The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as Animal Ethics Committees (AECs), on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice and allow for the flexibility that is required in research and teaching activities using animals.

### GENERAL ANAESTHESIA OF MICE AND RATS

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and endorsed by the University of Melbourne Animal Welfare & Ethics Committee.

| V1 Date of Approval: | 4 April 2016 | Date of Review: | 4 April 2019 |

1. **ASSOCIATED STANDARDS**

   This standard should be read in conjunction with the following University of Melbourne Animal Care & Use Standards:

   - Balanced analgesia
   - Surgery and aseptic technique of mice and rats

2. **SUMMARY**

   - General anaesthesia involves loss of sensation, loss of consciousness and usually muscle relaxation. Sedation involves the induction of a calmer state.
   - Anaesthesia must be used for procedures that would normally require anaesthesia in veterinary or medical practice.

3. **BENEFITS & RISKS**

   3.1 Anaesthesia involving loss of sensation also provides loss of pain sensation during procedures. It does not generally provide analgesia beyond the anaesthetic duration, so this may still be required for recovery procedures.
   3.2 General anaesthesia can provide immobilisation for delicate procedures allowing precision related to anatomical locations whilst minimising the chances of trauma.
   3.3 Potential side effects will vary depending on the animal and drugs. General anaesthesia often suppresses the cardiorespiratory system.
   3.4 Keratitis and irritation of the eye can occur if eye lubricant is not used under general anaesthesia

4. **PROCEDURE/PROTOCOL**

   4.1 Training

      4.1.1 Anaesthesia must be performed only by personnel with appropriate training and experience and who are approved as competent. Training for anaesthesia should include clinical examination, dosing/dilutions, pharmacology (side effects, body system responses, agent selection), cardiorespiratory physiology, monitoring, supportive care, and responding to emergencies. Training in anaesthesia must be under the direct and constant supervision of competent trainers. All new anaesthetists must undergo OREI online anaesthetic training. An acceptable skill level must be
demonstrated on more than two consecutive occasions before people are permitted to perform anaesthesia without supervision.

4.1.2 Practical trainers should provide the content, format and/or competency assessment sheets to the Animal Welfare Officer (AWO) prior to commencement of training. Trainers must have completed OREI online anaesthetic training and they should have been observed delivering training to an appropriate standard as determined by the AWO or a registered veterinarian.

4.2 Prior assessment and preparation

4.2.1 The choice of anaesthetic is determined by multiple factors including:
   - species/strain, weight, sex, age and health status of the animal;
   - anticipated duration of the procedure and whether it is to be recovery;
   - appropriateness for the procedure, including possible interaction with other substances used and control of anaesthetic depth;
   - availability of the equipment required to administer agent(s);
   - safety for the worker; and
   - reliability of the anaesthetic agent to produce a steady-state between anaesthesia/analgesia, muscle relaxation and optimum physiological function.

4.2.2 Consultation with the Animal Welfare Officer is advisable when anaesthetists are considering new agents or administration to animals with a higher risk profile.

4.2.3 General anaesthesia can be either by inhalation (eg. isoflurane) or injectable. Inhalational anaesthesia allows improved dose control. Tables I1-7 in the NH&MRC Guidelines provide dose rates for some anaesthetics.

4.2.4 Animals that have been transported from an external facility should be allowed to acclimatise for at least 24 hours before anaesthesia. Rodents do not require withholding of food or water prior to anaesthesia.

4.2.5 A health assessment of animals should be performed prior to anaesthesia and this must include body weight where the procedure is greater than 10 seconds. Weight will assist in determining pharmaceutical doses and post-operative monitoring. Lighting, stimulation and handling of animals should be minimised prior to anaesthesia as stress may adversely impact on anaesthesia.

4.2.6 Care of the eyes with lubricant at the onset of anaesthesia and protection from direct exposure to high heat light sources is recommended to prevent keratitis and eye irritation.

4.3 Isoflurane

4.3.1 Isoflurane is the recommended method of anaesthesia for mice and rats, due to the improved control of anaesthetic depth.

4.3.2 The recommended procedure is to use an anaesthetic machine with a controlled oxygen flow rate and anaesthetic vapour flow concentration. Induction can be done in a chamber with the aforementioned oxygen and vapour controls and is usually started at a rate of around 4% until loss of the righting reflex occurs.

4.3.3 Maintenance rates are usually 1-2.5% but variability is expected and rates should be based on anaesthesia monitoring. Usually a mask is used for maintenance although intubation can be performed allowing improved airway control.

4.3.4 Oxygen may be set at flows of 0.8-1L/min for maintenance depending on the anaesthetic machine and patient size. Higher rates may be utilised for induction and recovery.

4.3.5 The use of a ‘bell jar’ without accurately adjustable flow rates and concentrations should be avoided due to the potential to overdose, underdose, or release vapours into the room.
5. **MONITORING & INTERVENTION**

5.1 Monitoring and responding during anaesthesia

- Surgical depth of anaesthesia in rodents is predominantly assessed by loss of pedal reflexes. This should be done at least every 5 minutes.

- Observations during anaesthesia should include colour of mucous membranes (particularly for abnormal pallor or darkness/cyanosis), pattern and rate of respiration, and blood loss. Pulse oximetry is a common method of detecting oxygen saturation of haemoglobin and the devices are often placed on the tail, ears or footpads.

5.1.1 Procedures which last > 10 seconds

- Body temperature must be maintained with heatpads and insulation (cotton wool/aluminium or bubble wrap), to reduce the risks of hypothermia which can be a cause of anaesthetic death. A thermometer should be used to ensure temperature of warming devices is 27-30 °C. Heatpads should be tested for accuracy at least every 6 months.

- Subcutaneous 0.9% saline or Hartmann’s solution, often warmed to body temperature, should be given at the onset of anaesthesia at around 3-4% of body weight or up to 5% in acute blood loss.

Table 1: Physiologic data without anaesthesia

<table>
<thead>
<tr>
<th></th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (per min)</td>
<td>570</td>
<td>350</td>
</tr>
<tr>
<td>Respiratory rate (per min)</td>
<td>180</td>
<td>80</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.4</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 2: Physiologic data under isoflurane (Constantinides, Mean, & Janssen, 2011; Hacker, White & Black, 2005)

<table>
<thead>
<tr>
<th></th>
<th>C57/BL6 Inbred Mouse</th>
<th>CD® IGS Outbred Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (per min)</td>
<td>478 +/- 11.4</td>
<td>390</td>
</tr>
<tr>
<td>Respiratory rate (per min)</td>
<td>40 +/- 10</td>
<td>62</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>36.3 +/- 2.6</td>
<td>Not available</td>
</tr>
</tbody>
</table>

5.2 Post-procedure monitoring and care

5.2.1 Where possible procedures should not be performed on Fridays as most animal facilities have reduced staff and animal observations on weekends.

5.2.2 Paper towel or tissue should be used instead of sawdust for recovery to prevent obstruction of airways.

5.2.3 Heat should be provided in half the enclosure and animals kept individually housed until the depressant effects of anaesthesia have resolved.

5.2.4 Animals should not be returned to the holding area until they have regained the righting reflex.

5.2.5 Where an animal’s ability to access food and water may be affected in the post-anaesthetic period, food must be moistened or flavoured and placed with water in a more accessible location such as the cage floor to improve nutrition and hydration.

5.2.6 A method of humane euthanasia should be provided in any AEC application that includes anaesthesia, in order to address any potential complications that may arise.

5.2.7 Post-anaesthesia monitoring records should be kept in the room where the animals are housed.

6. **ADDITIONAL INFORMATION**

Not applicable
7. **ENFORCEABLE REQUIREMENTS**

7.1 Performance of the procedure by competent personnel or those under supervised tuition only

7.2 For procedures of > 10 seconds, weighing the animal prior to anaesthesia

7.3 Pedal reflex, respiration and mucous membranes records of animals under anaesthesia kept on a monitoring sheet.

7.4 Use of a heat pad for general anaesthesia.

7.5 Animals observed until righting reflex regained.

7.6 Acceptable skill level must be demonstrated on more than two consecutive occasions before people are permitted to administer general anaesthesia without supervision

8. **EXEMPTIONS**

Where adherence to this Standard conflicts with proposed work, the University’s AECs may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the University’s AWO.

9. **UNEXPECTED ADVERSE INCIDENTS**

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the University’s AWO and relevant Animal Facility Manager must be notified of the event. The AWO will advise researchers of the appropriate response.

10. **GLOSSARY**

<table>
<thead>
<tr>
<th>Scientific Term</th>
<th>Lay Description</th>
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</thead>
<tbody>
<tr>
<td>Cardiorespiratory system</td>
<td>Includes the heart, veins, arteries, lungs, and airways (ie. windpipe, mouth, nose)</td>
</tr>
<tr>
<td>Consciousness</td>
<td>Awareness</td>
</tr>
<tr>
<td>Hartmann’s solution</td>
<td>A solution that has the same salt concentration as blood and is used for supporting blood volume.</td>
</tr>
<tr>
<td>Mucous membranes</td>
<td>Visible areas of mucous membranes in rodents include the mouth (gums) and eyes (conjunctiva). Normal colour is pale pink.</td>
</tr>
</tbody>
</table>

11. **REFERENCES & RESOURCES**

The following source material contributed to the development of this Standard:

- NHMRC. 2008. Guidelines to promote the wellbeing of animals used for scientific purposes.