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.

Handling and Restraint of Mice and Rats

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and approved by the University of Melbourne’s Animal Ethics Committees.

V1 Date of Approval: 7 March 2017
Date of Review: 7 March 2020

1. ASSOCIATED STANDARDS

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

- Administration of substances by oral gavage in mice and rats
- Blood collection in mice and rats
- Humane killing of mice and rats
- Training in non-surgical procedures
- General anaesthesia of mice and rats
- Analgesia

2. SUMMARY

2.1 Mice and rats in the care of animal facilities will frequently require handling or restraint as part of routine husbandry, research or teaching purposes.

2.2 It is essential that handling and restraint techniques be appropriate to the species and given procedure, to ensure that minimal distress is experienced by the animal. Correct handling and restraint techniques benefit both animals and their handlers. Reducing the pain and distress that is experienced by the animal reduces the likelihood of stress-induced physiological changes that may impact research outcomes.

2.3 Restraint is often required to enable safe administration of substances, particularly by injection, or for blood collection. It is essential that trained, competent handlers or those under direct supervision undertake these activities, in order to lessen the negative impact on the mice or rats.

3. BENEFITS & RISKS

3.1 Benefits

3.1.1 Mice and rats can be conditioned to accept gentle handling and appropriate restraint. By creating a positive association with these procedures and the humans involved, they experience less distress, and an improvement in their overall wellbeing.

3.1.2 Correct handling and restraint improves the safety of handlers by preventing bites and scratches, and makes it more likely that procedures can be carried out correctly and safely for the animal.
3.2 Risks

3.2.1 Improper handling or restraint builds a negative association that may result in an unpleasant and distressing experience for the animal. The impact on their welfare is magnified if the negative experience occurs repeatedly, and may cause chronic stress.

3.2.2 Mice or rats that develop an aversion to handling may become aggressive or excessively stressed afterwards and need to be removed from a study group, increasing the total number of animals that are needed to conduct a given project. This directly conflicts with the goal of “Reduction”.

4. PROCEDURE/PROTOCOL

4.1 General principles

4.1.1 Animals should be approached using a calm and confident manner, and sudden or exaggerated movements should be avoided (e.g. waving hands and arms). The environment should be quiet and free of loud or high pitched noises, and handlers should use soft tones and a quiet voice when talking.

4.1.2 When selecting a method of restraint, investigators should ensure it is appropriate for the species and given procedure. Investigators should aim to minimise the length of time an animal is restrained in order to limit the distress or discomfort that it may experience.

4.2 Considerations for handling and restraint

4.2.1 If handling or restraint is likely to cause harm, including pain and distress to the animal, then use of chemical restraint such as sedatives or anaesthesia should be considered. The impact on the animal associated with restraint for administration of the sedative agent should thus be less than the impact of the procedure itself with no sedation.

4.2.2 Whenever possible, animals should be conditioned to the methods used prior to beginning any research, in order to reduce distress associated with the handling or restraint.

4.2.3 If any adverse impact is detected, such as breathing difficulty or loss of consciousness, the animal must be released, or the method of restraint must be modified to minimise that impact.

4.2.4 If prolonged restraint, confinement or isolation of an animal is required as part of a project, including after a procedure, methods used must consider the animal’s physiological and behavioural needs, and ability to exercise. Prior conditioning to these activities may help to negate some of the potential negative impact on the animal, and may include the use of treats (e.g. sunflower seeds).

4.2.5 For procedures that require prolonged restraint or confinement, these must only be performed by competent investigators. In addition, the animals must be assessed regularly by a person with veterinary, or other appropriate qualifications, who are independent of the project. The AEC may request that the Animal Welfare Officer (AWO) or representative be invited to observe the procedures where there is potential to severely impact the welfare of the animals involved.

4.3 Minimising the impact on animals during handling and restraint

4.3.1 In order to minimise the pain and/or distress associated with handling, restraint and procedures, investigators should consider the following:

(i) Select procedures that cause the least amount of tissue damage to reduce the potential painful stimuli.

(ii) Ensure staff are suitably skilled and trained before undertaking work on live animals including blood collection techniques, injections and handling (see JOVE Video in supplementary resources).

(iii) Handle animals regularly, including during routine husbandry, to familiarise them with interaction with humans.

(iv) Consider the use of sedation or anaesthesia to reduce the associated impact of procedures.
4.4 Training

4.4.1 Appropriate training in restraint and handling techniques minimises the pain and distress experienced by the animals. This includes reducing:

(i) Unintentional needle stick injury to internal organs (for the intraperitoneal route)
(ii) Additional irritation and pain at an injection site
(iii) Injury to the animal and the handler
(iv) Incorrect or inaccurate delivery sites, thereby improving animal welfare, human safety and research outcomes.

4.4.2 Trainees must be assessed as competent in handling and restraint of mice and/or rats prior to commencing unsupervised animal work. Competency can be assessed by the project supervisor, Animal Facility Manager (AFM), AWO, or other identified practical trainer (refer 4.6.3, below). Any areas of a project including procedures and general handling/restraint that investigators may require training for should be identified in the ethics application and discussed with the project supervisor.

4.4.3 Practical trainers may provide the content, format and/or competency assessment sheets to the AWO prior to commencement of training. Trainers should have been observed delivering training to an appropriate standard as determined by the AWO or AFM.

4.5 MICE

4.5.1 Handling

4.5.1.1 All investigators and staff should be aware of potentially lower impact or non-physical restraint methods that can reduce animal stress when physical restraint is not required. These include cupping the mouse between both hands, or use of a handling tunnel or similar equipment. It may be preferable to use these types of techniques over more physical contact approaches to handling. Care and time must be taken to allow the animal to acclimatise to the handler and the equipment being used, which will facilitate ease of handling. When more physical methods are needed, mice should be physically restrained with great care (see Restraint section 4.5.2).

4.5.1.2 Investigators must be aware that handling and lifting mice by their tail may induce unnecessary anxiety and stress in the mice, which may alter or affect research outcomes (see J Hurst & R West (2010) Taming anxiety in laboratory mice. Nature Methods 7 (10), 825-842).

4.5.1.3 In special cases of specific pathogen free or immune-compromised mice, forceps can be used to grasp the scruff at the base of the neck and between the shoulders. A 25-30 cm long set of rubber-protected atraumatic forceps can be used.

4.5.1.4 When there is a need to physically handle, visually examine or quickly move a mouse, great care must be taken to ensure both animal and handler safety. Acceptable methods include cupping of the hands or handling the mouse by the base of the tail. Mice should not be lifted and held by the tail for longer than 2-3 seconds.

4.5.2 Restraint

4.5.2.1 At all times the oral mucous membranes and respiration rate should be monitored during and after restraint. If there is any doubt to the respiratory rate or colour of the animal, it should be immediately released and monitored prior to attempting to restrain the animal again (See Video in supplementary resources).

4.5.2.2 If a more detailed examination or procedure is required, the mouse may be picked up individually by gently but firmly grasping the base of the tail and immediately placing the mouse on a surface it can grasp onto (i.e. grid cage top). The mouse is stretched out by gently and slowly pulling from the base of the tail, and the thumb and forefinger of the other hand is used to grasp the skin at the base of the neck (scruff). The hold should be sufficient to prevent the mouse from turning its head to bite, but not so tight as to restrict the mouse from breathing.

4.5.2.3 The technique described above can also be used for more invasive procedures such as intraperitoneal injection or oral gavage. Once the mouse has been scruffed, the body is positioned so the back of the mouse rests in the palm of the hand with the head and belly facing the
It is important the body is fully supported and in a straight and comfortable position for
the mouse.

4.5.2.4 Various injections or substances can be administered once the mouse is comfortable and
secured in the appropriate position (4.5.2.1 or 4.5.2.2 – see Videos in supplementary
resources and other standards on REI website).

4.5.3 Venepuncture

4.5.3.1 Refer to the “Blood collection in mice and rats” Standard

4.5.4 Chemical use or restraint

4.5.4.1 Where a procedure requires prolonged restraint or is expected to cause minor, transient pain
or distress to the animal, investigators should consider the use of sedative or anxiolytic
agents. This includes agents such as acepromazine, xylazine, medetomidine,
bendodiazapines (diazepam, midazolam) or ketamine. It is important to note that many agents
that cause sedation or even anaesthesia do not provide pain relief, so this may still be
required, depending on the procedure. All such procedures on animals must be approved by
the AEC.

4.5.5 Anaesthesia

4.5.5.1 Refer to the “General anaesthesia of mice and rats” Standard

4.6 RATS

4.6.1 Handling

4.6.1.1 Rats may be picked up by gently but firmly grasping the rat around the shoulders. The
handler’s thumb can then be placed under the rat’s mandible (jaw) and the front legs of the
animal allowed to gently cross in front. Using the other hand, gently scoop and offer support of
the hindlimbs. The time that feet are unsupported should be minimised (2-3 seconds) and the
animal should be placed on the arm or another surface as soon as possible.

4.6.1.2 Alternative methods that can reduce animal stress when restraint is not required include
handling tunnels or similar equipment.

4.6.1.3 Due to their larger size and weight, rats should have their body adequately supported when
handled or moved. The handling and movement of rats solely by the tail or tail base
should not be performed, due to the risk of tail and de-gloving injuries.

4.6.2 Restraint

4.6.2.1 If examination or procedures are required, gently but firmly grasp the rat around the shoulders.
The handler’s thumb can then be placed under the rat’s mandible (jaw) and the front legs of the
animal allowed to gently cross in front of each other. Using the other hand, gently scoop
and offer support of the hindlimbs. For greater restraint during examination or procedures,
place the hand over the back of the animal with the thumb and forefinger placed over the area
of the neck and close to its ears. Gently proceed to scruff the animal from the neck down the
back, as long as the length of the handler’s palm. The scruff should be sufficient to prevent the
rat from turning its head to bite, but not so tight as to restrict the rat from breathing.

4.6.2.2 The oral mucous membranes and respiration rate should be monitored during and after
restraint. If there is any doubt to the respiratory rate or colour of the animal it should be
immediately released and monitored prior to attempting to restrain the animal again (See
Video in supplementary resources).

4.6.2.3 Handlers must avoid handling and manipulating rats by the base of the tail. Rats must never
be handled, restrained or lifted by the distal (end) portion or of the tail.

4.6.3 Venepuncture

4.6.3.1 Refer to the “Blood collection in mice and rats” Standard
4.6.4 Chemical use or restraint

4.6.4.1 Where a procedure requires prolonged restraint or is expected to cause minor, transient pain or distress to the animal, investigators should consider the use of sedative or anxiolytic agents. This includes agents such as acepromazine, xylazine, medetomidine, benzodiazepines (diazepam, midazolam) or ketamine. It is important to note that many agents that cause sedation or even anaesthesia do not provide pain relief, so this may still be required, depending on the procedure.

4.6.5 Anaesthesia

4.6.5.1 Refer to the “General anaesthesia of mice and rats” Standard

5. MONITORING & INTERVENTION

5.1 Monitoring during restraint and handling

5.1.1 Investigators should monitor animals for general signs of physiological and psychological distress, including (but not limited to):
- Ruffled and unkempt fur
- Abnormal posture/hunching
- Reduced food intake
- Weight loss
- Teeth grinding
- Increased vocalisation
- Changes in movement and behaviour
- Self-isolation
- Abnormal respiratory pattern or effort
- Porphyrin (red) tear production in rats

5.1.2 Animals displaying signs of illness prior to being handled are at greater risk of stress and should be handled with greater care.

5.1.3 An adverse response to handling or restraint may be displayed as signs of pain or distress in rats and mice. In particular, increased vocalisation, biting or aggression toward humans and/or conspecifics, increased avoidance of handlers and demonstrable escape and ‘freezing’ behaviours may be displayed.

5.2 Monitoring

The following are potential undesirable or adverse effects following restraint:
- Increased respiratory and heart rates from stress
- Absence or decrease of normal behaviors (i.e. grooming, eating, sleeping, play)
- Trauma with bruising/swelling/fracture
- Strangulation leading to respiratory distress
- Death due to trauma, stress or prolonged respiratory distress

5.2.1 Monitoring must be done for the first 5 minutes immediately after handling, restraint and procedures such as blood collection and include: assessment for further bleeding, expected side effects post-experimental procedures, activity, movement/locomotion, skin colour (blue membranes indicate cyanosis) and respiration rate. If abnormal signs occur during handling, restraint or procedure the animal should be immediately released.

5.2.1.1 If abnormal signs occur the animal must be monitored for an additional 5 minutes. Animals should then be checked ideally twice more during the day.

5.2.1.2 If abnormal signs persist or recur, then increased monitoring may be required, and the AFM, AWO and/or the Project Supervisor should be contacted.
5.2.2 Monitoring for Anaemia

5.2.2.1 Monitoring techniques for animals that may be prone to anaemia from blood loss can include: haematology (packed cell volume, haemoglobin, red cell count, reticulocyte count), blood pressure, mucous membrane colour, weight loss (chronic), activity levels and respiration rate. Animals prone to anaemia would include those subjected to repeat sampling within 10% of the NHMRC maximum amounts and frequencies (eg. 6.3-7% blood volume collected weekly, 9-10% blood volume collected every 2 weeks) and those animals where greater than normal or anticipated blood loss occurred.

5.2.2.2 Monitoring of these animals must be a minimum of twice weekly including the day after blood collection. Twice daily monitoring should be performed for those with expected clinical signs of anaemia.

5.2.2.3 Animals with moderate signs of anaemia should be given parenteral fluids and monitored twice daily. For rodents this can be 3-4 mL of warm sterile fluids (0.95% NaCl or Lactated Ringer’s Solution) per 100 g body weight by subcutaneous injection. Animals with severe signs should be euthanised.

6. ADDITIONAL INFORMATION

6.1 Research Ethics and Integrity (REI) provides Animal Welfare and Ethics training to all new researchers which offers an overview of the animal welfare considerations that may apply to their project. Investigators should consider how handling, restraint and procedures may impact an animal’s wellbeing.

6.2 One of the AECs roles is to consider the cumulative burden that repeated procedures, including handling and restraint, may have on the animal. It may be of use to investigators to map out the individual experience of each animal (or group of animals) to better understand the potential impacts of their project. This information should be included in the ethics application where appropriate.

7. ENFORCEABLE REQUIREMENTS

7.1 Performance of the procedure by competent investigators or trainees under the direct supervision of competent investigators.

7.2 Contact the AFM or AWO if three unsuccessful attempts on an individual animal.

7.3 Monitor the animals as above (Section 5) for a minimum of 5 minutes immediately after handling, restraint and procedures, and further monitoring of the animal as required.

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 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 AWO and relevant AFM must be notified of the event. The AWO will advise researchers of the appropriate response.

In addition, a necropsy must be performed on any animal whose illness or death constitutes an unexpected adverse event. The body of an animal found deceased, or humanely killed as a consequence of an unexpected adverse event, must be refrigerated and the necropsy performed in a timely manner to provide for accurate and reliable results. A full necropsy report as well as any relevant photographs and external laboratory results should be submitted to the AWO alongside the adverse event report.
10. GLOSSARY

<table>
<thead>
<tr>
<th>Scientific Term</th>
<th>Lay Description</th>
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<tbody>
<tr>
<td>Anaemia</td>
<td>Decrease in the amount of red blood cells or hemoglobin in the blood to below normal levels</td>
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<tr>
<td>Anxiolytic Agents</td>
<td>A drug used to relieve anxiety</td>
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<tr>
<td>Atraumatic</td>
<td>A medical or surgical procedure causing minimal tissue injury</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>A bluish discoloration of the skin or mucous membranes due to poor circulation or inadequate oxygenation of the blood</td>
</tr>
<tr>
<td>Haematology</td>
<td>The study and treatment of the blood</td>
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<tr>
<td>Mucous Membranes</td>
<td>Abbreviated as 'mm'. Typically refers to the gums or underside of the lips, which is normally pink. Colour may change and become pale or dark in various conditions.</td>
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<tr>
<td>Parenteral</td>
<td>Administration of a substance in the body other than the mouth and alimentary canal</td>
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<tr>
<td>Porphyrin</td>
<td>The excessive production and accumulation of pigmented (rust-colored/ orange-red/pink) eye and nasal secretions</td>
</tr>
<tr>
<td>Scruff</td>
<td>The skin and surrounding area around the back of an animal's neck</td>
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11. REFERENCES & RESOURCES

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

- National Centre for the Replacement, Refinement & Reduction of Animals in Research. https://www.nc3rs.org.uk/handling-and-restraint#references
- National Health and Medical Research Council (NHMRC). 2008. Guidelines to promote the wellbeing of animals used for scientific purposes.
- Chapter 3 Handling the Laboratory Rat by George J. Krinke accessed 14/07/21/016 https://www.usp.br/bioterio/Artigos/Procedimentos%20experimentais/HandlingThe_Laboratory_Rat-By_George_J_Krinke.pdf
- Chapter 31 Handling the Laboratory Mouse by George J. Krinke accessed 14/07/2016 https://www.usp.br/bioterio/Artigos/Procedimentos%20experimentais/Handling-3.pdf

The following resources may provide additional or supplementary information:

- Blood and Biochemistry reference levels for laboratory animals https://www.ahc.umn.edu/rar/refvalues.html
- Chapter 3 Handling the Laboratory Rat by George J. Krinke accessed 14/07/21/016 https://www.usp.br/bioterio/Artigos/Procedimentos%20experimentais/HandlingThe_Laboratory_Rat-By_George_J_Krinke.pdf
- Chapter 31 Handling the Laboratory Mouse by George J. Krinke accessed 14/07/2016 https://www.usp.br/bioterio/Artigos/Procedimentos%20experimentais/Handling-3.pdf