LAB_015 Rodent Anaesthesia- SomnoSuite Low-Flow Isoflurane in Mice (Expiry: Sept 2028)

I. OBJECTIVE

To describe the procedure for monitoring and supporting rodents during anaesthesia using a SomnoSuite low-flow isoflurane system within UQBR facilities. The low-flow isoflurane system is equipped with precision syringe pump and integrated digital vaporizer which uses room air or compressed oxygen to deliver anaesthesia at low flow rates proportionate to the rodent's size. The use of low-flow isoflurane vaporisers presents a viable alternative to the traditional high-flow vaporising method, offering enhanced safety measures for rodents, and minimizing researchers' potential exposure to waste gases. The SomnoSuite low-flow isoflurane system adds surgical functionality by providing the ability to utilise either atmospheric air or 100% oxygen as the carrier gas during specific procedures as required. For example, surgeries that require soldering should not use oxygen due to safety and welfare concerns.

Animal ethics applications **:

If using this SOP, you must describe the following under variations to the use of the SOP:

1. Oxygen is preferred to air as the carrier gas since it supports oxygenation of the animals whilst under anaesthesia and reduces the risk of anaesthetic related death and other complications. If you are unable to use oxygen, you must provide a justification for using room air.
2. If requesting to use room air instead of oxygen as the carrier gas, this must only be for very short, low impact procedures.
3. If you need to use room air as the carrier gas, you must confirm that arterial blood oxygen saturation (SpO2) monitoring will be performed and describe a management plan for hypoxia in the animals.

NB: The use of (*) indicates this statement is dependent on the Facility procedures
NB: The use of (**) indicates this statement is dependent on AEC Approvals

II. COMMENTS / RECOMMENDATIONS

- Isoflurane anaesthesia has many advantages over injectable anaesthetics: minimal rodent handling, wide margin of safety, S4 (prescription) categorisation (compared to S8 category drugs which are controlled drugs and much more tightly regulated), short recovery time, and ease of anaesthetic titration.
- The low-flow isoflurane system minimises the leakage of anaesthetic gases into the atmosphere and reduces the risk of exposure to lab personnel from waste anaesthesia gas.
- Isoflurane does not provide analgesia (i.e. pain relief). AEC approved analgesia should be used for any procedure suspected to cause pain (see Guideline: Anaesthesia & Analgesia for Survival Rodent Surgery, for analgesic options).

1.1. Competent - the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments (*The Code*).

- The anaesthetic period should be as short as possible and not exceed 3 hours in duration. Long anaesthesia requires additional consideration of rodent support, particularly related to maintaining body temperature, fluid status, and eye protection.
- Workstations must allow rodents to be in your visual field for the duration of their anaesthetic period.
- An assistant is strongly recommended to be present for all surgical procedures.
- Anaesthetic monitoring equipment should be used to supplement manual/ visual monitoring (e.g. use of specialised blood pressure, pulse oximetry or respiratory rate monitoring equipment).
- Procedures involving the use of anaesthesia should be done early in the day to allow an adequate time period of observation during recovery.
- Complications associated with anaesthetic and surgical procedures should be referred to the UQBR veterinarians for support (see LAB_022 UQBR Veterinary Care Program) and must be reported to AEC if there was the potential for impact on animal welfare .
- Users must keep anaesthesia monitoring records, and surgical records (reference Appendix A and B for an anaesthetic monitoring record template and example). Contac the UQBR Veterinarians or Animal Ethics Unit Veterinary Officer for further advice if needed.
- Any associated experimental compounds or medications (including your anaesthetic protocol) must be detailed within the AEC application.
- Wherever possible, active heating (e.g. a heat mat) should be used at all times to help maintain normal body temperature of the anaesthetised rodent. If active heating is not to be used, justification must be provided to the AEC**
- In the event of equipment failure, or anaesthetic recovery mid-surgery, “alleviating unanticipated pain and distress must take precedence over an individual animal reaching the planned endpoint of the project, or the continuation or completion of the project. If necessary, animals must be humanely killed without delay” (Clause 2.4.18, Australian code for the care and use of animals for scientific purposes 8th Ed., 2013).

III. SAFETY AND COMPLIANCE

1. The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken, and risk assessments have been performed. If unsure, consult your supervisor. Facility protocols should be followed.
2. This SOP must be read in conjunction with 2.70.16 Working Safely with Isoflurane.
3. This procedure has particular risks of:
    - Exposure to isoflurane fumes or waste anaesthetic gas – follow safety procedures including use of appropriate equipment (e.g. exhaust ventilated workspaces); some personnel, notably pregnant personnel, may be particularly susceptible and must individually assess their risk of exposure
    - Mouse bite injury – take appropriate care
    - Musculoskeletal injury when performed regularly – consider suitable ergonomic design wherever possible
4. Facility and procedure appropriate PPE use is essential when handling laboratory rodents.
5. All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form.
6. In the event of a spill follow facility emergency spill procedures relative to SDS details (available online, and as hard copies within UQBR facilities) *

IV. TRAINING CONSIDERATIONS

- All users must have completed the online module training through UQBR Learning Management System (LMS) before using this equipment: UQBR - Introduction to Gaseous Anaesthesia in Rodents using Isoflurane
- Following completion of the online training, users must contact the Facility manager to organise and receive competency training in the proper use of this equipment
- All unsupervised procedures must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.

V. EQUIPMENT

- PPE*: Minimum PPE is gloves, gown, mask and safety glasses; additional PPE may be required based on facility or additional risk e.g. working with infectious animals.
- Low-Flow Isoflurane unit equipped (*)
- Induction chamber, transparent Perspex (*)
- Compressed oxygen supply
- Breathing circuit (*) i.e., nose cone and relevant connections
- Timing device
- Eye care lubricant (sterile and aqueous e.g. Lacrilube®, Lacri-lube©, Visco-tears© gel)
- Weighing scales
- Heating equipment (*) (must include active heating e.g. a heat mat)
- Anaesthetic Monitoring Record (see appendices A and B)
- Anaesthetic monitoring equipment (*) (e.g. Physiosuite®, MouseSTAT®, pulse oximeter, rectal temperature probe)
- Liquid isoflurane – as per AEC approved protocol (**)

VI. PREPARATION

1. Perform a pre-anaesthetic assessment of the rodent's general physical condition (including measurement of pre-anaesthetic body weight). It is ideal to use the project scoresheet as a checklist and record of this assessment. If the rodent appears unwell it should not be anaesthetised.
2. Label the Anaesthetic Monitoring Record with relevant details (e.g. rodent identification, pre-anaesthetic physical condition, procedure to be performed).
3. Ensure the heating equipment in both the procedure and recovery areas is turned on in advance and all anaesthetic equipment is set up within the appropriate workspace (*).
4. Ensure the timing device (timer, watch, wall clock) is accessible within the workspace (*).
5. Check the anaesthetic unit is ready for use:
   - Ensure all components of the anaesthetic unit are appropriately connected, oxygen supply is turned on if connected and appropriate.
   - Ensure the isoflurane syringe contains sufficient liquid isoflurane.
   - Ensure exhaust ventilation at the workstation is turned on and working

VII. PROCEDURE

The operation of the low-flow isoflurane system differs from that of a conventional system, so operational steps and a diagram are detailed below. Facility specific manuals or 'how to guides' should be referred to in combination with this SOP.

Low-flow Isoflurane Rig Setup

1. Ensure that the tubing is properly arranged to deliver isoflurane from the anaesthetic rig to the induction chamber and nose cone.
2. Additionally, ensure that waste isoflurane gas is efficiently removed from the induction and nose cone to the suction port through the anaesthetic rig, as illustrated above.
3. Securely place an isoflurane-filled syringe into the syringe holding block on the rig. Then, attach the anaesthetic delivery line to the syringe.
4. Turn the Machine on and set the gas (100% oxygen or atmospheric air) flow rate for induction chamber and nose cone.
5. Adjust the anaesthetic gas flow rate for induction chamber at 1000 mL/min and 60 – 100 mL/min for nose cone.
6. Fully turn on the scavenger ports for induction chamber and nose cone on the wall. Redirect the isoflurane to the induction chamber by closing the tube clamp on the nose cone and clicking "Induction" tab on the isoflurane rig.
7. Gently collect the rodent and place them into the induction chamber and close the lid.
8. Turn the anaesthetic concentration knob to set the concentration of isoflurane as 4% (similar to the conventional isoflurane machine). Make sure "Deliver" light is on and green when delivering isoflurane.
9. From this point, until anaesthetic recovery, the rodent must be continuously monitored (in your visual field).
10. Monitor the rodent for appropriate level of anaesthetic induction: there should be loss of the righting reflex (LORR) and unconsciousness (see table 1 and 2 for details).
11. This should take only 1-2 minutes. Upon exposure to isoflurane rodents should display an initial period of hyper-excitability, followed by sedation, unconsciousness, and progressive loss of reflexes.
12. Gently collect the rodent and place it onto the pre-warmed workspace with its nares (nostrils) positioned within the anaesthetic nose cone.
13. Attach animal monitoring equipment such as Physiosuite®or MouseSTAT®.
14. At the same time transfer the gas supply from the induction chamber to the nose cone by pressing "Nose Cone" tab on the isoflurane rig, opening the tube clamp for the nose cone and closing the clamp for the induction chamber tubing. Reduce the concentration of isoflurane to ~2% by adjusting the anaesthesia concentration knob.
    It is critical that rodents do not remain in the induction chamber, exposed to high levels of isoflurane, for prolonged periods (i.e. >10 minutes), or else the anaesthetic may go too "deep" and complications, including anaesthetic mortalities, are likely to occur. Table 2 gives an indication of behaviour that indicates the rodent may be too "deep", too "light", or appropriately anaesthetised, when using isoflurane.
15. Apply eye lubrication.

This should be done with clean technique, using a small amount of eye lubricant applied to a fresh cotton tip. When applying the lubricant to the eyeball's surface the cotton tip itself should not actually contact the eyeball.

16. The use of anaesthetic monitoring equipment, such as pulse oximetry is highly recommended when using any gaseous anaesthetic and essential if room air is used as the carrier gas to monitor for any hypoxia in the rodent during the surgical procedure.
17. Before proceeding, ensure the rodent has reached an appropriate depth of anaesthesia (see tables 1 & 2):

    i. **Light plane of anaesthesia –** for minor procedures only (e.g. non-surgical/non-invasive, minor intervention requiring immobilization):
    - Muscle tone must be loose/weak
    - Skin pinch reflex should be absent, i.e. superficial pain should be absent.

    ii. **Deep plane of anaesthesia –** for major procedures (e.g. surgical/invasive, major intervention).
    - Muscle tone must be loose/weak.
    - Skin pinch reflex must be absent, i.e. superficial pain is absent.
    - Toe pinch reflex must be absent, i.e. deep pain is absent.

18. Perform the AEC approved procedure (**).
19. Once the procedure is completed, touch "Remove" tab on the screen to remove the syringe pusher block to the end. Remove and empty the syringe.
20. Touch "Menu" on the screen and then "Power Off" to turn off the low flow machine.
21. Turn off the scavenge ports.
22. Place the rodent into an individual cage within the pre-warmed recovery area (recovering rodents should not be placed with non-anaesthetised rodents - refer to UQBR Guideline 2 Rodent Heating Procedures).
    If there is any concern about the anaesthetic (e.g. unstable anaesthesia, prolonged anaesthesia) the rodent should remain on the nose cone and be provided pure oxygen gas in the anaesthetic recovery period – only removing the rodent from the nose cone once restraint would be required to keep it in place (i.e. once the rodent is moving).
    If the rodent experiences hypoxia (i.e., a drop in pulse oximetry less than 90%) when room air is used as a carrier gas during the surgical procedure, stop the surgery and manually administer 100% oxygen without stopping isoflurane supply.
23. Continuously monitor the rodent (the mouse should be in eyeline at all times) until it has recovered its reflexes, is normally responsive to external stimuli, and is able to ambulate, eat, and drink and toilet normally.
24. Continue to provide analgesics as described by the approved AEC activity**. Refer to Guideline – Rodent Analgesia (Procedure Specific).
25. Heated ventilated chambers (similar to a humidicrib) may be used to support the rodent over 12-24 hours post procedure.

VIII. REFERENCE INFORMATION

Table 1: Basic reflexes in rodents, their method of assessment, and significance in anaesthetic monitoring.

Reflex	Method of assessment	Significance
Righting reflex	The animal is gently rolled onto its back. The righting reflex is lost when the animal is unable to regain an upright posture (standing or lying down).	Loss of the righting reflex (LORR) is correlated with a loss of consciousness
Skin pinch reflex (panniculus reflex)	The loose skin over the animal's dorsal surface is pinched. This reflex is lost when the animal does not visibly respond (e.g. by flinching).	Loss of this response is correlated with loss of superficial pain.
Toe pinch reflex (pedal withdrawal reflex)	One of the hind limbs is gently extended, and then the footpad is firmly pinched. The toe pinch reflex is lost when the animal does not respond by withdrawing the extended limb.	Loss of this reflex is correlated with loss of deep pain.

Table 2: Measures of anaesthetic depth in rodents under isoflurane anaesthesia. Please note: these parameters should be considered only as a guide to maintaining appropriate anaesthetic depth.

Too light	Appropriate		Too deep
	Light plane of anaesthesia	Deep plane of anaesthesia	
▪ Loss of the righting reflex (LORR) but muscle tone is still present ▪ Reflexes present ▪ Rapid and shallow respiratory rate	▪ Muscle tone loose/weak ▪ Skin pinch reflex absent ▪ Toe pinch reflex variably present ▪ Rhythmic, but shallow, respiratory rate	▪ Muscle tone loose/weak ▪ Skin pinch reflex absent ▪ Toe pinch reflex absent ▪ Reduced respiratory rate, but still rhythmic	▪ Respiratory rate may be erratic, abdominal breathing has developed ("see-saw" breathing)

IX. IMPORTANT LINKS

LAB_022 UQBR Veterinary Care Program

2.70.16 Working Safely with Isoflurane.

Guideline – Rodent Analgesia (Procedure Specific)

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

BIBLIOGRAPHY

1. Flecknell, P. (2009). Laboratory Animal Anaesthesia, 3rd Edition. Academic Press: London.
2. NHMRC. (2008). Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes: The Assessment and Alleviation of Pain and Distress in Research Animals. National Health and Medical Research Council (NHMRC). Retrieved from https://www.nhmrc.gov.au/about-us/publications/guidelines-promote-wellbeing-animals-used-scientific-purposes
3. NHMRC. (2013). Australian code for the care and use of animals for scientific purposes, 8th edition. National Health and Medical Research Council (NHMRC). Retrieved from https://www.nhmrc.gov.au/about-us/publications/australian-code-care-anduse-animals-scientific-purposes

Version #	AEC	AEC Review Date	Outcome Status
1.2	Anatomical Biosciences AEC	August 2025	Approved with comment (admin)
1.2	Molecular Biosciences and Health Sciences AECs	August 2025	Approved
1.2	Laboratory Biomedicine AEC	September 2025	Approved

Appendix A | Anaesthetic monitoring record for rodent anaesthesia. This template may benefit from adjustments to suit individual, specific anaesthetic procedures.
[Mucous membrane (MM) colour should be light pink; Temperature should not drop below 35.5°C; Respiratory rate (RR) will vary (60-220/min) but rapid drops should be of concern; Arterial blood oxygen saturation (SpO2) should be >95%; Heart rate and blood pressure is not listed but should be added if equipment is available that can accurately measure these parameters]

General details	Animal details	Anaesthetics and analgesics details
Date of anaesthesia:	Species/strain/sex:	Drug dose and route: / Drug concentration (and volume injected): / Time:
AEC approval #:	Rodent ID:	
Procedure:	Animal's condition:	
Experimental details:	Body Weight:	
Personnel (and roles):	Notes:	

Duration (min) /Time	Position	Righting Reflex [s]	Muscle tone [s]	Skin pinch [s]	Toe/tail pinch [s]	MM colour	Temp (°C)	RR	SpO2	Comments [*anaesthetic start and end; †surgery start and end]	Iso. (%)

Those criteria marked with "[s]" may be assessed as: absent (-); only mildly present (+); present but dull (++); present/normal conscious response (+++); LORR = Loss of righting reflex; Iso. (%) = Isoflurane (%)

Comments:

Conditions:
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Appendix B | Example of a completed anaesthetic monitoring record from isoflurane anaesthesia (and surgical procedure) performed in mice.

General details	Animal details	Anaesthetics and analgesics details
Date of anaesthesia: 17/11/2020	**Species/strain/sex:** Mouse: CBA/J (male)	
AEC approval #: 465/21	**Rodent ID:** #7	
Procedure: Surgery - Tumour resection (flank)	**Animal's condition:** Bright, alert, normally responsive	
Experimental details: 1x10^6 RMA/S injected SC (03/11/2020)	**Body Weight:** 25g	
Personnel (and roles): TB (surgery); KS (assistant)	**Notes:** 1 of 6 tumour resections today	

Drug dose and route:	Drug concentration (and volume injected):	Time:
Meloxicam 2mg/kg SC	0.5mg/mL (=100uL)	09:19 (+1 repeat dose in 24hours)
Buprenorphine 0.05mg/kg SC	0.03mg/mL (40uL)	09:45
Isoflurane 1-5% (via vaporiser)	1-5%	09:15 to 09:40

Duration (min) /Time	Position	Righting Reflex ^{s}	Muscle tone ^{s}	Skin pinch ^{s}	Toe/tail pinch ^{s}	MM colour	Temp (°C)	RR	SpO2	Comments [***anaesthetic start and recovery/end; †††surgery start and end]	Iso. (%)
0 / 09:15	ambulatory (a)	+++	+++	+++	+++	Pink (p)	conscious (c)	(c)	(c)	***, 9:16 LORR, 9:18 moved to nose cone, 9:19 meloxicam	5 à 2
5 / 09:20	Lateral	-	-	+	+	(p)	38.8			surgical skin prep commenced	2
10 / 09:30	Lateral	-	-	-	-	(p)		120	95%	†††	2
15/ 09:35	Lateral	-	-			(p)		128	95%		2
20 / 09:40	Lateral	-	-	-	-	(p)				†††, ***, returned to recovery cage, buprenorphine	2 à 0
30 / 09:50	(a)	+++	+++	+++	+++	(p)	conscious (c)	(c)	(c)	Quiet, normally responsive	
90 / 10:50	(a)	conscious (c)	(c)	(c)	(c)	(p)	(c)	(c)	(c)	Bright, alert, normally responsive	
150 / 11:50	(a)	(c)	(c)	(c)	(c)	(p)	(c)	(c)	(c)	Bright, alert, normally responsive	

Those criteria marked with "^{s}" may be assessed as: absent (-); only mildly present (+); present but dull (++); present/normal conscious response (+++); LORR = Loss of righting reflex; Iso. (%) = Isoflurane (%)

Comments:	Procedure performed routinely. Repeat dose: 2mg/kg meloxicam (100uL SC) to be administered at 09:00, 18/11/2020 by TB

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