

ANIMAL CARE AND USE STANDARD

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, 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.

ADMINISTRATION OF SUBSTANCES BY ORAL GAVAGE IN 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.

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1. ASSOCIATED STANDARDS

There are no associated Standards.

2. SUMMARY

- Oral gavage is the administration of a substance via the oral cavity, using a feeding needle or tube, into the lower oesophagus or stomach. Once introduced, the substance to be dosed is slowly expelled, and the tube is withdrawn slowly.
- A commonly used method of administration, it is generally safe if performed with care by competent persons.

3. BENEFITS & RISKS

- The main advantage of oral gavage over other methods of oral administration is that it is the most accurate and reliable method for administering substances into the gastro-intestinal tract, as it eliminates risks of variability in intake between individual animals (which may arise when substances are administered through delivery in food and/or water).
- Compared with other methods of oral administration, it is more invasive, more difficult and stressful (handling, restraint and introduction of equipment into the animal is required). This is associated with a higher risk of complications, especially if repeated or chronic dosing is necessary.

4. PROCEDURE/PROTOCOL

4.1 Restraint

- Generally manual restraint of the animal is sufficient to enable the procedure to be carried out. The benefit of performing oral gavaging in conscious animals is that the swallowing reflex can be easily observed as the tube is passed, which helps indicate that the tube has entered the correct anatomical tract.
- Successful execution of the procedure requires acclimatisation of the animals to handling before the study and investigator training to competency in manual handling and restraint of animals and in the procedure itself. This will ensure that any stress experienced by both the animal and the person carrying out the procedure is minimised. Additional training is required where animals are pregnant, young or have a condition which may impact the method of gavaging.

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- The use of general anaesthesia for restraint of an animal during gavage introduces an additional procedure to the animal that carries its own risk. Therefore in line with the 3Rs, this should be avoided where possible, unless express approval has been given by the AEC and the AWO.

4.2 Gavage equipment

- Good preparation and maintenance of equipment is essential.
- A range of sizes and types of equipment can be acceptable, as follows:
- Both stainless steel gavage needles and flexible catheters are acceptable, provided they are of a suitable size and length for the size of the animal.
- Both metal gavage needles with or without bulbs at the end are acceptable, again as long as they are of a suitable size and length for the size of the animal. Note that gavage needles with bulbs are generally considered safer because they are rounded and smoothed, and may therefore help to minimise risk of accidental tissue trauma as it is passed through the oesophagus.
- For repeated dosing, flexible tubes may be safer than metal tubes, although both are acceptable.
- Both curved and straight gavage needles are acceptable.
- In mice, 18-22 gauge gavage tubes are generally used, with smaller tubes (higher gauge) used for smaller mice.
- In rats, 18 gauge soft gavage tubes are generally used with a range of 16-20 gauge depending on the size of the rat.
- Checking the appropriateness of the length is also important to make sure it reaches the stomach and not just the oesophagus. The tube should extend from the tip of the nose to the last rib. Marking the tube in advance can be helpful; use of cadavers for this purpose before the study may be helpful. Whilst inserting the tube advancement must immediately be halted if resistance is felt regardless of how deep the tube has been inserted.
- Stainless steel gavage tubes must be checked before use to ensure that sharp scratches have not developed in the surface over time from use, which may predispose to oesophageal trauma.
- Stainless steel gavage tubes must be cleaned and sterilised by autoclaving prior to the day of use. Stainless steel tubes must also be disinfected with alcohol between cohorts on the same day.
- Flexible gavage tubes must only be used on a maximum of one cohort on the same day before disposal. Where flexible tubes are used on more than one animal, they must be checked closely between animals and discarded if any damage is visible.

4.3 Substance

- The smallest possible volume of substance should be used. In any case, the maximum volume should not exceed 10ml/kg (10µl/g).
- The properties of the substance, including possible toxicity and irritancy, should be known from previous in vitro and/or in vivo studies and must be reported in the animal ethics application. Refrigerated substances must be warmed to body temperature or, less preferably, room temperature, where this doesn't impact the stability of the substance.

4.4 Dosing Frequency, and Repeated or Prolonged Administration Periods

- The gavage needle must be inserted gently avoiding excessive force. Where an animal is excessively struggling or the gavage needle is unable to be easily advanced, the animal must be replaced into its home cage and observed until there are no signs of pain or distress.
- In line with the 3Rs, the fewest possible number of doses should be used, particularly because the risk of complications are increased with repeated use of this technique in the same animal.
- It is acknowledged that there is currently no evidence-based recommendation available for maximum number of gavage doses that can be safely given per day on an individual animal, nor the maximum number

of days an animal can be gavaged.

- However, as a general guide for the University, it is recommended that animals should not be gavaged more than twice a day for more than a week, or once a day for more than a month.
- For repeated gavaging, the gavage needle should be dipped into a concentrated solution of sucrose (approx. 1g/ml) before each use as a refinement measure to reduce stress-related reactions. An exemption will be granted for projects where this will not be appropriate due to experimental result or health impacts, eg. in diabetic animals. Food rewards following the procedure are strongly recommended as another method of positive reinforcement.
- In cases where higher frequencies of dosing within a day, or over a prolonged period is required, alternative methods of oral dosing such as incorporation into food or water should be considered as alternative options. Osmotic minipumps may also be considered where intravenous or subcutaneous routes can be substituted for oral administration.

5. MONITORING & INTERVENTION

- Animals should be monitored closely for at least 15 min after an oral gavage and should also be checked 4-12 hours after the procedure on the same day and again the following day. After this, routine monitoring may be resumed.
- Complications may be acute (eg. choking) or damage may lead to signs that do not appear until much later.
- Complications may include:
 - Inadvertent tracheal administration
 - Oesophageal trauma or perforation
 - Oesophageal impaction
 - Gastric rupture
 - Aspiration pneumonia
- Signs associated with complications can range from general signs such as those listed on the Office for Research Ethics & Integrity (OREI) monitoring sheet template to specific signs. The above complications may lead to: dyspnoea, blood on the gavage tube, melena, abdominal pain (eg. belly pressing), or throat pain.
- Adverse signs such as severe dyspnoea or blood on the gavage tube should lead to immediate euthanasia. If mild dyspnoea does not resolve within 10 minutes the animal must be euthanased. For chronic problems, adverse signs must prompt input from the Animal Facility Manager and the Animal Welfare Officer.

6. ADDITIONAL INFORMATION

- N/A

7. ENFORCEABLE REQUIREMENTS

- Performance of the procedure by competent investigators or trainees under the direct supervision of competent investigators
- Performance of the procedure must be on un-anaesthetised animals
- Use of appropriate equipment as described above
- Limitation to the volume of liquid delivered (<10ml/kg)
- Limitation of the frequency and duration of gavage to those recommended above
- The use of sucrose for repeated gavage protocols
- Adherence to monitoring described above

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's Animal Ethics Committees 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

| Scientific Term | Lay Description |
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| Aspiration | Material inadvertently entering the lungs |
| Aspiration pneumonia | Pneumonia developing as a result of aspiration |
| Direct supervision | In the same room and visually observing the procedure |
| Dyspnoea | Difficulty breathing which may include changes in the rate or depth |
| Oesophageal impaction | A blockage in the oesophagus due to local inflammation |
| Oesophagus | A muscular tube connecting the throat with the stomach |
| Sepsis | A life-threatening whole-body inflammatory response to an infection |
| Trachea | Windpipe, a tube that connects the throat to the lungs, allowing the passage of air |

11. REFERENCES & RESOURCES

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

- Harkness JE, Turner, PV & Vandewoude S (2010). Harkness and Wagner's biology and medicine of rabbits and rodents. John Wiley & Sons (pp187-8)
- Machholz E, Mulder G, Ruiz C, Corning BF & Pritchett-Corning KR (2012). Manual restraint and common compound administration routes in mice and rats. *Journal of Visualised Experiments*, 67

The following resources may provide additional or supplementary information:

- Newcastle University. Oral gavage in the mouse. Available: <http://www.procedureswithcare.org.uk> (video)
- Newcastle University. Oral gavage in the rat. Available: <http://www.procedureswithcare.org.uk> (video)
- OREI Monitoring sheet template (excludes specific signs associated with gavaging). Available: <http://www.orei.unimelb.edu.au/content/forms-templates-guidance-documents>