LAB_060 Rodent Anaesthesia - Isoflurane

I. OBJECTIVE

To describe the procedure for monitoring and supporting rodents during isoflurane anaesthesia (using an induction chamber and nose cone) within UQBR facilities.

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 animal handling, wide margin of safety, S4 categorisation (compared to S8), short recovery time, and ease of anaesthetic titration.
- Isoflurane does not provide analgesia (i.e. pain relief). Sufficient and appropriate pain relief is a requirement of any procedure suspected to cause pain (see Guideline: Anaesthesia & Analgesia for Survival Rodent Surgery, for analgesic options).
- The anaesthetic period should be as short as possible, and not exceed 3 hours in duration. Long anaesthesia requires additional consideration of animal 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.
- Wherever possible 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).
- Complications associated with anaesthetic and surgical procedures should be are referred to the UQBR veterinarians for support (see LAB_022 UQBR Veterinary Care Protocol).

In relation to human safety:

- This SOP must be read in conjunction with 2.70.16 Working Safely with Isoflurane
- Facility and procedure appropriate PPE use is essential when handling laboratory rodents
- All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form. 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
- In the event of a spill follow facility emergency spill procedures relative to SDS details (available online, and as hard copies within UQBR facilities) *

III. EQUIPMENT

- PPE (*)
- Anaesthetic unit (see Guideline: Use of Anesthesia Machine and Circuit) **
    - Anaesthetic machine
    - Oxygen source (wall, portable cylinder, concentrator)
    - Isoflurane vaporiser
    - Induction chamber (5L), transparent Perspex
    - Breathing circuit (non-rebreathing) and nose cone connection
    - Charcoal scavenger (passive)
- Exhaust ventilated work station (e.g. flume, or biosafety cabinet)
- Timing device
- Eye care lubricant (sterile and aqueous e.g. Lacrilube®)
- Weighing scales
- Heating equipment (must include active heating e.g. a heat mats)
- Anaesthetic Monitoring Record (see appendices A and B)
- Anaesthetic monitoring equipment (*) (e.g. Physiosuite®, Somnosuite®, pulse oximeter, rectal temperature probe)

IV. PREPARATION

1. Perform a pre-anaesthetic assessment of the animals' general physical condition (including measurement of pre-anaesthetic body weight). If the animal 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, and gas pressure can be maintained within the system (i.e. it is not leaking gas)
    - Ensure the isoflurane vaporiser contains sufficient liquid isoflurane (see Guideline: Use of Anesthesia Machine and Circuit)
    - Ensure exhaust ventilation at the workstation is turned on and working

V. PROCEDURE

1. Ensure the anaesthetic machine is set up to supply gas to the induction chamber.
2. At the anaesthetic machine, turn the oxygen flow meter to 1L/min and the vaporiser setting to 4-5% isoflurane delivery.
3. Gently collect the rodent and place them into the induction chamber, and close the lid.

From this point, until anaesthetic recovery, the rodent must be continuously monitored (in your visual field).

4. 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). 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.

5. Gently collect the rodent and place it onto the pre-warmed workspace with its nares positioned within the anaesthetic nose cone. At the same time transfer the gas supply from the induction chamber to the nose cone. Turn the oxygen flow rate down to 400mL/min and titrate isoflurane to the lowest dose required to enable your procedure (dependent on the procedure, 1-2% is often sufficient). **
   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), else the anaesthetic is likely to 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.

6. 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.

7. Apply any anaesthetic monitoring equipment to the rodent (e.g. pulse oximetry clip, blood pressure cuff)
8. Before proceeding, ensure the animal 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
9. Perform the approved procedure (**).
10. Once the procedure is completed, turn the oxygen flow meter to 0mL/min and the isoflurane vaporiser setting to "OFF", and place the rodent into an individual cage within the pre-warmed recovery area (recovering animals should not be placed with non-anaesthetised animals - 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 provided pure oxygen gas (without isoflurane) 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).

11. Continuously monitor the rodent until it has recovered its reflexes, is normally responsive to external stimuli, and is able to ambulate, eat, and drink and toilet normally.
12. Heated ventilated chambers (similar to a humidicrib) may be used to support the animal over 12-24 hours post procedure.

VI. 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	▪ Muscle tone loose/weak ▪ Skin pinch reflex absent ▪ Toe pinch reflex absent ▪ Respiratory rate may be erratic, abdominal breathing has developed ("see-saw" breathing)

VII. BIBLIOGRAPHY

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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).

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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:

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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 → 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 → 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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