The SWMS Mouse – Anaesthesia and Analgesia contains the following sections:

- Legislation
  - University Policy
  - Local Policy
  - Safe Work Method Statement
  - Personal Protective Equipment Required
  - Hazards and Controls
  - Before Work Commences
  - General Information
- NHMRC/ Flecknell P 2015/ AWO Anaesthesia and Analgesia Guidelines
- Anaesthesia Settings and Setup
- Induction and Maintenance of Anaesthesia
- Monitoring Depth of Anaesthesia
- Recovery from Anaesthesia and Sedation
- Medications Associated with Anaesthesia and Surgery
Legislation

- Australian Code for the Care and Use of Animals for Scientific Purposes 8th 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

University Policy

- Work Health and Safety Policy 2013
- Responsible Conduct of Research Policy 2016
- NHMRC Guidelines

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 (via the Animal Ethics Review Sub-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-economics/animal-economics_home.cfm](http://www.flinders.edu.au/research/researcher-support/ebi/animal-economics/animal-economics_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 Statement for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Animal Facility Manager.

Related SWMS documents include:
- SWMS 1.0 Mouse- Sexing, Handling, Restraint and Ear Notching
- RA 1.0 Mouse- Sexing, Handling, Restraint and Ear Notching
- SWMS 1.7 Mouse -Transportation
- RA 1.7 Mouse –Transportation
- SWMS 1.9 Mouse – PC1, PC2 and Infectious Containment Husbandry
- RA1.9 Mouse – PC1, PC2 and Infectious Containment Husbandry
- SWMS 10.2 - Emergency Contingency
- RA 10.2 - Emergency Contingency

Personal Protective Equipment Required

- Gloves
- Gown
- Mask
- Hair Net
• Shoe Covers

### Hazards and Controls
- Anaesthetic Inhalation may produce health damage.
- Cumulative effects may result following exposure.
- Do not breathe gas/fumes/vapour/spray.
- Use only in well ventilated areas and use scavenge systems.
- Remove all contaminated clothing immediately if spilt on body.
- If a spill occurs, vacate the area, close doors, ring 33#, alert others in the vicinity.
- Animal bites and scratches are prevented by training, demonstration of competency, adhere to SWMS.
- Needle Stick- DO NOT recap needles, dispose immediately into sharps containers, adhere to the relevant SWMS.

### Before Work Commences
Ensure that you are aware of the locations of the following:
- Spill Kit
- Fire Extinguisher
- Eye Wash
- Exits

**Risk Assessment and SDS (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

**List Of Equipment Needed**
- Isoflurane
- Induction Chamber
- Filler Key
- Heat Mat
- Anaesthetic machine
- Heat mat
General Information

- All procedures are to be performed by trained competent staff.
- Training is available from senior animal house staff or Animal Welfare Officer.
- Evidence of training is available in the “Staff Training Needs Analysis” and the Competency Skills Register administered by the Research Services Office.
- **Women of childbearing age** should be warned that exposure to Isoflurane may have an effect on fertility and foetal development. Women who are planning pregnancy, or who are pregnant, may wish to request to be removed from tasks involving direct exposure, such as filling Isoflurane machine.
### Anaesthesia

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>3 - 5% Induction</td>
<td>Inhalation</td>
</tr>
<tr>
<td></td>
<td>1 - 4% Maintenance</td>
<td>Inhalation</td>
</tr>
<tr>
<td>Servoflurane</td>
<td>5-8% Induction</td>
<td>Inhalation</td>
</tr>
<tr>
<td></td>
<td>2.5 - 4% Maintenance</td>
<td>Inhalation</td>
</tr>
<tr>
<td>Ketamine +</td>
<td>75 - 100mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td>Acepromazine, or +</td>
<td>2.5 - 5 mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td>Buprenorphine or +</td>
<td>0.05 mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>0.5 – 2.0 mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td>Ketamine +</td>
<td>75 - 100mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td>Medetomidine</td>
<td>0.5 – 1.0 mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td><strong>Reverse medetomidine with</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>atipamezole</td>
<td>1 mg/kg</td>
<td>IP or SC</td>
</tr>
<tr>
<td>Ketamine +</td>
<td>100mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td>Xylazine</td>
<td>10 mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td><strong>Reverse xylazine with</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>atipamezole</td>
<td>1 mg/kg</td>
<td>SC</td>
</tr>
<tr>
<td>Ketamine +</td>
<td>100mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td>Diazepam</td>
<td>5 mg/kg</td>
<td>IP</td>
</tr>
<tr>
<td>Pentobarbitone</td>
<td>40 - 50 mg/kg (adult)</td>
<td>IP</td>
</tr>
<tr>
<td></td>
<td>10 - 20 mg/kg (young)</td>
<td>IP</td>
</tr>
<tr>
<td>Alphaxalone</td>
<td>10 - 15 mg/kg</td>
<td>IV</td>
</tr>
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</table>

### Analgesia

<table>
<thead>
<tr>
<th>Drug-NSAID</th>
<th>Dose and frequency</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carprofen</td>
<td>5 mg/kg, 24 hourly</td>
<td>SC</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>1-5 mg/kg, 24 hourly</td>
<td>SC or per os</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug-Opioid</th>
<th>Dose and frequency</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.05 - 0.10 mg/kg, 12 hourly</td>
<td>SC</td>
</tr>
<tr>
<td>Buprenorphine oral</td>
<td>1 mg/kg in Nutella (give 125 mg to a 25 g mouse of 0.2 mg/g nut paste) 24 hourly as per Abelson et al 2012</td>
<td>PO</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>1.0 - 2.0 mg/kg, 4 hourly</td>
<td>SC</td>
</tr>
<tr>
<td><strong>Reverse µ opiates with</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>butorphanol</td>
<td>1.0 – 2.0 mg/kg</td>
<td>SC</td>
</tr>
<tr>
<td><strong>Reverse Opiate and Opioid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with Naloxone</td>
<td>3mg/kg</td>
<td>IP, IM or IV</td>
</tr>
</tbody>
</table>

### Sedation

| Acetylprimazine only, or with | 2.5 - 5.0 mg/kg | IP |
| Butorphanol or              | 0.5 – 2.0 mg/kg | IP |
| Buprenorphine               | 0.05 mg/kg      | IP |

### Local Anaesthesia (Animal Welfare Officer Recommendations)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignocaine</td>
<td>Dilute to 0.5%, recommended incisional volume is 50 µL, Waite et al, BJA, 104 (6): 768 – 73, maximum dose is 10 mg/kg, fast onset short acting (&lt;1 hour)</td>
<td>Infiltration</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>Dilute to 0.25%, recommended incisional volume is 50 µL, Waite et al, BJA, 104 (6): 768 – 73, maximum dose is 3 mg/kg, total dose q1-6h infiltration and epidural and q8-24h plexus or nerve</td>
<td>Infiltration</td>
</tr>
<tr>
<td>Lignocaine/bupivacaine mix</td>
<td>Mix 1% lignocaine with 0.25% bupivacaine as a 50:50 mixture. Dilute this mixture with an equal quantity of water for injection. Infiltrate the incision with a maximum of 50µL (Flecknell, 2015)</td>
<td>Infiltration</td>
</tr>
</tbody>
</table>
Suggested indications and protocols for researcher/technician training by the AWO and Animal Facility staff:

- Mice sedation for moderately invasive procedures such as gavage, cheek bleed training etc, either:
  - acetylpromazine/butorphanol at 2.5 to 5 mg/kg⁻¹/1.0 to 2 mg/kg⁻¹ i.p or s.c – For the maximal dose mix 1.25 mLs of acetylpromazine (2 mg/mL) with 0.075 mLs of butorphanol (10 mg/mL) and add sterile Normal Saline for injection up to 5 mLs – dose at 0.1 mL / 10 g bodyweight.
  - acetylpromazine/buprenorphine at 2.5 to 5 mg/kg⁻¹/0.05 mg/kg⁻¹ i.p or s.c – For the maximal dose mix 1.25 mLs of acetylpromazine (2 mg/mL) with 0.08 mLs of Temgesic (300 mcg/mL) and add sterile Normal Saline for injection up to 5 mLs – dose at 0.1 mL / 10 g bodyweight.

- Mice sedation alternative to the above using xylazine at 10 mg/kg⁻¹ – Mix 0.5 mL of xylazine (20 mg/mL) and add sterile Normal Saline for injection up to 10 mLs – dose at 0.1 mL / 10 g bodyweight.

- Mouse non-recovery anaesthesia, for invasive procedures, after previous acetylpromazine/butorphanol or acetylpromazine/buprenorphine sedation, Zoletil at 40 to 60 mg/kg⁻¹ by i.p or s.c injection – Mix 0.4 to 0.6 mLs of Zoletil (100 mg/mL) and add sterile Normal Saline for injection up to 10 mLs – dose at 0.1 mL / 10 g bodyweight.

- Mouse non-recovery anaesthesia for invasive procedures without pre-medication, Zoletil/medetomidine at 40 to 60 mg kg⁻¹ Zoletil /0.5 mg kg⁻¹ medetomidine i.p or s.c injection – Mix 0.4 to 0.6 mLs of Zoletil (100 mg/mL) and 0.5 mLs of Domitor (1 mg/mL) and add sterile Normal Saline for injection up to 10 mLs – dose at 0.1 mL / 10 g bodyweight.

- Mouse recovery anaesthesia of short term duration, ketamine/xylazine at 100 mg kg⁻¹/ 10 mg kg⁻¹ xylazine by i.p or s.c injection – Mix 1 mL of ketamine (100 mg/mL) and 0.5 mLs of xylazine (20 mg/mL) and add sterile Normal Saline for injection up to 10 mLs – dose at 0.1 mL / 10 g bodyweight.

NOTE: A “Permit to Possess Poisons and Controlled Drugs” must be obtained from SA Health Drugs of Dependence Unit before being able to use Schedule 8 Controlled Drugs. All drugs must be kept in a lockable cabinet when not in use, and a register of use with balance remaining maintained.

**Anaesthesia Settings and Setup**

- Check the level of Isoflurane and top up using the method below if required.

  **Method:**
  1. To fill the vaporiser you must have an Isoflurane filler key, as this is the only way the vaporiser can be filled.
2. Attach the key filler to the top of the Isoflurane bottle by matching up gaps on the key with the collar on the bottle, and screw on firmly.

3. Open the fill port by undoing the screw and removing the port plug, and place the key in the port. Once fully inserted, lock the key in place by tightening the screw.

4. Raise the Isoflurane bottle above the level of the port to fill the vaporiser. The Fill window shows the level of Isoflurane in the vaporiser. To cease filling the vaporiser, lower the bottle of Isoflurane below the level of the port when the level of Isoflurane is level with the top line in the fill window.

5. Release the key loosening the screw, remove the filling key, replace the fill port plug, and retighten the screw. Remove key filler from the Isoflurane bottle, and recap the bottle firmly.

- **Gaseous Anaesthesia: Isoflurane**
  - Induction setting range is between 2 - 5%. A routine induction setting is 3% for most animals.
  - Maintenance setting range is 1- 4%. Most animals can be maintained between 1.5 and 3%.
  - Oxygen flow rate (flow meter): For induction in chambers - the oxygen flow rate is recommended to be set to fill the chamber as rapidly as possible. When using non-re-breathing mask systems the flow rate is recommended to be a minimum of 500 mLs/minute.

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**Induction and Maintenance of Anaesthesia**

**NOTE:** An anaesthetised animal must never be placed into a cage with a non-anaesthetised animal.

**NOTE:** All animals must be continually monitored until fully recovered and mobile following procedures.

**The Midget 3 anaesthetic machine can be used as an open circuit, non-re-breathing system with a mask for maintenance anaesthesia of small laboratory animals.**

1. Rodents should be placed on warm mats for any anaesthetic lasting more than 5 minutes. Turn on the warm mat quarter of an hour before it is required. Ensure it is insulated from the table top.
2. Turn on the oxygen at the wall, if using the mains oxygen plumbed into the theatres, or if using an oxygen bottle, check that enough pressure remains in the bottle. Set the flow meter to deliver the maximum flow rate possible to the chamber. Ensure the scavenger is attached and turn on at the correct setting.

3. Ensure flow is directed to the chamber or mask.

4. Place the rodent in the chamber and ensure it is sealed. To provide pre-oxygenation, allow the animal to stay in the chamber in the oxygen flow for up to 5 minutes prior to turning on the vaporiser. Mice receiving injectable anaesthetics should be exposed to 100% oxygen in the latter manner immediately after injection until procedures commence.

5. To induce anaesthesia, turn on the Isoflurane vaporiser to between 2.5 to 5%.

6. Once the animal is deemed to be sufficiently anaesthetised, transfer the animal to a non-rebreathing mask-circuit and set the isoflurane to between 1.5 to 2.5 %. An ophthalmic ointment, such as Lacrilube, should be applied to the eyes of the animal and 1 to 1.5 mLs of Sterile Normal Saline for Injection administered by the subcutaneous or peritoneal route unless these agents are contraindicated with regard to the research model or clinical condition of the animal.

7. Continue to monitor the animal and adjust the vaporiser as necessary.

8. On completion of the procedure, turn the Isoflurane off and keep the animal on 100% oxygen until voluntary movement makes maintaining supplemental oxygen impossible without animal restraint. If an animal has received an injectable anaesthetic, provide it with 5 minutes of 100% oxygen after the end of the procedure. Providing pre and post oxygenation will reduce the incidence of adverse events during anaesthesia, and is recommended as routine practice.

**NOTE:** The suction should be connected to the mask to minimise leakage of gases and to reduce carbon dioxide build up.

*When using a mask, the suction is to be kept low so as not to remove the gas before the animal can inhale it.*
Monitor Depth of Anaesthesia

**NOTE:** Mouse should be kept on a heat mat during the procedure and kept in an ambient temperature of 30-35°C until the animal recovers righting reflex.

1. **Induction** - Animal will begin to lose its righting reflex.

2. **Adequate Anaesthesia** - Tail Pinch and pedal withdraw reflex (lack of movement following firm pinch of the tail and the webbing between the feet digits). A Firm pinch with plain forceps, which does not break the skin or cause any deep tissue damage, is sufficient to show if the animals is too light. Any observed movement (withdrawing the tail or foot) indicates that the animal is not sufficiently anesthetized to do surgery. Respiration rate should be regular. Mucous membranes and exposed tissues should be bright pink to red.

3. **Anaesthesia Inadequate** - Any movement of the mouse whilst under anaesthesia indicate inadequate depth, so therefore slightly increase the amount of Isoflurane delivered. Rapid, shallow respirations usually indicate the animal is too "light".

4. **Anaesthesia too deep** - Respiration Rate may be deep, shallow, decreased or irregular. Mucous membranes and exposed tissues should be bright pink to red if the colour changes to grey or blue colour anaesthesia is too deep. Spasmodic gasps with dilated pupils and/or blue/grey muzzle and extremities is associated with anoxia and excessively deep anaesthesia. Decease or turn off the Isoflurane being delivered and flush the circuit with pure oxygen.

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**Recovery from Anaesthesia and Sedation**

- 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 should be taken:
  1. Place two to three food cubes into a cup cake container, and add sufficient water to soak the food, and leave this on the cage substrate at the opposite end of the cage to the nest.
  2. Administer a volume of body-temperature Normal Saline for Injection, equivalent to 5% of bodyweight, by subcutaneous injection with a maximum volume of 0.5 mLs in each injection site.
  3. 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.
  4. Monitor clinical signs of recovery and complete Clinical Record Sheets as specified in the AWC approved project details.
  5. If animals have not recovered full mobility by close of business, contact the Animal Welfare Officer and follow veterinary advice.
Medications Associated with Anaesthesia and Surgery

- Surgical practice should be conducted, as far as possible, in an aseptic manner. Antibiotics should not be used as an alternative to aseptic technique. The use of prophylactic antibiotics should be restricted to single injections of short-acting narrow to broadspectrum agents, or long-acting narrow spectrum agents.

- Antibiotics which are recommended to prevent or treat bacterial infections associated with surgery are:
  (i) soluble ampicillin at 50mg/kg by subcutaneous or intraperitoneal injection;
  (ii) enrofloxacin (25 mg/mL or less) at 10 mg/kg. Where possible, based on AWO advice, alternatives to enrofloxacin should be used; or
  (iii) alternative antibiotics as recommended by the AWO.

- These agents should be administered prior to the start of surgery, in order to obtain therapeutic tissue levels of the drugs in the peri and post-operative periods. If postsurgical infection occurs, the AWO should be consulted for treatment advice.

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.

<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
<th>Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manager Animal Facility</td>
<td>Roxanne Collingwood</td>
<td>8204 4380</td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="mailto:roxanne.collingwood@flinders.edu.au">roxanne.collingwood@flinders.edu.au</a></td>
</tr>
<tr>
<td>Animal Welfare Officer</td>
<td>Lewis Vaughan</td>
<td>0450 424 143</td>
</tr>
<tr>
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<td></td>
<td><a href="mailto:awo@flinders.edu.au">awo@flinders.edu.au</a></td>
</tr>
</tbody>
</table>

Useful References
http://www.nhmrc.gov.au - Guidelines to promote the wellbeing of animals used for scientific purposes, NHMRC, 2008


http://www.adelaide.edu.au/ANZCCART/


Flecknell PA (2015). Laboratory Animal Anaesthesia, Elsevier


Any questions regarding the above guidelines and any technical advice/assistance required can be directed to Animal Facility Manager or the Animal Welfare Officer.