

Injecting Mice

Basic things to remember when injecting mice

- A new needle should be used for each animal, since this will reduce discomfort caused by the procedure and also reduce the risk of any injection-site infection.
- If repeated injections of material are needed, consider alternatives such as the use of minipumps.
- If possible, material to be injected should be non-irritant, have a near-neutral pH, and not be excessively hypertonic or hypotonic.
- When repeated doses of material are needed, varying the site of injection can help reduce the likelihood of local skin reactions.
- It is not usually necessary to try to sterilise the skin with antiseptics – their use is almost always ineffective and they simply prolong the duration of restraint needed and may cause additional disturbance to the animal.
- The volume to be injected should be the lowest volume possible and not exceed the current recommended guidelines
- All substances for injection should be sterile since contamination can cause infection and irritation at the site of injection and cause clinical illness in the animals and affect research results.
- Warm substances to room or body temperature since injection of cold substances can cause discomfort and drop in body temperature (if this does not damage the material being used).

Deciding where should you inject a mouse

The selection of a particular route of administration must balance a number of factors – for example, the volume and physicochemical properties of the substance, the required speed of onset, and other factors such as the degree of tissue irritation that could be caused.

These topics are discussed in more detail in [guidelines from LASA \(1998\)](#), [BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement \(2001\)](#) and [Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider](#).

Deciding how much to inject in mice

Table 1 below shows the maximum volumes you should be administering to mice and rats together with the recommended needle size.

Species	Needle Gauge	Volume	
Mouse	25-27g	< 10 ml/kg	i.e.: for a 25 gram mouse, the maximum volume would be 0.25 ml
Rat	23-25g	< 10 ml/kg	i.e.: for a 250 gram rat, the maximum volume would be 2.5 ml

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

Where should you inject mice?

Mice can be injected in four different areas, depending on the research being performed. The four ways are as follows:

- Subcutaneous injection (SQ or SC);
- Intraperitoneal injection;
- Intravenous injection (IV); or
- Oral gavage

For more information on these different administration routes please see below.

Injecting mice subcutaneously

What is the subcutaneous injection for mice?

Subcutaneous means “under the skin”. The most usual site for subcutaneous injection in mice is over the shoulders, into the loose skin over the neck, but other sites with loose folds of skin can also be used, for example over the flank. Photograph 1 below shows a mouse receiving a subcutaneous injection over the shoulders.



Photograph 1: subcutaneous injection in a mouse

Key Points to consider when performing subcutaneous injections in mice

- Subcutaneous administration of material often causes minimal pain or discomfort

Subcutaneous Injection Technique in mice

It is possible to inject under the skin when holding the animal in a neck-skin hold: either from the front at the base of the skin fold between the thumb and index finger (be careful not to stick your fingers), or beneath the lateral skin in front of the hind leg. The needle must be introduced very obliquely beneath the lateral skin, and in order to ensure the correct location of the needle tip you can lift the skin slightly with a sideways movement of the needle before injection.

A mouse placed on a surface may also be injected above the pelvis. The mouse should be pulled slightly backwards by the tail so that it will remain still. The tail should be held with the other fingers, while the thumb and index finger are used to lift the skin on the pelvis. With the other hand, the needle will then be introduced under the skin fold. This technique requires some more practice and it may be difficult especially if the mouse puts up a lot of resistance.

When the injected substance enters the correct location, there is no resistance in the plunger, and the skin may exhibit a bump depending on the point and amount of the injection.

Intraperitoneal injection (i.p.)

What is the intraperitoneal injection for mice, and where does it occur?

An i.p. injection is made into the right lower quarter of the abdominal cavity. Photograph 2 belows the correct insertion site for an intraperitoneal injection in mice.



Photograph 2: a mouse receiving an intraperitoneal injection

Key points to consider when performing an intraperitoneal injection in mice

Although widely used as a means of administering substances, particularly injectable anaesthetics, this is an inherently unreliable technique, since inadvertent injection of some material into the gut, abdominal fat and subcutaneous tissues is a relatively frequent occurrence.

For this reason, it may be preferable to use other routes such as subcutaneous or oral administration.

Intraperitoneal Injection Technique in mice

It has been recommended in related literature that the animal be kept head downwards, in which case the organs will be pressed more cranially in the abdominal cavity and so “out of the way” when doing the injection. In practice this does not matter much, as long as the procedure is performed correctly.

The needle should be injected from the caudal direction, at a slight angle to the abdominal wall, with a quick and firm movement, but only so far that the tip just barely pierces the abdominal wall. Before the injection you should slightly pull on the plunger: if any fluid enters the syringe, you must inject again with a clean needle and syringe.

Regardless of how skilfully the procedure is done, i.p. injections have a degree of risk of failure. Therefore other injection routes are recommended, unless there is a specific need to inject into the abdominal cavity.

Intravenous injection (i.v.)

What is an intravenous injection in mice?

An intravenous injection is one that is into the lateral tail vein of a mouse



Photograph 3: a mouse receiving an intravenous injection

Key points to consider when performing an intravenous injection in mice

- The injection is difficult especially in mice due to the small size of the vein, and this technique requires practice.
- Warming the tail or the whole animal dilates the tail veins and significantly facilitates the procedure.
- Good lighting is important.
- In the main it is best to use an immobilisation device.

Technique

The tail is pulled straight so that it is crossways on the index finger, and the thumb presses the tip of the tail behind the index finger. The tail may also be on the edge of a table. The needle should be held parallel to the tail and inserted into the vein at the exact point where the tail starts to bend downwards. The needle bevel should be pointed upwards. The needle is inserted 2-4 mm inside the vein. If the needle is correctly placed inside the vein, no resistance can be felt in the plunger when injecting and you can see through the skin how the injected substance advances in the vein. After the injection is finished, the normal colour of the vein returns when the blood circulates again. You must press your finger on the point of injection when pulling out the needle, and for a moment afterwards, to prevent bleeding.

Other possible IV injection points are the dorsal metatarsal vein (on the dorsal side of the tarsus), the jugular, femoral, sublingual and penile vein.

Intragastric administration or gavage

What is an oral gavage for mice?

Oral gavage in mice is where material is administered orally, normally using a cannula or catheter to dose directly into the stomach.



Photograph 4: a mouse receiving an oral gavage

Key points to consider when performing an oral gavage

- Although gavage can be undertaken using rigid dosing cannulae, flexible catheters or tubes are preferred, and these are less likely to cause oesophageal trauma.
- Inadvertent dosing into the lung may occur, and this usually results in the animal showing immediate signs of respiratory distress.
- If this is observed, then the animal should be humanely killed.
- If possible, feed the material via a gelatin mixture, or via a catheter in the mouth

Oral Gavage Technique in mice

For the gavage procedure, you should grab the mouse into a firm neck-tail-hold so that the head in particular is as immobile as possible. Optimally the neck would be straight so that the mouth, pharynx and oesophagus form a straight line, but gavage can be done also if the neck is not quite straight. Reaching the correct position can be facilitated if the neck-hold is made with your thumb and middle finger, and the index finger is used to turn the head up from the crown skin.

The mouse should be held in an upright position. The ballpoint probe is carefully inserted into the mouth, and the tip advances along the palate to the back of the mouth. At this point the probe should be in a parallel line with the oesophagus. The probe must not be pushed down the pharynx with force!

When the probe is correctly placed, it will go down easily when the mouse swallows. If the mouse is upright, gravity is enough to advance the probe into the stomach, without any pushing.

A rat should be held around the thorax so that the thumb or the index finger can be used to press the lower jaw, or alternatively the neck and back skin. The probe is inserted into the mouth between the teeth and across the tongue into the pharynx.

Dipping the probe in sugar water right before the oral gavage will facilitate and accelerate the procedure. The animal will resist less and swallow the probe more easily when it tastes sweet.

Possible complications are gavage into the lungs, tearing the oesophagus, and damage to the pharynx. No unnecessary force must be used in the procedure. The animal should be allowed to swallow the probe without forcing it down. A probe of an appropriate size will easily be swallowed up to the base, the tip all the way down to the stomach; in the trachea the probe will not travel as far. When the probe travels through the trachea, your fingers may feel a “vibration” caused by the tracheal rings. If the probe does not travel easily deep enough or the animal starts to cough, gag or otherwise resist once the tip has passed the pharynx, the probe must be pulled out. After a successful gavage the animal will behave normally.