The purpose of this Standard Operating Procedure (SOP) is to describe the method of oral gavage in rats.

1. **Introduction and Definitions:** To administer precise amounts of liquid diet, drugs or test compounds orally to rats using the gavage method. To minimize stress to the animal the person must be skilled in the gavage technique before starting a study.

   The choice of whether to use a rigid or flexible gavage needle or to use a straight or curved gavage needle is according to operator preference and the needs of the study. Gavage needles are available in disposable plastic or reusable stainless steel. All gavage needles have a ball or pear-shaped smooth rounded tip to prevent injury to the esophagus and other tissues.

2. **Materials:**
   - Appropriate feeding needles (also known as gavage needles or feeding tubes)
   - Sterile syringe (1-10ml)
   - Scale
     - The volume to be administered will depend on the weight of the animal. The volume should not exceed 1% (10ml/kg) of the animal’s body weight (e.g. 500g = 5ml).
   - Marker
   - Isoflurane anesthetic as needed

<table>
<thead>
<tr>
<th>Weight range (g)</th>
<th>Gauge</th>
<th>Length (inches)</th>
<th>Ball diameter (mm)</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-75g</td>
<td>20</td>
<td>1”, 1 ½”</td>
<td>2 ¼ mm</td>
<td>Straight, curved</td>
</tr>
<tr>
<td>75-100g</td>
<td>18</td>
<td>1”, 1 ½”</td>
<td>2 ¼ mm</td>
<td>Straight, curved</td>
</tr>
<tr>
<td>100-200g</td>
<td>18</td>
<td>2”, 3”</td>
<td>2 ¼ mm</td>
<td>Straight, curved</td>
</tr>
<tr>
<td>200-300g</td>
<td>16</td>
<td>2”, 3”</td>
<td>3 mm</td>
<td>Straight, curved</td>
</tr>
<tr>
<td>&lt;300g</td>
<td>16</td>
<td>3”, 4”</td>
<td>3 mm</td>
<td>Straight, curved</td>
</tr>
</tbody>
</table>

3. **Procedures:**
   - Set up work surface with materials.
   - Draw the desired amount of liquid compound into the syringe with the gavage needle in place.
   - Identify the rat. Depending on the size, pick it up with one hand and restrain the rat against your body using the “v-hold” ensuring that its head and neck are stabilized. Smaller animals can be held using standard scruff technique.
Take care that you are not restraining the rat so tightly that its breathing is impaired.

Prior to performing the procedure, measure the distance from the oral cavity to the tip of the xyploid process. This is where the stomach lies. Mark this distance on the feeding needle. Do not advance the needle further than this point. When gavaging, the tip of the needle is to be positioned just below the stomach’s cardiac sphincter.

With the rat’s head moderately extended in vertical alignment, gently insert the needle into the lateral side of the mouth behind the teeth.

The needle is then advanced gently along the upper palate towards the back of the throat. Slight pivoting of curved needles will help feed the needle past the epiglottis and into its correct midline placement (esophagus).

The rat may exhibit a swallowing reflex at this point.

Once the esophagus is reached, gravity should be used to help guide the needle as it slip down into the esophageal tract.

Forcing the needle can cause damage to the esophageal wall or force the needle into the trachea. If the animal is struggling, it may not be inserted properly and should be carefully removed. Allow the animal to rest before trying the procedure again. No more than three attempts are allowed.

Once in position, inject the fluid slowly to prevent it from coming back up into the oral cavity or rupturing the esophagus. If the animal starts to cough or choke, stop injecting, remove the needle and allow the animal to recover in its cage. Do not attempt again for a minimum of 24 hours.

Once administered, remove the needle gently, following the same angle as insertion.

Place animal back in cage and monitor for 10 minutes.

Photo credit: UBC Animal Care Guidelines

**Serial daily gavage may include brief isoflurane anesthesia for the animal prior to needle insertion and compound administration. Animal must have righting and swallowing reflex before return to home cage.**
4. Complications
Improper gavage technique can lead to several complications, acute or delayed. These may include:
- Esophagitis (inflammation of the esophagus)
- Perforation of the esophagus, trachea or lungs
- Damage to the cardiac sphincter (upper stomach sphincter)
- Insertion of needle and solution into the lungs/inadvertent tracheal administration
- Lung perforation
- Damage to the oral cavity
- Aspiration of solution into the lungs from regurgitation (needle is too short)
- Traumatic injuries related to improper restraint
- Gastric rupture
- Esophageal impaction
- Aspiration pneumonia

5. Clinical Signs of Complications
Requiring close monitoring and possible euthanasia if not resolved within a few hours, or at the recommendation of the Veterinarian team:
- Respiratory distress/dyspnea (increased respiratory rate and effort)
- Blood on the needle
- “Noisy” breathing or clicking when breathing
- Pale or blue extremities
- Hunched appearance
- Squinted eyes
- 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

References:
2) https://iacuc.wsu.edu/documents/2016/06/wsu_sop_10.pdf/
3) Vol 55, No 6 Journal of the American Association for Laboratory Animal Science November 2016, Carissa P Jones,* Kelli L Boyd, and Jeanne M Wallace, Evaluation of Mice Undergoing Serial Oral Gavage While Awake or Anesthetized

Revised: February 28, 2019 / February 28, 2022