Oral Dosing (Gavage) in Adult Mice and Rats SOP

Purpose
This Standard Operating Procedure (SOP) describes the procedure of orally dosing rats and mice. This procedure will administer the compound into the distal esophagus where it will then enter the stomach. This SOP follows the UBC and CCAC guidelines for acceptable oral dose volumes in rodents.

Responsibility
Trained persons listed on an approved Animal Care Committee protocol performing the procedure.
All animal users orally dosing rodents must have successfully completed the UBC Animal Care Services (or equivalent) Rodent Biology and Husbandry course.

References
Canadian Council on Animal Care (CCAC) guidelines (www.ccac.ca)
Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189662/)

Materials
Correctly sized metal gavage/feeding needles (see chart below)
Correctly sized plastic gavage/feeding needles (see chart below, ball diameter n/a)
Red Rubber Flexible Feeding Tube - 8fr (Rats only)
Appropriate sized syringes
Solution/compound to be administered
Optional: towel for wrapping rats if using flexible red rubber feeding tubes
**Recommended Gavage Needle Size and Maximum Volume of Administration***

<table>
<thead>
<tr>
<th>Species</th>
<th>Gauge</th>
<th>Length</th>
<th>Ball Diameter</th>
<th>Volume</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>24-20G</td>
<td>2.5 - 3.8 cm (1.0-1.5&quot;)</td>
<td>1.25-2.25 mm</td>
<td>&lt;10 ml/kg</td>
<td>For a 25 gm mouse, the maximum volume would be 0.25 ml</td>
</tr>
<tr>
<td>Rat</td>
<td>20-16G</td>
<td>3.8 – 10 cm (1.5-4&quot;)</td>
<td>2.25-4.0 mm</td>
<td>&lt;10 ml/kg</td>
<td>For a 250 gm rat, the maximum volume would be 2.5 ml</td>
</tr>
</tbody>
</table>

*Greater than the recommended volumes should not be given unless justified and approved on the Animal Care Protocol and increased monitoring for complications implemented.

**PROCEDURE**

**a. Preparation**

1. Weigh the animal and calculate the maximum volume that can be administered (refer to table above).
2. Choose the gavage needle best suited for your use. There are metal and flexible plastic feeding needles in various sizes for mice and rats. Metal gavage needles are typically easier to use in mice since they cannot bite through the tube but can easily damage the esophagus if the mouse struggles. Straight or curved metal gavage needles are available and the choice depends on personal preference and experience. Flexible plastic gavage needles (mice or rats) or red rubber feeding tubes (for rats) have less chance of damaging the esophagus but animals can bite through them and they require some practice to use effectively.

![Metal Gavage Needles](image1.png) ![Plastic Gavage Needles](image2.png) ![Red Rubber Feeding Tube for Rats](image3.png)

3. Prior to performing the oral gavage procedure, measure the distance from the oral cavity to the end of the xyphoid process (caudal point of the sternum) with the feeding needle/tube on the outside of the restrained animal (see photos below). This will be the distance the needle will be inserted into the esophagus. Mark this distance on the needle using a permanent marker or a small piece of tape.
**NOTE:** If using a needle that is too long, there is a greater chance of injuring the animal by damaging the esophagus. Using a needle that is too short may cause the animal to aspirate the substance.

**Rat – measuring the distance from oral cavity to xyphoid process**

4. Pre-fill the syringe and gavage needle/tube with the correct volume of the compound to be administered. Wipe the outside of the needle/tube to remove any of the compound coating the outside of the needle/tube (this ensures correct dosing as well as prevents the animal from tasting potentially bitter compounds).
b. Restraint (see “Proper restraint and initial placement of Gavage Needles/tubes” pictures below).

5. **MICE**: Gently remove the animal from the cage and firmly restrain the animal in an upright position. Get a good scruff of skin over the mouse’s shoulders, so that the front legs are extended out to the side and the head and neck are immobilized. Ensure the animal can breathe freely (watch to see if the chest is moving).

**RATS**: Gently remove the animal from the cage and, if using a metal or plastic gavage needle, restrain the rat in an upright position, using either a v‐hold or crossover to immobilize the head and neck. Ensure the animal can breathe freely. If using a flexible red rubber feeding tube, the rat can be gently restrained by hand or by wrapping in a towel while they are sitting flat on a table or counter.

**Proper restraint and initial placement of Gavage Needles/tubes**

![Rat V‐Hold restraint of rat for passage of metal gavage needle. Gavage needle is inserted into the left side of the animal’s mouth and directed along the hard palate of the mouth to the back of the throat.](image)

![Gentle restraint of rat for passage of red rubber feeding tube. The rat could also be wrapped in a towel to make the control of the front legs easier. Gavage tube is inserted into the left side of the animal’s mouth and gently slid down the esophagus to the proper distance.](image)

![Mouse Gentle scruff of mouse for passage of the metal gavage needle. Gavage needle is inserted into the left side of the animal’s mouth and directed along the hard palate of the mouth to the back of the throat.](image)
c. Gavage procedure (see “Advancing the Gavage Needle down the Esophagus” pictures below).

6. **Gavage Needle (Metal or Plastic):** Slide the end of the gavage needle into the left side of the animals’ mouth behind the front teeth and in front of the first molar (the “diastema”), along the roof of the animal’s mouth slightly towards the animal’s left side (you may feel the ridges of the hard palate as you slide the needle back). Once the gavage needle is at the back of the mouth (the animal usually “gags” at this stage), gently tilt the head back towards the spine with gentle pressure from the gavage needle. This allows the esophagus to be in a straight line to the stomach. There should be no resistance when passing the gavage needle. The gavage needle should slide down the esophagus with gravity alone. The gavage needle may need to be twisted clockwise slightly as it passes the epiglottis and into the esophagus. Pass the needle into the esophagus until the pre-marked line reaches the mouth.

**Red Rubber Feeding Tube (rats only):** Wrap the rat loosely in a towel or hold the rat on a table or counter so that its front feet cannot grab hold of the feeding tube. Slide the feeding tube into the left side of the rat’s mouth, over the tongue to the back of the throat. The tube should easily slide down the esophagus, as the animal is either sitting flat on the counter or slightly elevated with the esophagus in a straight line with the stomach. Pass the needle into the esophagus until the pre-marked line reaches the mouth. The rat will often try to pull the tube out with its front paws, so gentle restraint of the front limbs is necessary.

**NOTE:** If there is any resistance or if the animal struggles excessively remove the gavage needle/tube immediately and ensure you have good restraint on the animal before attempting to pass the needle again. Do not force the needle/tube down the esophagus.

**Advancing the Gavage Needle down the Esophagus**

![Image of Gavage Needle in Rat](image1)

Mouse

![Image of Gavage Needle in Mouse](image2)

**Gavage Needle press**

Once the metal tube is at the back of the mouth, gently tip the animal's head back towards its spine so that the head and neck are in a straight line without resistance. Ensure the animal can breathe freely.
7. Once the feeding needle or tube is at the pre-measured distance, ensure the animal is breathing normally. If the needle or tube is in the trachea, the animal will struggle to breathe. If the animal is breathing normally, inject a very small “test” dose (~0.05 ml). If there is no change in breathing effort, then slowly inject the solution (over 2-3 seconds) to minimize the fluid coming back up the esophagus. If injecting an oily or viscous substance, use the smallest volume possible and inject more slowly (over 5 – 10 seconds) and remove the needle slowly so none of the substance is brought back up the esophagus into the back of the throat. Inhaling any oily substance into the lungs will result in the death of the animal.

**NOTE:** If the animal is not breathing normally, immediately remove the gavage needle from the esophagus and release the restraint.

8. When the entire substance is administered, remove the feeding needle slowly, in the opposite direction from insertion and return the animal to its cage.

9. Monitor the animal for at least 10 minutes, to observe for potential complications (see below). If there were any complications such as: obvious aspiration or excessive struggling, monitor the animal until it is acting normally before leaving it for the day.

d. **Cleaning instructions for Needles and Feeding Tubes:**

1. Metal gavage needles should be wiped with gauze between animals. Clean with soapy water, rinse well and allow to dry before storing.
2. Plastic gavage needles are usually meant to be single use. Discard after each animal.
3. Red Rubber Feeding tubes can be re-used. Wipe with gauze between animals. Rinse with water when finished using. Then soak in soapy water for ~ 20 minutes. Rinse again with water and allow tubes to dry before storing.

**NOTE:** Before using plastic tubes again, assess for any cracks or bite marks in the tube. Flush with water and if any leaks are seen, discard and do not use. Ensure water flows easily and there is no debris built up in the tube.

e. **Complications**

1. Insertion of needle and administration of substance into the trachea/lungs.
2. Aspiration of solution into lungs due to: needle length being too short, not pre-measuring to ensure the correct length of needle is inserted or regurgitation.
3. Perforation of the esophagus, trachea or lungs, due to improper restraint and excessive struggling by the animal.
4. Damage to the cardiac sphincter (tissue protecting opening into stomach).
5. Damage to the oral cavity.
6. Esophagitis (inflammation of the esophagus).
7. Other traumatic injuries related to improper animal restraint.
8. Death.
Clinical Signs of complications (requiring close monitoring and possibly euthanasia if signs do not resolve with a few hours):

- Respiratory distress/dyspnea (increased respiratory rate and effort)
- “Noisy” breathing or clicking when breathing
- Pale or blue extremities
- Hunched appearance
- Squinted eyes (eyes held partially closed)
- Piloerection
- Blood at nose or mouth
- Swelling of neck or under front legs (due to air or fluid escaping from damaged esophagus)
- Loss of weight due to inability to swallow