kg).
3. If the heart stops (cardiac arrest), compress the chest gently with thumb and forefinger at the region over the heart at a rate of about 90 compressions/minute (slightly faster than once per second). Most rodents will not recover from cardiac arrest.

If you detect signs that your animal is at too light a plane of anesthesia (return of reflexes, HR suddenly increasing, limb movement):

1. Stop all surgical stimulation.
2. Check whether all tubing is connected and has no kinks, that the flowmeter is at a minimum of 0.8 L/min, and that the vaporizer window shows that there is sufficient isoflurane.

3. Check that the animal's nose has not become displaced from the nose cone.
4. Increase the % isoflurane.
    a. If the only sign is a mild positive toe pinch reflex +/- a mild increase in heart rate, increase isoflurane in 0.5% increments and monitor every 1-2 minutes until the reflex vanishes.
    b. If you are doing surgery and the toe pinch is a strong positive, the tail reflex returns, HR increases significantly, or you see movement, increase isoflurane by 1-3% and monitor closely. You will need to decrease isoflurane % again in the next few minutes to prevent respiratory depression with larger increases.
5. Once the animal has returned to a surgical plane of anesthesia, continue to monitor closely. Many animals require a higher % of isoflurane at the start of surgery or during stimulating activities but will become too deep if kept at this % for the entire procedure.

RECOVERY FROM ANESTHESIA:

1. Turn the isoflurane % to 0 but keep the flowmeter open to continue to oxygenate the animal.
2. Monitor the tail and toe pinch reflexes. Once positive, remove the temperature probe and pulse oximeter.
3. When the animal shows initial signs of waking (stiffening or movement of the limbs), remove the animal from the nose cone and place in the prepared recovery cage on its stomach or back, ensuring the head and neck are extended.
4. Watch the animal directly until it is awake and ambulating. The animal may still show signs of sedation.
5. Monitor every 3-5 minutes in the heated recovery cage/incubator until the animal is fully recovered (i.e., ambulating and acting normally).
6. If anesthetizing another animal, keep the flowmeter open and open the tubing to the induction chamber to flush any remaining isoflurane.
7. Return the animal to a cage with clean bedding but with nesting material from the previous cage (to retain familiar scents). Supplemental heat may still be beneficial.
8. Provide nutritional supplementation with food that is energy-dense and highly palatable (e.g., Clear H_2O gel cups, strawberry milkshake, Bio-Serv Bacon Softies) and offer a mash option of the animal's regular pelleted diet. Any novel item should be introduced prior to anesthesia.
9. Provide careful and regular monitoring for 3-7+ days (as per the assessment sheet and AUP), depending on the level of invasiveness of the procedure and whether sutures/staples remain.
10. Pay careful attention to other sources of stress: post-operative pain and dehydration, noise and light intensity, thermal comfort, social stress (social housing is the default).
11. Provide supportive care (fluids, heat, mash, nutritional supplementation) and contacting ACS veterinarians promptly if evidence of infection or illness is observed.

5. References

University of Texas San Antonio: Rodent Survival Surgery Policy
https://research.utsa.edu/_files/pdfs/compliance-integrity-pdf-folder/iacuc-forms/IACP-Policy-004-Rodent-Survival-Surgery-Review-approved-11-12-2021.pdf
Advanced Anesthesia courses *[MOOC].* Research animal training. https://researchanimaltraining.com/courses/

Appendix 1 – Checklist to Prepare for Anesthesia

Before anesthesia:

☐ Dedicated room/area for the procedure, where clutter, noise, and activity are minimized, and surgical area is away from drafts/vents.

☐ Cages with conscious animals draped for transportation.

☐ Animal appears externally healthy and baseline weight recorded.

☐ Analgesia provided (if being given to the awake animal)

☐ Induction chamber and procedure area on a down draft table and scavenge canister(s) weighed.

☐ Tubing connections are secure, and gas is being sent to the correct place

☐ Isoflurane and oxygen levels adequate and are flowing when the flowmeter is opened +/- oxygen flush valve working correctly.

☐ Fluids/analgesia drawn up, soaps and solutions prepared, and both placed on a heating pad.

☐ Sterile field, instrument area, and equipment prepared as needed for aseptic surgery.

☐ Induction chamber clean, warmed, and floor covered with a towel or fleece.

Steps for induction

☐ Pre-oxygenate animal in induction chamber at 1.5 – 2 L/min oxygen for 2-3 minutes.

☐ Induce animal with 4-5% isoflurane.

☐ Check for righting reflex and watch for decreased rate of respiration.

☐ Switch flow of oxygen and isoflurane from chamber to nose cone.

☐ Transfer animal from chamber to nose cone.

☐ Check reflexes and reduce vaporizer to 1-2.5% and oxygen to 0.8 – 1 L/min.

☐ Apply ophthalmic ointment.

☐ Administer analgesics.

☐ Administer warmed fluids (0.5 – 1 mL baseline for mice, 5 – 10 mL baseline for rats).

☐ Attach temperature probe and pulse oximeter.

Maintenance

☐ Observe core temperature, respiratory rate (RR), SpO_2, heart rate (HR), colour (mucous membranes, feet, ears, nose, tail) every 3-5 minutes and record these parameters on an anesthetic monitoring sheet every 10 minutes. Check pedal withdrawal reflex if other parameters suggest changes in depth.

☐ Continue to adjust the vaporizer in 0.25-0.5 % increments based on monitoring parameters.

Recovery

☐ Turn the isoflurane % to 0 but keep the flowmeter open to continue to oxygenate the animal.

☐ Monitor the tail and toe pinch reflexes. Once positive, remove the temperature probe and pulse oximeter.

☐ When the animal shows initial signs of waking (stiffening or movement of the limbs), remove the animal from the nose cone and place in the prepared recovery cage on its stomach or back, ensuring the head and neck are extended. Move cage to incubator if using.

☐ Watch the animal directly until it is awake and ambulating. The animal may still show signs of sedation.

☐ Monitor every 3-5 minutes in the recovery cage until the animal is fully recovered (i.e., ambulating and acting normally)

☐ Return the animal to a cage with clean bedding but with nesting material from the previous cage (to retain familiar scents)

☐ Provide nutritional supplementation with food that is energy-dense and highly palatable (e.g., Clear H_2O gel cups, strawberry milkshake, Bio-Serv Bacon Softies) and offer a mash option of the animal's regular pelleted diet.

☐ Provide careful and regular monitoring for 3-7+ days (as per the assessment sheet and AUP), depending on the level of invasiveness of the procedure and whether sutures/staples remain.

Before the next animal

☐ Purge isoflurane from the induction chamber by allowing oxygen alone to flow through for 1-2 min.

☐ Replace towel in induction chamber if soiled or if animal will be from a different cage.

☐ Wipe rectal probe with alcohol swab and re-check temperature of heat pads.

☐ Check isoflurane and oxygen levels and F-air canister weight if previously borderline.

APPENDIX 2 – TEMPLATE ANESTHESIA RECORD

AUP #:	**Investigator:**	**Student:**
Animal ID:	**Pre-surgical Weight:**	**Veterinarian:** ACS VETS **Email:** acsvets@uoguelph.ca

Surgeon:
Procedure date and time:

Pre-op analgesic therapy (drug/dose/route/time)
1. NSAID:
2. Opioid:
3. Local:

Pre-op checklist:
- ☐ Check Iso and O_2 levels
- ☐ Weigh F-air
- ☐ Check temperature of heat pad
- ☐ Pre-oxygenate animal
- ☐ Eye lube
- ☐ Fluids warmed

Recovery notes:

Time (every 3-5 mins)	**RR** (RPM) *60-80*	**HR** (BPM) *M=300-600 R=300-350*	**SPO2** *>95%*	**Colour** *Pink*	**Temp** *37.5˚C*	**O2**	**Iso %**	**Reflex** (++ / + / -)	**Comments/Observations/Treatments:**	Init.

APPENDIX 2 – TEMPLATE ANESTHESIA RECORD

Time **(every 3-5 mins)**	**RR** **(RPM)** *60-80*	**HR** **(BPM)** *M=300-600 R=300-350*	**SPO2** *>95%*	**Colour** *Pink*	**Temp** *37.5˚C*	**O2**	**Iso %**	**Reflex** **(++ / + / -)**	**Comments/Observations/Treatments:**	Init.