THE UNIVERSITY OF TOLEDO
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

SUBJECT: Administration of Substances Guideline

DATE: February 19, 2025

Administration of Substances – Volume and Route

This guideline establishes safe practices for the administration of substances to laboratory animals. Procedures such as administration of substances must be described in the approved Institutional Animal Care and Use Committee (IACUC) protocol, including the route and volume of agents. Any exceptions to these guidelines, such as increase in volume, must be scientifically justified in the protocol.

The appropriate route of administration and volume will depend on the animal species, age/size of the animal, and experimental needs. Researchers should take measures to minimize stress associated with the substance administration, using the least painful method possible. This will also help to minimize experimental variables and confounding influences on research data. Excessive volumes by any route can cause pain, necrosis, and changes in absorption as well as leakage from the site of injection. Large volume injections should be injected as smaller volumes in multiple sites. Researchers should contact DLAR for injection technique training[1,2].

<u>General information</u>

1. Needle size
    a. Use the smallest diameter needle possible to minimize pain and tissue trauma.
    b. Mice, rats, and voles: 22-27g, 1/2-3/4 in
    c. Rabbits: 20-27g, 1/2-3/4 in
    d. Safety needles and syringes must be used when possible
    e. Do not recap needles.
    f. Dispose of used needles and syringes in a designated sharps container.
    g. Needles cannot be re-used between animals. Contact IACUC for exceptions.

2. Subcutaneous (SC)
    a. Location: scruff (dorsal neck-shoulder area), flank
    b. Slow rate of absorption
3. Intraperitoneal (IP)
    a. Location: lower abdominal quadrants
    b. Quickly absorbed
    c. Aspirate before injecting to avoid inadvertent administration into the bladder or gastrointestinal tract.
    d. Repeated daily intraperitoneal dosing for up to one month is well-tolerated in rodents. Doses should be administered to alternating sides of the abdomen.
    e. Administration of irritating substances may cause ileus (stasis of the gastrointestinal tract) and peritonitis (inflammation of the abdominal cavity).
4. Intravenous (IV)
    a. Location:

i. Rodents: tail vein, retro-orbital sinus
ii. Rabbits: lateral ear vein, cephalic vein

b. Warm the rodent tail in warm water or place the rodent cage in a DLAR incubator 30 minutes prior to needle insertion to dilate the blood vessels.
c. Give smaller volumes by bolus, up to 1 ml/kg max. Larger quantities should be injected slowly over time (3-10 minutes).
d. A syringe pump may be used.

5. Oral (PO, per os)
   a. Voluntary consumption
      i. Substances may be mixed with the daily diet, flavored water, or other palatable items to encourage consumption. Habituate animals to any novel food items or water flavoring before adding drug. Food or water bottle may be measured to quantify amount animals consumed. Animal weight or body condition should be monitored to ensure an appropriate daily caloric or water intake. Food or water containing additives should be clearly labeled and disposed of properly.
   b. Gavage
      i. Oral gavage ensures that a defined quantity of substance is administered to the animal.
      ii. Bulbed gavage needle size:
         1. Mouse, Vole: 18-22g, 2-3cm long
         2. Rat: 15-18g, 6-8cm long
      iii. Rubber feeding tube size:
         1. Rat: 8 Fr.
         2. Rabbit: 18-22 Fr.
      iv. Proper training in technique is essential to prevent injury or death due to tissue trauma or fluid aspiration.

6. Intradermal (ID)
a. This method is not recommended unless required by experimental model.
b. Location: Base of tail or hock are recommended intradermal locations. Footpad is not recommended unless justified in the protocol.
7. Intramuscular (IM)
   a. Location: quadriceps, epaxial (rabbit)
   b. Intramuscular injection may be painful and is not recommended in small rodents.
8. Intranasal (IN)
   a. Performed under anesthesia using a micropipette.

Table 1. Acceptable Volumes by Route

Species	Gavage (PO) (ml/kg)	Intravenous (IV) (bolus) (ml/kg)	Intravenous (IV) (infusion) (ml/kg/hr)	Intraperitoneal (IP) (ml/kg)	Subcutaneous (SC) (ml/kg)	Intradermal (ID) (ml/inj)	Intramuscular (IM) (ml/kg/site)	Intranasal (IN) (ml/inj)
Mouse, Voles	5 - 10	1 - 5	2 - 4	1 - 10	1 - 40	0.05 - 0.1	0.05	0.03 - 0.05
Rat	5 - 10	1 - 5	2 - 4	1 - 10	1 - 5	0.05 - 0.1	0.05	0.03 - 0.05
Rabbit	5 - 10	1 - 5	2 - 4	1 - 10	1 - 5	0.05 - 0.1	0.05	0.2 - 0.5

(If a range is provided, the first dose is ideal. If a single value is listed, it is the maximum allowable dose.

References

1. PV Turner, T Brabb, C Pekow, MA Vasbinder. 2011. Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider. J Am Assoc Lab Anim Sci 50(5): 600-613.
2. Diehl, K.H., Hull, R., Morton, D. Pfister, R., Rahemampianina, Y., Smith, D., Vidal, J-M., and Vorstenbosch, C. 2001. A Good Practice Guide to the Administration of Substances and Removal of Blood Including Routes and Volumes. Journal of Applied Toxicology. 21, 15-23.
3. Southam DS, Dolovich M, O'Byrne PM, Inman MD. Distribution of intranasal instillations in mice: effects of volume, time, body position, and anesthesia. Am J Physiol Lung Cell Mol Physiol. 2002 Apr;282(4):L833-9. doi: 10.1152/ajplung.00173.2001. PMID: 11880310.
4. University of Wisconsin, Animal User Requirement #2: The Reuse of Needles in Research Animals https://www.rarc.wisc.edu/policies/aur_2_the_reuse_of_needles.html
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