

Utarbeidet av AÅ Godkjent av	Standard operating procedure Guidelines for the administration of substances to rodents	Versjon: 1,0 Utarbeidet: 01.11.19 Revidert: 14.11.23
---------------------------------	---	--

Objective

The objective of this SOP is to provide guidance on the administration of substances to rodents, to minimize pain and distress.

Background

Dosing of experimental animals is necessary in a variety of scientific protocols. These guidelines are designed to highlight the necessary considerations, to refine the technique and secure good animal welfare.

General recommendations

The volume to be injected should be the least amount possible. Highly viscous should be avoided as they are more difficult to inject and require a larger needle size.

NB: all injections, substances and volumes must be approved in your FOTS protocol.

Recommended Injection Volumes

Species	Route and Volumes (ml)				
	Oral	SC	IP	IM	IV (slow iv)
Mouse (30 g)	0,2	2-3	2-3	0,05	0,2
Rat (250 g)	1,0	5-10	5-10	0,2	0,5

Notes: The subcutaneous site does not include Freund's adjuvant administration.

Vehicles for administration

- Vehicles should ideally be biologically inert, have no effect on the biophysical properties of the compound and have no toxic effects on the animals.
- Compounds include aqueous isotonic solutions, buffered solutions, co-solvent systems, suspensions, and oils.
- For non-aqueous injections, consideration should be given for time of absorption before re-dosing.
- Whenever possible, use sterile medical grade compounds with valid expiration date to avoid confounding side effects.

Recommended needle sizes for administration of substances

Species	Intradermal	Subcutaneous	Intramuscular	Intravenous	Intraperitoneal
Mouse	27G	25G	27G	26-28G	25-27G
Rat	27G	25G	25G	25-27G	23-25G

Administrative Routes

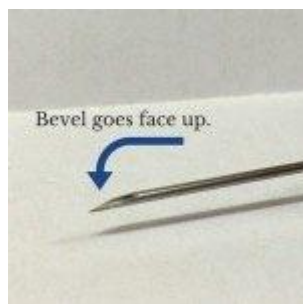
Oral Route (PO)

- If accuracy of PO dosing is very important, liquids should be administered by gavage. This technique is associated with potential adverse consequences and requires extensive experience. At CoMed, gavage is mainly performed by the animal technicians, but the researchers can also learn the technique and perform the dosing themselves if preferred. If oral gavage is relevant for you, contact the facility for advice.
- Formulations in pelleted diet or in water can be custom made, either by a commercial company or in house. It is important to check for palatability before planning the study, by performing a literature search and/or discussing with colleagues. If little information is to be found, consider performing a pilot study.
- Rodents cannot be fed powdered diet for prolonged periods (over 2 weeks) as their teeth will grow continuously and mal occlusion will occur.
- Some compounds can be formulated into small jelly cubes.

Parenteral Routes

General recommendations:

- The dose volume, stability of the formulation, the pH (4.5-8.0), viscosity, osmolality, buffering capacity, sterility and biocompatibility of the formulation are all factors to consider.
- The smallest needle size should be used, considering the dose volume, viscosity of injection material, speed of injection and species.
- Volume and frequency of administration should be kept to a minimum.
- Warm substance to room or body temperature immediately before administration.
- Inject with the bevel pointing upwards.



Subcutaneous (sc)

- This route is frequently used and is relatively easy to perform.
- Usually given in the scruff (access skin) of the neck.
- Restrain the animal and lift the skin with your thumb and index finger to create a skin fold in the shape of a tent. Inject into the fold.
- Subcutaneous sites should be limited to 2 to 3 sites per day.



Intraperitoneal (ip)

- This route is frequently used but can lead to complications.
- There is a possibility of injecting into and damaging internal organs, and irritant materials may cause peritonitis.
- Make sure to immobilize your animal properly, hold the animal head downwards and inject into the lower right quadrant.
- For rats, we recommend a two-person technique: one person immobilizes the animal, and another performs the injection.
- First, perforate the skin in an angle almost in level with the skin. Then point your syringe down towards the muscular layer and press it carefully through the subcutis until you feel resistance from the muscular layer. Push the needle a very short distance through the muscular layer with a firm movement and stop as soon as you are through. Inject your compound and withdraw your syringe.
- If repeated injections are necessary, it is recommended to switch sides.



Intramuscular (im)

- Intramuscular injections are painful because muscle fibers are placed under tension by the injected material. Consider alternative routes if possible.
- If intramuscular injections really are necessary, they can be made into the front or back of the thigh in all small rodents.
- Proper restraint is very important.
- Consider using anesthesia instead of physical restraint.
- Aspirate briefly with the syringe before injection. If blood reverses, stop the procedure.
- Sites should be rotated for multiple dose studies.
- No more than 2 intramuscular sites should be used per day.



Intravenous administration (iv)

- For this route, distinctions are made between bolus injection, slow intravenous injection, and intravenous infusion.
- In most studies, the test substance is given over a short period, approximately 1 minute (bolus injection). Such relatively rapid injections require the test substance to be compatible with blood and not too viscous.
- For mice, use the lateral tail vein. For rats and rabbits, the tarsal vein can also be an alternative.
- Consider using anesthesia if injecting on an awake animal will cause great distress or discomfort to the animal.
- If anesthesia is not an option, physical restraint is necessary. There are commercial restraint devices on the market. The facility might have some as well, please contact us for advice.
- Warm the tail with warm water or a heating pad of about 40-45°C.
- Start your injection at the lower portion of the tail, about 1/3 from the tip.
- Insert needle bevel up while pulling back the plunger carefully. If blood appears, stop the movement, and inject your compound. If you feel resistance or observe a bubble, stop, remove your needle, and apply light pressure to stop bleeding. A maximum of three attempts per vein is recommended.



Intradermal (id)

- This site is typically used for assessment of immune, inflammatory, or sensitization response, and should only be restricted to cases of absolute necessity.
- It is difficult in mice due to the very thin skin.
- Anesthetize the animal, shave the area, clean it and wipe with ethanol.
- Hold the skin tight and inject the needle bevel up. You should feel resistance both as the needle is advanced and as you inject your compound.

-
- You should see a hard bleb afterwards.
 - Volumes of up to 0.05 can be used dependent upon the thickness of skin.

**For more information and tutorials,
please visit the website:**

<http://www.procedureswithcare.org.uk/>

Reference:

1. A good practice guide to the administration of substances and removal of blood, including routes and volumes. Karl-Heinz Diehl, Robin Hull, David Morton, Rudolf Pfister, Yvon Rabemampianina, David Smith, Jean-Marc Vidal, Cor Van De Vorstenbosch . J Appl Toxicol 21 15-23, 2001.
2. Refining procedures for the administration of substances. Report of the BVAAWF/ FRAME/ RSPCA/ UFAW joint working group on refinement. D.B. Mortom, M. Jennings, A. Beckwell, R. Ewbank, C. Godfrey, B. Holgayte, I. Inglis, R. James, C. Page, I. Sharman, R. Verschoyle, L. Westall and A.B. Wilson. Laboratory Animals 35, 1-41, 2001.