

UBC ANIMAL CARE COMMITTEE

TECH 09a - Oral Dosing (Gavage) in Adult Mice SOP

Last date revised: February 2021

Date approved:

Version No. 2

PURPOSE:

- To describe the procedure for the administration of substances by the oral dosing (gavage) route in adult mice following the Canadian Council on Animal Care (CCAC) current guidelines for acceptable volumes and administration routes in rodents.

RESPONSIBILITY:

- All animal users performing oral dosing in rodents must have successfully completed the UBC Animal Care Services (or equivalent) Introduction to Working with Rodents in Research (IWRR), Rodent Restraint/SQ/IP injections (RSCIP) and Rodent Oral Dosing/Gavage courses.
- Only trained persons who are listed on an approved Animal Care Committee (ACC) Animal Care Protocol are responsible for performing oral dosing according to this SOP.

MATERIALS: *(can be purchased from Animal Care Services).*

- Weigh Scale
- Appropriately sized metal or plastic gavage tube (see Table 1 below)
- Appropriately sized syringes for volumes to be administered (see Table 1 below)
- Solution/compound to be administered
- 2" x 2" Gauze

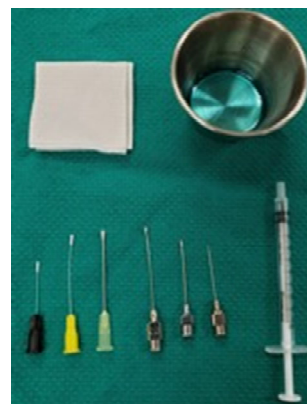


Table 1 -Recommended Gavage Needle/Tube Size and Maximum Volume of Administration

Metal Gavage Needles/Tubes				
Species	Gauge	Length	Ball Diameter	Maximum Volume*
Mouse	< 14 gm: 24 G	< 14 gm: 2.5 cm (1 inch)	<14 gm: 1.25 mm	10 ml/kg
	>14 gm: 22-20 G	>14 gm: up to 3.8 cm (1.5 inch)	>14 gm: up to 2.25 mm	
	For most adult mice 22 G is most common	For most adult mice 3.8 cm is most common	For most adult mice, 2.25 mm is most common	
Flexible Plastic Gavage Tubes				
Species	Gauge	Length	Tip Diameter	Maximum Volume*
Mouse	< 14 gm: 24 G	< 14 gm: 2.5 cm	<14 gm: 1.2 mm	10 ml/kg
	>14 G: 22-20 G	>14 gm: up to 3.8 cm	>14 gm: up to 1.6 mm	
	For most adult mice 22 G is most common	For most adult mice 3.8 cm is most common	For most adult mice, 2.25 mm is most common	

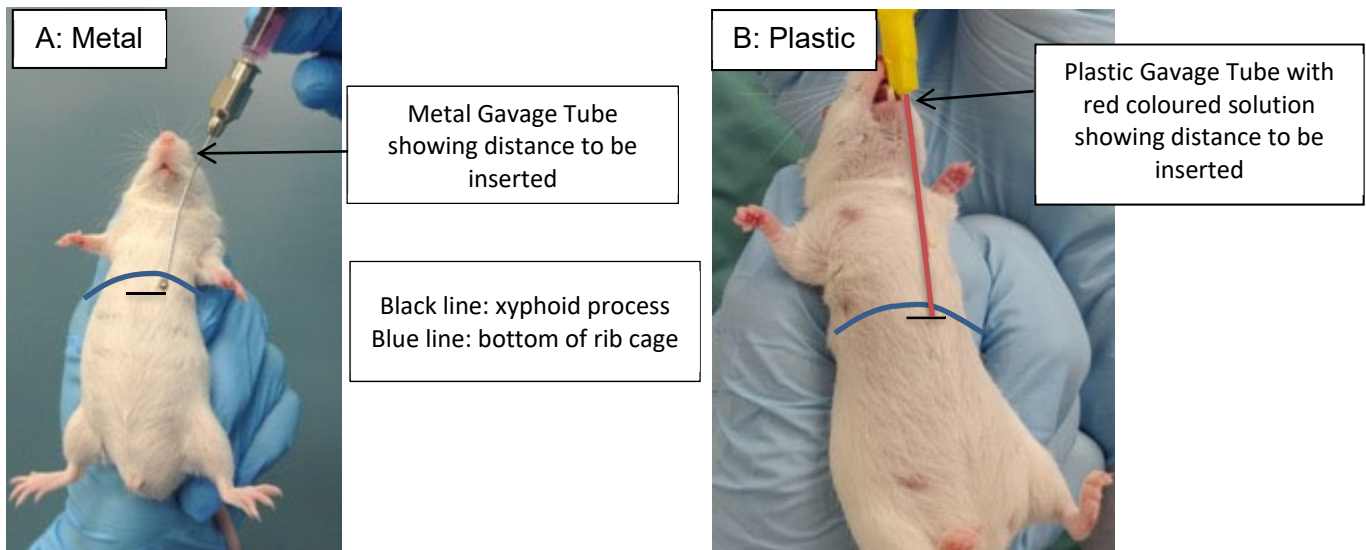
*The lowest volume possible should be administered. Greater than the recommended volumes of drugs should not be given unless justified and approved on the Animal Care Protocol and increased monitoring for complications implemented.

In the following procedures, the words feeding needle, gavage needle and gavage tube are synonymous.

PROCEDURE:

1. Weigh the animal and calculate the volume to be administered (refer to Table 1 for maximum recommended volume and Page 5 for how to calculate volume).
2. Measure the distance from the oral cavity or tip of nose to the end of the xiphoid process (caudal point of the sternum) or last rib with the gavage tube placed on the outside of the restrained animal. This will be the distance the tube will be inserted into the esophagus. See Figures 1A and 1B.
 - a. Tip: Mark this distance on the tube using a permanent marker or a small piece of tape so this distance can be used on all mice of the same strain and weight.
 - b. **NOTE:** *If using a gavage tube that is too long, there is a greater chance of injuring the animal if the tube is inserted fully. Using a gavage tube that is too short may allow the substance to enter the oropharynx and cause the animal to aspirate the substance into the lungs.*

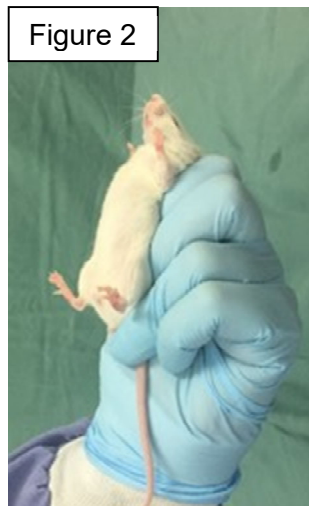
Figure 1



3. Pre-fill the syringe and gavage tube with the correct volume of the compound to be administered.
4. Wipe the outside of the gavage tube with gauze to remove any of the compound coating the outside of the tube (to ensure accurate dosing and prevent animal from tasting potentially bitter compounds).

****Tip:** If permitted on the study protocol, dipping the end of the gavage tube in a sweet substance, such as sugar water, can stimulate licking and swallowing to improve the gavage process so rodents better tolerate the procedure, especially with repeat gavaging.
5. Restrain the mouse in an appropriate manner.
 - a. "Scruff" the mouse (gather the skin) with your non-dominant hand and firmly restrain the animal in an upright position facing the handler.

- b. Ensure an adequate restraint is achieved so that the front legs are extended out to the side and that the **head and neck are immobilized** (cannot move up or down or side to side). Ensure the animal can breathe freely (observe if the chest is moving and the nose and feet remain pink). See Figure 2.



6. If restraint has taken more than a few minutes, immediately before performing the gavage, ensure the plunger in the syringe is moving freely. Pull back on the plunger then eject any air out of the gavage tube.
7. Insert the end of the gavage tube into the left side of the animal's mouth in the gap behind the upper incisors and in front of the first molar (the "diastema"). For curved metal gavage tubes, ensure the concave curve of the tube is facing the mandible (lower jaw) of the mouse so that it will follow the curve of the oropharynx. See Figures 3A and 3B.

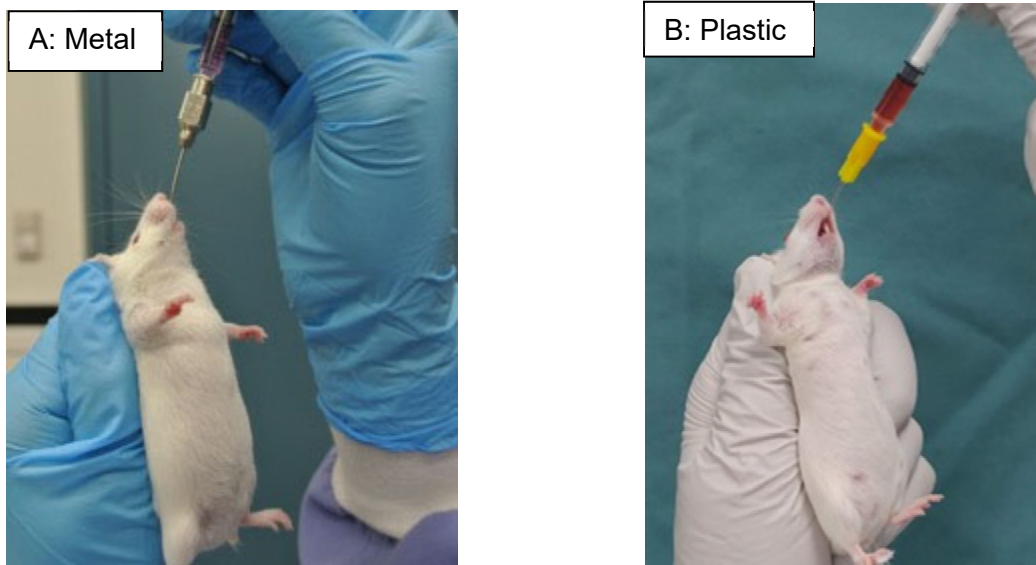
Figure 3



8. Advance the gavage tube along the roof of the animal's mouth slightly towards the animal's left side all the way to the back of the mouth and tongue.
- a. You may feel the ridges of the hard palate as you slide the gavage tube back.
 - b. The animal usually "gags" at this stage – opens its mouth wide and extends tongue.
9. Once the gavage tube is at the back of the mouth/behind the tongue:

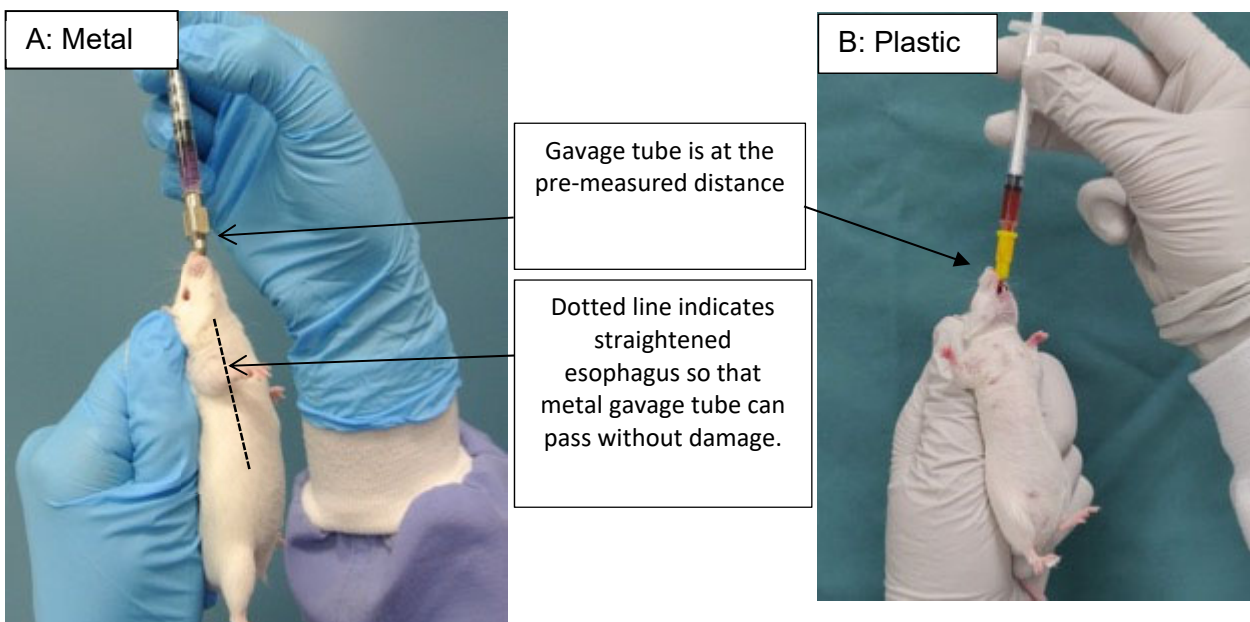
- a. Metal gavage tube: tilt the head back by gently tipping the syringe up and back towards the animal's spine. This aligns the esophagus in a straight line to the stomach. See Figure 4A.
- b. Plastic gavage tube: tilting the head back is not necessary because the tube is flexible and will follow the curve of the oropharynx. See Figure 4B.

Figure 4



10. With gentle guidance, allow the gavage tube to pass down the esophagus to the pre-determined distance. There should be no resistance. See Figures 5A and B.
 - a. If the metal gavage tube does not easily slide down the esophagus, continue gently tilting the head back as above. Metal gavage tubes may also need to be slightly twisted clockwise as it passes the epiglottis and into the esophagus.
 - b. Flexible plastic tubes usually pass easily down the esophagus.

Figure 5



NOTE: *If there is any resistance or if the animal struggles excessively, remove the gavage tube immediately and ensure you have good restraint on the animal before attempting to pass the gavage tube again. **Never force the gavage tube down the esophagus.***

11. Ensure the animal is breathing normally. If the gavage tube is in the trachea, the animal will struggle to breathe and the tongue will turn dark red or blue.
12. 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).

NOTE: *If the animal is **not breathing normally**, do not administer the substance and immediately remove the gavage tube from the esophagus and release the restraint. Monitor animal’s breathing closely. Do not attempt the procedure again until the animal is behaving and breathing normally.*

13. Once the entire substance has been administered, remove the gavage tube slowly (over 1-2 seconds), in a smooth arcing motion, and return the animal to its cage. Removing the gavage tube slowly helps ensure none of the substance is brought back up the esophagus into the back of the throat. Inhaling any substance into the lungs will likely result in the death of the animal.
14. Return the animal to its cage and observe for any complications (see below). Monitor immediately after gavage for 10 minutes, at the end of the day and the next day (or as described in the approved Animal Care Protocol). Do not leave animal for the day unless you are satisfied the animal is behaving normally.
15. Note procedure (drug, dose, route, volume and any complications) on cage card/monitoring records.

→CALCULATING VOLUME (IN ML) TO BE ADMINISTERED:

- Convert animal’s weight from grams to kilograms
 - Divide the weight in grams by 1000
 - E.g. 25g mouse ÷ 1000 = 0.025kg
- Calculate the volume to give in ml
 - Volume (ml) = dose (ml/kg) x weight of animal (kg)
 - E.g. For a 25g mouse getting 10 ml/kg
Volume (ml) = (10 ml/kg x 0.025 kg) = **0.25 ml**

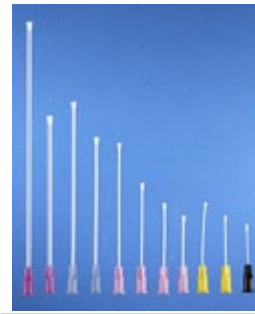
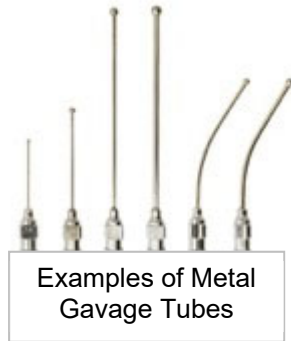
CLEANING INSTRUCTIONS FOR METAL GAVAGE TUBES:

- Between animals, metal gavage tubes should be flushed with water and wiped dry with gauze. When finished, clean with soapy water, rinse well and allow to dry before storing.
- Plastic gavage tubes are single use and should be discarded after the procedure. There are almost always some bite marks or rough spots on the tube or tip, which could cause irritation of the esophagus if used again and a higher chance of leakage and aspiration for the next animal.

IMPORTANT NOTES:

- Never lose control of the gavage tube during the gavage procedure. Hold the syringe so that the plunger can be depressed without needing to adjust grip on syringe. (See Appendix 1).
- Oral gavage administers substances into the distal esophagus, where it then enters the stomach. The goal is not to put the tube into the stomach.
- Use the tube gauge and length appropriate for the size of mouse and the viscosity and volume of substance being administered.
- Before gavaging, it is important to determine the appropriate insertion length of the gavage needle.
- Excellent restraint is important, especially when using metal gavage needles. Even when using plastic gavage tubes, the head must be immobilized and neck straightened without impeding respiration so the animal cannot turn its head and bite the tube.
- NEVER force the gavage needle down; allow the animal to swallow and gravity to assist.
- Ensure animal is breathing normally before administering substance.
- Administer substance slowly; do not pull needle out until finished administering.
- Substance given must be in suspension or solution at room to body temperature; give the smallest volume possible.
- STOP procedure if animal shows any signs of respiratory distress (e.g. skin turns blue, gasping, coughing, struggles, fluid comes out of nose).
- Metal Gavage tubes:
 - Available curved or straight, different lengths, and different ball sizes and shapes
 - Pros:
 - Mouse cannot bite through the tube
 - Reusable and autoclavable
 - Cons:
 - Can damage the oropharynx or esophagus since they are not flexible
 - Must be washed properly
 - Need excellent restraint of the mouse
 - Cannot see if there are any bubbles in the tube
- Plastic Gavage Tubes:
 - Available in many different sizes and are flexible
 - Pros:
 - Less likely to damage the oropharynx or esophagus
 - Disposable so no need to wash
 - Translucent so can see if any bubbles are in tube
 - Cons:
 - Mouse can bite through the tube

- More costly since disposable and not reusable



COMPLICATIONS:

- **Aspiration of solution into lungs**
 - **Due to:** tube length being too short from not pre-measuring to the correct length resulting in accidental administration into trachea or regurgitation.
 - **Clinical Signs:** Varying degrees of respiratory distress/dyspnea (increased respiratory rate and effort) – may see severe gasping, “noisy” breathing or clicking when breathing.
 - **Response:** Place the animal in a cage, administer oxygen if available and monitor breathing closely. If the solution was aqueous, it may recover. If the substance was oily or viscous, the animal should be euthanized. Monitor closely and if any signs of respiratory distress continue after 10 minutes, the animal should be humanely euthanized.
- **Perforation of the esophagus, trachea or lungs**
 - **Due to:** improper restraint and excessive struggling by the animal or forceful insertion of gavage tube.
 - **Clinical Signs:**
 - Pain (hunched, piloerection, squinted eyes) within minutes or hours of gavage.
 - Swelling of neck or under front legs (due to air, fluid, food escaping from damaged esophagus). May appear immediately or within days after gavage.
 - Significant loss of weight due to inability to pass food down esophagus.
 - Varying degrees of respiratory distress/dyspnea (increased respiratory rate and effort) – may see severe gasping, “noisy” breathing or clicking when breathing
 - **Response:** Humane euthanasia
- **Chewing off a piece of the tubes (plastic tubes only):**
 - **Response:** If this happens, keep the mouse restrained and use atraumatic forceps to attempt to grasp the portion of the tube that is in the esophagus and gently remove it.
 - **Response:** If swallowed, monitor animal closely over the next few days to ensure it is behaving normally and is not losing weight and that the piece has not resulted in a perforation or blockage. Look for it passing in the feces. Contact the facility veterinarian if animal is not doing well.
- **Damage to the oral cavity:**
 - **Due to:** improper restraint and excessive struggling by the animal or forceful insertion of gavage tube.

- **Clinical Signs:** Blood in mouth or nose
- **Response:** If any blood is seen in the animal's mouth or on the gavage tube, monitor closely to ensure bleeding does not persist. Re-check animal's mouth after a few minutes. If any bleeding is seen, consult the facility Veterinarian since the animal may need to be humanely euthanized. If no blood is seen, check the animal again at the end of the day to ensure they have recovered.
- **Esophagitis (inflammation of the esophagus):**
 - **Due to:** irritation of the esophagus by the substance administered or the gavage tube (e.g. rough surfaces on a plastic tube, animal struggles during gavage, or tube advanced too forcefully)
 - **Clinical Signs:** may not be seen for a few hours to days later
 - Hunched appearance
 - Squinted eyes (eyes partially closed)
 - Piloerection
 - Significant loss of weight due to inability to pass food down esophagus or animal's unwillingness to eat.
 - **Response:** Consult the facility Veterinarian for treatment options.
- **Other traumatic injuries related to improper animal restraint:**
 - **Clinical Signs:** Torn toe nails, bulging eyes, dyspnea, cyanosis (blue extremities), respiratory distress, etc.
 - **Response:** Dependent on severity of symptoms: humane euthanasia or consultation with facility Veterinarian for treatment options.

REFERENCES:

- UBC ACC SOPs and Guidelines <https://animalcare.ubc.ca/planning-your-research/sops-guidelines>
- Canadian Council on Animal Care (CCAC) guidelines (www.ccac.ca)
- A good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes; Diehl, K et al. 2001
<http://onlinelibrary.wiley.com/doi/10.1002/jat.727/abstract>
- Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider; Turner, Pekow, Vasbinder, Brabb, 2011
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189663/>
- Routes of Administration: The Handbook of Experimental Animal, The Laboratory Mouse; Shimizu, 2004
<http://www.usp.br/bioterio/Artigos/Procedimentos%20experimentais/Routeadministration-4.pdf>
- A Spoonful of Sugar Helps the Medicine Go Down: A Novel Technique to Improve Oral Gavage in Mice. Hoggatt, A. F., et al. Journal of the American Association for Laboratory Animal Science 49: 329-334 (2010).
- Instech Labs Mouse Oral Gavage via Plastic Needle - video
<https://www.instechlabs.com/mouse-oral-gavage-watch>
- Gavage-related Reflux in Rats: Identification, Pathogenesis, and Toxicological Implications (Review). Damsch et al. Toxicologic Pathology, 39:348-360, 2011.

Appendix 1: Example of way to hold syringe for gavage

Use forefinger to inject. Keep finger away from plunger until tube is fully inserted and ready to inject the substance.

