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### Legislation

- NHMRC, 2013, Australian Code for the Care and Use of Animals for Scientific Purposes 8<sup>th</sup> Ed.
- Animal Welfare Act 1985
- Animal Welfare Regulations 2012
- Gene Technology Act 2000 (the Act)
- Gene Technology Regulations 2001
- Work Health and Safety Regulations 2012

### Policies and Guidelines

- Flinders University, 2017, Work Health and Safety Policy
- NHMRC, 2018, Australian Code for the Responsible Conduct of Research
- NHMRC, 2008, Guidelines to promote the wellbeing of animals used for scientific purposes The assessment and alleviation of pain and distress in research animals.

### **Local Policy**

Use of the College of Medicine and Public Health Animal Facilities by all staff and researchers of the College of Medicine and Public Health, Flinders University, is subject to awareness of, and adherence to the following:

# **Research Involving Animals:**

□ The University holds a licence for the use of animals for teaching and research purposes. To satisfy the requirements of the licence, anyone wishing to undertake teaching and research using animals must submit a proposal to the Animal Welfare Committee. No work with animals may commence until written approval has been received from the Animal Welfare Committee. Standardised application forms for Research and Teaching can be found on the Flinders University website listed below. It is your responsibility to regularly check this site for updates to guidelines, forms etc

http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics home.cfm

- All staff and students involved in animal research must complete Animal Ethics Online Training (AEOT) and must also regularly attend Animal Researcher Information Sessions (ARIS).
- All personnel working with Genetically Modified Animals (GMO) or working with in a PC1 or PC2 facility must attended a Biosafety Training Day every 3 years

# Safe Work Method Statement

Refer to Risk assessments, Safe Work method Statements for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Animal Facility Manager

SWMS 1.3 Mouse – Anaesthesia and Analgesia

SWMS 1.7 Mouse Transportation

RA 1.7 Mouse Transportation

SWMS 10.2 - Emergency Contingency

RA 10.2 - Emergency Contingency

# Personal Protective Equipment Required

- Gloves
- Gown
- Mask

- Hair Net
- Shoe Covers

### **Hazards and Controls**

- > Animal bites- training, demonstrate competency, adhere to SWMS.
- Animal Scratches- training, demonstrate competency, adhere to SWMS.
- Needle Stick- DO NOT recap needles, dispose immediately into sharps containers, adhere to SWMS.
- Chemical exposure- wear PPE and goggles.

## **Before Work Commences**

# Ensure that you are aware of the locations of the following:

- Spill Kit
- Fire Extinguisher
- Eye Wash
- o Exits

<u>Risk Assessment and SDS</u> (Safety Data Sheet) - Ensure that you have read and understood for all the substances being used.

## **Equipment**

- Check for safety and electrical compliance
- Ensure that you have read and understood the Risk Assessment and Safe Work Method Statements
- Obtain training before using any equipment

#### General Information

- All procedures are to be performed by competent staff registered on the Competency Skills Register.
- Training is available from the Animal Welfare Officer or senior animal house staff who may act on the behalf of the AWO (Animal Welfare Officer).

## Recovery from Anaesthesia and Sedation

- 1. Once a procedure has been completed, and anaesthetic gases have been turned off, animals should be retained on 100% oxygen by mask as long as possible, and need to be continuously monitored until they have regained righting reflex. During this period they should be maintained on a small animal heat mat (set a 28 to 32°C).
- 2. Check mucous membrane/extremity/muzzle colour, hydration by the skin pinch test, transparency of the cornea, and breathing rhythm and speed. If there are problems, contact a senior researcher, AWO (via 0404 721 720), or the Animal Facility Manager to assist you.
- 3. For recovery purposes, if animals have received injectable anaesthesia/sedation, or have not regained full mobility within 15 minutes after the termination of gaseous anaesthesia, the following actions, depending on AWO advice, should be taken:
  - Place two to three food cubes into a cup cake container, and add sufficient water to soak the food. Leave this on the cage substrate at the opposite end of the cage to the nest. A small amount of Sustagen powder may be sprinkled on the surface of the soaked food. This will encourage the animals to start eating soon after recovery, and

raise blood sugar levels. Check with the Primary Applicant of the project to determine if Sustagen may add to confounding variables before using it. Water gel packs with or without additional electrolytes can be used to promote hydration during the recovery period. See:

https://www.ablescientific.com.au/product/index/ASNECTAR\_ABLE\_NECTAR\_MOISTURE\_SACHETS\_75G\_100BOX

- Administer a volume of body-temperature Normal Saline for Injection, equivalent to 3% of bodyweight, by subcutaneous injection with a maximum volume of 0.5 mLs in each injection site.
- o Place the cage on a thermostatically controlled warm mat, with the mat in contact with half of the external floor of the cage. Leave the cage on this warm mat until the animals are capable of independent purposeful movement. Consider leaving them in an IVC cage on a bench overnight in their holding room, and do not return to the ventilated IVC rack. This will provide additional warmth and comfort overnight. Check with the AWO or Animal Facility Manager before doing this.
- Monitor clinical signs of recovery and complete Clinical Record Sheets (CRSs) as specified in the AWC approved project details.
- Always check that the sipper tube of the bottle is functioning and that there is a free flow of water occurs when your gloved finger touches the end of the sipper tube.
- If animals have not recovered full mobility by close of business, contact the AWO and follow veterinary advice.
- If animals have not recovered sufficiently by the next morning, consult the AWO and consider continuing the above interventions.

## **Animal Monitoring**

- 1. Follow the requirements for monitoring described in the AWC approved conditions for the project.
- 2. The default monitoring regime is:
  - Monitor animals continuously while recovering from anaesthesia until they have regained righting reflex and are fully mobile.
  - o Recheck in 2 hours.
  - Recheck at the end of the day, just prior to dusk.
  - o Recheck early next morning, no later than 9:30 AM (the earlier the better).
- 3. Continue to check the animals twice daily for the next 2 days using individual Clinical Record Sheets. Ensure the times of the checks are well spaced out, early in the morning and late in the afternoon.
- 4. If animals are showing adverse clinical signs or have lost more than 10% weight loss, when compared to pre-operative weight, continue to check once to twice daily (depending on CRS requirement) for 5 more days using individual Clinical Record Sheets. After this, if animals do not show adverse signs they can go onto a Maintenance Monitoring Sheet, which involves daily cage checks.
- 5. Schedule surgeries and procedures that require protracted recovery to occur early in the week to avoid animals recovering over weekends when there are minimal staff members available.

### **Post-Operative Duties**

- 1. Check the whole cage, water bottle, bedding, substrate, and all the animals as a group prior to examining individuals.
- 2. Clinically score individual animals using the criteria described in the Clinical Record Coversheet and record the scores on the Clinical Record Sheet.
- 3. Provide sufficient time for animals to wake up and commence moving, and allow the animals to leave the nest prior to commencing to score. Remember, they sleep during the day are sleepy and take time to wake up. Often animals may look slightly ruffled and reluctant to move during this waking period.
- 4. Check water bottle levels with every check. If the bottle has less than 100 mLs fill it up to 150 mLs.
- 5. Ensure the nest has copious, dry, and well-lofted material. This provides up to 3 degrees of extra warmth for the animals. If nest material is dirty or wet, remove it and replace with new material. Attempt to always leave a bit of residual nesting material since this promotes group harmony.
- 6. If the substrate has concentrated wet and soiled patches, remove these and replace with new bedding.
- 7. Ensure that soaked food is completely changed every day. Do not over load soaked food containers. 3 to 4 pellets is adequate for up to 5 mice.
- 8. Check incision lines. Ensure sutures or wound clips are intact and holding the wound together. If wounds are open and there is suture failure, re-suture under general anaesthesia. If wound edges are more than 24 hours old, the edges need to be trimmed to exposure fresh tissue prior to repair. If there are signs of inflammation (heat, pain, redness, or swelling) contact the AWO and follow advice regarding treatment.

### Post-Operative Clinical Monitoring

• The default clinical scoring criteria on Clinical Record Coversheets are:

MONITORING CRITERIA (Clinical Criteria Columns 1 -5 are recommended for all projects subject to AWO advice)

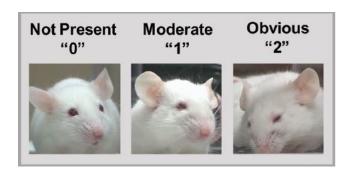
		Normal	Moderate abnormality	Significant/sustained abnormality
	Score	0 (leave blank)	1	2
CRS Column	Clinical Criteria			
1	Lack of, or abnormal movement	Active spontaneous free movement	Lack of spontaneous movement/hyperactivity/occasional ataxia /circling/dragging legs	Reduced movement on stimulation/frequent ataxia, circling, locomotion defects
2	Hunching	Extended back, normal movement and gait	Slight arching on movement	Arched on movement
3	Ruffled coat	Smooth sleek coat	Slight ruffling	Significant ruffling over entire coat
4	Abnormal breathing	Normal breathing in character and frequency	Increased respiratory rate or slight increased effort	Marked increase in respiratory rate with marked effort
5	Skin crinkling/tenting/sunken eyes	Soft, pliable, elastic, bright eyes (> 75% open)	Skin crinkled, slight tenting when skin lifted	Skin tenting and sunken eyes

	Incision site or Injury	No evidence of	Slight scratching	Significant		
6		incision damage	biting/inflammation/redness	biting/scratching/inflammation		
		or injury		/redness		
	Optional additional criteria are shown below – These should be included if applicable to the project, or interchanged with project specific					
criteria. Example: tumour-scoring criteria for tumour studies.						
	Abnormal discharge from	No discharges	Slight discharge (blood/pus etc)	Significant discharge		
7	orifices		from any orifice	(blood/pus etc) from any		
			-	orifice -		
0	Profuse/bloody diarrhoea	Well-formed stools	Profuse with cage smeared with	Bloody & profuse diarrhoea		
0			diarrhoea			

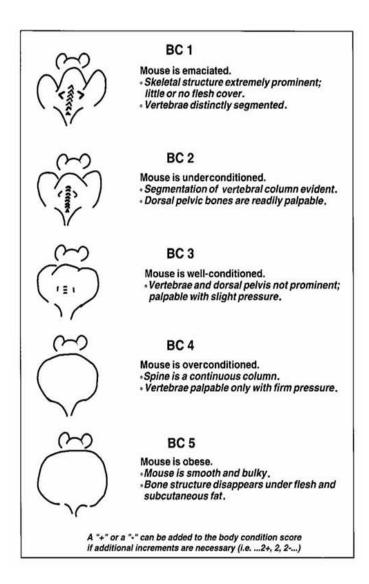
- Check the Clinical Record Coversheet of your approved project to confirm any variations to the above clinical criteria.
- The default actions in response to the clinical scores above are:

### **ACTIONS AND INTERVENTIONS**

ACTIONS AND INTERVENTIONS				
Score	Assessment	Actions/Interventions		
0	Animal within normal limits	No interventions required.		
1	Animal demonstrates slight or moderate deviation from normal	Approved refinement measures to be instituted.		
2	Animal demonstrates significant or sustained deviation from normal	<ul> <li>Immediate consultation with the AWO or immediate euthanasia.</li> <li>Notify the AWO.</li> <li>Complete a Running Mortality Sheet if euthanised.</li> <li>If animal welfare compromise or mortality rates fall outside of approved conditions (section 3.7 of the application), an Unexpected Adverse Event (UAE) Report must be submitted.</li> </ul>		
Accumulative score of 3 or greater	Animal demonstrates significant or sustained deviation from normal	<ul> <li>Immediate consultation with the AWO and follow AWO advice, or immediate euthanasia.</li> <li>euthanised.</li> <li>Notify the AWO.</li> <li>Complete a Running Mortality Sheet.</li> <li>If animal welfare compromise or mortality rates fall outside of approved conditions (section 3.7 of the application), an Unexpected Adverse Event (UAE) Report must be submitted.</li> </ul>		
Absolute humane endpoint criteria: Laboured breathing BCS < 2/5	Animal exhibits respiratory distress – gasping, blue membranes. BSC < 2/5 signifies sharp vertebral processes, body is emaciated	Immediate humanely kill or consult AWO, follow advice		
Weight loss %	Weight loss exceeds 10%, but is less the 15%	Correct sipper water flow, give soaked food Increase monitoring frequency to twice daily. Consult senior facility technician, notify the AWO depending on technician advice. If 10% weight loss occurs within 24 hours then consult AWO and follow advice or immediately humanely kill.		
Mouse/Rat grimace score (See illustration at end of document)	Mouse grimace "moderate" or "obvious" as per Matsumiya et al 2102, JAALAS	If within 48 hours of acute trauma administer pain relief. If beyond 48 hours consult AWO and follow advice.		
BCS < 2/5	BSC < 2/5 signifies sharp vertebral processes, body is emaciated	Immediate euthanasia or consult AWO and follow advice		



From Matsumiya et al, 2012, Using the Mouse Grimace Scale to Reevaluate the Efficacy of Postoperative Analgesics in Laboratory Mice, *JAALAS*, Vol 51, No 1, January 2012, pp 42 – 49



From Ullman-Culleré M H and Foltz C J, 1999, Laboratory Animal Science, Vol 49, No 3, June 1999

### Signs of Pain in the Mouse

- Signs of acute pain related to surgical trauma documented in mice include:
  - Lack of movement.
  - o Decrease food consumption, rapid loss of bodyweight, and body condition score.
  - o Facial grimace (see above illustration).
  - o Ruffled coat due to lack of self-grooming, or piloerection due to fever.
  - Signs related to dehydration due to an inability to reach the sipper tube (skin crinkling, skin tenting, or sunken eyes).
  - Change in posture, hunching on movement, writhing, rear-leg lift, belly-press, or flinching.
- Remember that signs of pain in mice may vary depending on time of day/night (more signs of pain at night), gender of observer (mice show less signs of pain to men), and familiarity with carer (mice show less pain to unfamiliar observers).

### SWMS Review

This SWMS currently applies to the animals housed in the College of Medicine and Public Health Animal Facility. This SWMS will be reviewed 3 yearly, but also updated more frequently as policies, techniques and animal care requirements change.

Position	Name	Contact Details
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Any questions regarding the above guidelines and any technical advice/ assistance required can be directed to Animal Facility Manager.