THE SWEDISH 3Rs CENTER

Date
2025-12-01

Diarienummer
5.2.17-17729/2025

# Refined Methods for Administering Medicinal Products or Other Substances

The administration of medicinal products or other substances is a common procedure performed on animals in research. For example, this may involve the administration of a substance tested in a study, or of medication after surgery. To enhance animal welfare and ensure the quality of research results, it is essential to select an appropriate method of administration and to carry out the procedure correctly.

Whether you are experienced in planning substance administration or doing so for the first time, it is important to reflect on how the procedure should be conducted. Several factors must be considered, such as the properties of the substance and its intended target within the body, the species you plan to work with, and the purpose of the administration. In some cases, one or more of these factors will determine which method must be used. In other cases, multiple methods may be available – where this occurs, it is important to choose the method that is most gentle for the animals and that provides reliable research results.

Below is information intended to support your choice of administration method. We have chosen to focus on oral administration and injections, which are the most commonly used routes of administration.

This information is primarily intended for those working with research animals kept by humans, in research facilities or otherwise. However, parts of the information may also be relevant to those conducting research involving wild animals.

If you already have an ethical approval and discover a way to refine your procedures without negatively affecting animal welfare, contact your Animal Welfare Body to get an assessment of whether they can help you amend your ethical approval.[1]

## Selecting the Administration Route

Choosing an administration route can be more or less challenging depending on whether you are working with a well-known or a novel substance. For well-established medicinal products or substances, relevant information is likely available, and standard procedures may already be in use at your facility. If, however, you are testing a new substance, you may need to consider several different factors when making your choice. Regardless of whether the procedure

[1] 5 ch. 6 § SJVFS 2025:28.

involves a familiar or unfamiliar substance, you should always strive to make improvements that enhance animal welfare and the quality of your research.

Factors to consider when selecting the route of administration include:

- The physical and chemical properties of the substance.
- How the substance is absorbed, distributed, metabolised and excreted by the body.
- Whether the substance is intended to have a local or a systemic effect.
- Whether you need to know the exact dose administered to each animal.
- Whether you need to know the exact time at which each animal receives its dose.
- Whether the substance will be administered once or on multiple occasions.
- The species you plan to work with.
- The health status and other specific characteristics of the animals in question.

Different species and routes of administration allow for different dosing volumes. The links below provide information on recommended volumes.

If you can choose between several routes or methods of administration, you should select the one that is most gentle for the animals. To further improve animal welfare and simplify the procedure, you may use refined handling methods and incorporate training of the animals.

Different routes and methods of administration may place varying demands on your skill level. Do not hesitate to seek assistance from more experienced colleagues if you feel uncertain.

Further information on the administration of medicinal products or other substances to animals in research

Recommendations for administration of substances to laboratory animals | GV-SOLAS (gv-solas.de)

CCAC guidelines: Administration of substances and biological sampling | CCAC (ccac.ca)

Guidelines on Administration of Substances to Laboratory Animals | University of Michigan (umich.edu)

Further information on handling and training of animals for procedures such as administration of medicinal products or other substances

Refined methods for handling, habituation and training | Swedish 3Rs Center (jordbruksverket.se)

Handling and training of mice and rats for low stress procedures | NC3Rs (nc3rs.org.uk)

## Oral Administration

Administering medicinal products or other substances orally – via the mouth – is a common and practical route of administration, particularly for long-term treatment. There are several different methods for oral administration, which vary in terms of precision and the extent to which handling or restraint of the animals is required. If you can choose a method where the animals voluntarily ingest the substance, you should do so rather than restraining the animal and administering the substance directly into its stomach via an oral dosing tube.

When a substance is administered orally, it will pass through the body's first-pass metabolism in the gastrointestinal tract and liver before reaching the bloodstream. This means that factors such as food intake, enzyme activity, and gastric pH can influence how much of the active substance enters the circulation.

### Voluntary Oral Administration

Voluntary oral administration is based on the animal willingly ingesting the substance, either independently or with the aid of gentle handling. It is a refined form of administration that can reduce stress and improve animal welfare, particularly when the substance is to be given repeatedly or over an extended period.

Voluntary oral administration can be carried out in several ways – using a micropipette, syringe, as a pill or capsule, in a treat placed in the cage, or mixed into feed or drinking water. The method you choose may depend on the properties of the substance and the species you are working with.

To encourage voluntary ingestion, you can mask the taste of the substance and increase the animal’s interest by mixing it with something palatable. The choice of flavouring agent may depend on the solubility characteristics of the substance, the species involved, and the purpose of the study. Examples to consider include condensed milk, fruit juice, hazelnut spread, peanut butter, banana, soft feed, and gelatine. It is important to first test the flavouring agent without the substance to ensure the animals do not avoid the taste. Consider conducting a pilot study to evaluate your approach to training, flavour masking and dosing.

If your substance is bitter, animals may become less willing to ingest it over time. In such cases, you may try increasing the proportion of flavouring agent in the mixture or using a lower concentration of the substance in a larger volume. You may also explore whether products developed for taste masking of medicines in human healthcare could be suitable for use.

### Micropipette or Syringe

The most precise method for voluntary oral dosing may be to use a micropipette or syringe and allow the animals to drink from it. This provides control over both the dose and the timing of administration. You can habituate the animals to drink from a pipette or syringe by offering them a palatable substance in the dosing device. If any of your animals remain hesitant to drink from the dosing device despite habituation, you may use gentle restraint to assist the animal while it drinks.

More information on voluntary oral dosing using a micropipette

Refining Oral Treatments in Laboratory Rodents | Rodent MDA (rodentmda.ch)

Examples of protocols for voluntary oral dosing using a micropipette

Oral dosing Rats and Mice | The 3Hs Initiative (3hs-website.cdn.prismic.io)

Standard Operating Procedure Micropipette-guided Drug Administration | University of Zurich (uzh.ch)

### Pills or Capsules

Voluntary oral dosing using pills or capsules is primarily used for larger animal species. You must ensure that the animal swallows the entire dose and does not spit out or chew the pill. Therefore, you should always observe the animal after administration and document any anomalies.

If the animal does not swallow the pill voluntarily, you may need to place it towards the back of the oral cavity using tweezers or a dosing stick. In such cases, hold the animal securely but gently, and avoid causing discomfort or injury to the mouth or throat. Once the pill is placed at the back of the mouth, you can gently stimulate the swallowing reflex by stroking the animal's throat, lightly blowing on its nose, or offering a small amount of water or something palatable via syringe directly into the mouth.

### Treats, Feed or Drinking Water

In some cases, administration via drinking water or feed is possible but the method presents challenges. It is difficult to guarantee accurate dosing for each animal, control the timing of administration, or monitor individual intake. Furthermore, if the substance alters the taste or smell of the food or water, it may disrupt normal consumption.

A similar approach is to mix the substance into a treat or another form of flavour masking and place it in the cage. In this case, you may be more confident about the timing of ingestion, particularly if you draw the animals' attention to the treat. To increase the likelihood that each animal receives the correct dose, you may temporarily separate them during administration.

### Gavage

Gavage, or oral dosing via a feeding tube, involves delivering the substance directly into the oesophagus or stomach. If the experiment require administration of an exact oral dose at a specific time and voluntary oral dosing is not possible, this method may be appropriate.

You may use a soft tube made of plastic or silicone, or a rigid metal tube to administer the substance. This procedure carries a risk of injury to the oesophagus, stomach, or nearby organs. It is therefore essential to use the correct tube size and to be confident in how to perform the procedure.

If you intend to use gavage, refining your technique and training the animals can be highly beneficial in reducing the need for restraint and, as far as possible, avoiding stress. At the end of this document, you will find links to videos from RISE Research Institutes of Sweden demonstrating refined methods for performing gavage and other procedures on mice and rats.

## Injections

If you need to ensure the exact amount of active substance entering the animal's body and avoid first-pass metabolism, injection may be a suitable route of administration. The anatomical site of injection affects how quickly the substance is absorbed.

To facilitate the procedure, you can train the animals to become accustomed to your handling and the grips you intend to use. This reduces the need for restraint. For larger animals, training may involve standing still in a designated location or signalling readiness to receive an injection.

### Intravenous Injection

Intravenous injection – injection into a vein – is commonly abbreviated and referred to as IV. With intravenous injection, the target area is small, both in width and depth. Therefore, it is advantageous to inject into an accessible and clearly visible vein. By occluding the blood flow in the vein, a process known as stasis, the vein becomes larger and easier to access.

It is also easier to achieve accurate placement of the injection if the animal remains still. For this reason, animals are often restrained, and smaller animals may be placed in equipment known as restrainers. A restrainer is typically a transparent tube in which the animal is immobilised while the tail or a limb is extended for injection. Restraint is often stressful for animals. A gentler approach is to train them to be still during the injection. This may be more difficult when injecting into a vein in the ear, such as in pigs or rabbits, as they often resist having their ears held. It is generally easier to inject into a leg or tail, where the animal may be less sensitive and can see what is happening.

When planning the site for intravenous injection, you must also consider what you will do if the first attempt fails. You should have the option to try again at a site further along the blood flow, closer to the heart. Attempting further away from the heart instead risks leakage of the substance into surrounding tissues or blockage at the initial injection site. For this reason, it is advisable to begin as far from the heart as possible, such as further down the tail in mice and rats. However, peripheral blood vessels are smaller, which may make the injection more challenging. Therefore, begin at a site where you are confident in your ability to perform the injection, while still allowing for two additional attempts.

You must also decide what to do if you have attempted three times and still have not succeeded – is it time to ask for assistance, are there other anatomical sites you can try, should the animal be removed from the study, or can you attempt the procedure again later in the day?

When performing an intravenous injection, the needle should be inserted at approximately a 25-degree angle, and then quickly levelled to follow the direction of the vein, avoiding excessive depth that could result in piercing through the vessel. When you observe blood entering the needle, you have reached the correct position and may attach the syringe to administer the substance. To confirm placement, you may also aspirate – gently pull back the syringe plunger after inserting the needle to check for blood return.

## Subcutaneous Injection

A subcutaneous injection, abbreviated SC, is an injection administered beneath the skin. For this type of injection, it is common practice to lift the skin into a tent-like shape. The needle should then be inserted just under the skin, taking care to avoid injury to your own fingers or the animal's underlying tissues. A substance intended for subcutaneous delivery but inadvertently injected into another tissue may produce different effects, potentially compromising your research results. Additionally, there is a risk of tissue damage.

In animals with loose, stretchable skin – such as mice and rats – subcutaneous injections can be administered almost anywhere on the body. Common sites include the neck and upper back, or the rump and thighs. Conversely, it is more difficult to administer a subcutaneous injection in animals with tight skin, such as adult pigs, which also have thick layers of fat across the body. If a subcutaneous injection is required in such animals, small areas where the skin can be lifted – such as the tail fold or behind the ears – may be suitable.

Once the needle has been inserted subcutaneously, it should be movable from side to side. If this is not possible, the needle may be incorrectly placed in another tissue, such as muscle or fat. When aspirating, no fluid should enter the syringe; instead, a vacuum bubble should form. Note that aspiration in muscle also produces

a vacuum bubble, so aspiration alone cannot reliably distinguish between these two injection sites.

There are differing views on how to manage the injection site following a subcutaneous injection. Some practitioners gently pat or rub the area to reduce pain from the needle or to reward the animal. Others argue that touching the site may risk expelling part of the substance or introducing infection. As far as we can determine, there is no conclusive evidence supporting either approach. Decide in advance how you will proceed, so that your actions are intentional. To reward the animal and signal that the procedure is complete, you could pat another part of the body.

## Intramuscular Injection

Intramuscular injections, abbreviated IM, involve administering a substance directly into a muscle. When a needle is inserted into a muscle and fluid is injected, the muscle cells are forced apart, which can cause pain.

Larger muscles have a greater capacity to receive an injection than smaller ones. For this reason, intramuscular injections are not recommended for small animals such as mice and rats. If you do need to perform such an injection, only a very small volume should be administered.

In larger animals, intramuscular injections are more commonly used – for example, to administer a sedative to pigs via injection into a muscle in the neck or rump. When injecting pigs in this manner, restraint is usually not required. You only need to approach the pig for a few seconds to insert the needle. You can then step back and inject via a tube. The needle may also be withdrawn using the tube. It is important to ensure the needle is removed safely to avoid the risk of it breaking and remaining in the muscle.

Larger animals may also experience pain from intramuscular injections. If you need to administer larger volumes, it may be less painful to divide the dose into several smaller injections across different muscles rather than delivering a single large injection.

## Intraperitoneal Injection

An intraperitoneal injection, abbreviated IP, is an injection into the abdominal cavity. This type of injection is considered a surgical procedure, as the needle penetrates the peritoneum. The uptake of substances administered intraperitoneally is rapid, but absorption is more similar to that of orally administered substances than to other types of injections. This is because blood from the abdominal cavity passes through the liver before circulating throughout the body.

There are many potential complications associated with intraperitoneal injections. It is therefore essential that you know how to carry out the procedure, hold the

animal correctly, and ensure it remains completely still. Incorrect needle placement may result in puncturing the bladder, intestines or other organs, with consequences for both the distribution of the substance and the animal's welfare. If an alternative route of administration is suitable for your substance, it is recommended that you use that instead.

Several safety measures can be taken before and during an intraperitoneal injection:

- Ensure you receive practical training at the animal facility.
- Consult an animal technician or veterinarian to determine the appropriate injection site, both in terms of vertical and lateral positioning. Placement may vary depending on species and sex.
- Use a needle stop to prevent excessive insertion depth. You can create a stop by trimming the top of a needle cap and fitting it onto the needle. With such a stop, the needle can be inserted perpendicular to the animal's body.
- Hold the animal firmly with its head tilted slightly downward, so that the intestines shift towards the head, creating more space in the abdominal cavity for the needle.
- Aspirate before injection to confirm correct placement. A vacuum bubble should form, and no fluid should enter the syringe.

## Needles and Hygiene

All types of injections involve introducing a foreign object into the animal's body. This carries a risk of injury, either directly through improper needle use or indirectly through infection. It is therefore important to clean the injection site as thoroughly as possible, preferably using a suitable alcohol-based disinfectant. You may also need to remove hair around the injection site to ensure accurate placement. However, when working with smaller species, avoid unnecessary hair removal or excessive use of alcohol, as this may lead to significant heat loss.

Do not reuse needles that are not designed for multiple use. Single-use needles are intended for one-time application. After the first puncture, the needle tip is likely to become damaged and blunt. Reusing such needles can cause pain to the animal due to increased and uneven tissue damage. Reuse also increases the risk of contamination. Make it your practice to use single-use needles only once, regardless of how minor the procedure may seem.

## Further Information on Injections

Intravenous, subcutaneous and intraperitoneal injection in mice

Dosing mice by injection | The 3Hs Initiative (3hs-website.cdn.prismic.io)

Intravenous Injection in the Mouse | Research Animal Training (researchanimaltraining.com)

Subcutaneous Injection in the Mouse | Research Animal Training (researchanimaltraining.com)

Intraperitoneal Injection in the Mouse | Research Animal Training (researchanimaltraining.com)

Intravenous, subcutaneous and intraperitoneal injection in rats

Dosing rats by injection | The 3Hs Initiative (3hs-website.cdn.prismic.io)

Injection of rats | Norecopa (norecopa.no)

Intravenous Injection in the Rat | Research Animal Training (researchanimaltraining.com)

Subcutaneous Injection in the Rat | Research Animal Training (researchanimaltraining.com)

Intraperitoneal Injection in the Rat | Research Animal Training (researchanimaltraining.com)

Intravenous and subcutaneous injection in rabbits

Injection of rabbits | Norecopa (norecopa.no)

More information on single use of needles

Single use of needles | NC3Rs (nc3rs.org.uk)

## Further Information on Refined Handling and Training for Procedures such as Gavage and Injections in Mice and Rats

Housing, handling and training mice | RISE Research Institutes of Sweden (youtube.com)

Housing, handling and training rats | RISE Research Institutes of Sweden (youtube.com)

Handling and training of mice and rats for low stress procedures at RISE Research Institutes of Sweden | NC3Rs (nc3rs.org.uk)