Institutional Animal Care and Use Committee		UNT Health
Title: Recommendations for Substance Administration		
Document #: 038	**Version #:** 05	
Approved by IACUC Date: June 3, 2025		

A. BACKGROUND INFORMATION

This Standard Operating Procedure (SOP) describes the recommendations for safely administering substances to laboratory animals, including guidelines for selecting needle gauges and maximum injection volumes allowed by site in laboratory animal species. It also provides guidelines for ensuring sterility and effectiveness of drugs when secondary containers are used for compounding, diluting, or transferring drugs and compounds to be administered by injection to animals.

B. RESPONSIBILITIES

It is the responsibility of Principal Investigators, staff and students to follow these recommendations.

C. PROCEDURES

a. When writing your protocol or amendment, make sure to add all substances including all requested information (e.g., agent, dose, route of administration, anticipated adverse reactions, pharmaceutical grade, monitoring, etc…).

b. Certain routes of administration, such as subcutaneous, intraperitoneal, intramuscular, intravenously, or oral gavage, will require the animal to be restrained. The restraint method should be described within the protocol/amendment.

c. The Substance Administration Table, referenced below, provides the recommended needle gauges and maximum volumes (mL/kg or mL per site) injected subcutaneously, intraperitoneally, intramuscularly, intravenously, and by oral gavage for each listed animal species. Intra-ocular injections are approved on a case by case basis, with veterinarian consultation and approval. If following the recommendations of the table below, referencing this SOP in place of providing the needle gauge is acceptable. However, <u>going outside of the recommended gauge and volumes requires a full description and justification within the protocol/amendment</u>.

d. It is strongly discouraged to reuse needles on multiple animals. It can lead to dulling of the needle, increasing the discomfort associated with injections, and can lead to disease transmission and/or contamination of vials of material to be injected.
e. **Guidelines for compounding and secondary container use for injectable drugs:**
    i. Definitions:
        1. **Secondary Containers:** Vials, bottles, or tubes used when drugs or compounds are moved from their original container.
        2. **Transferred:** When drugs or compounds are taken out of the primary container and placed into a secondary container (e.g., drugs in glass ampules).
        3. **Diluted:** When drugs or compounds are mixed with diluent to achieve a working concentration (e.g., antibiotics or analgesics for use in rodents).
        4. **Compounded:** When drugs are mixed with one or more drug or diluents (e.g., mixture of ketamine with xylazine and diluent).
    ii. **Types of Secondary Containers:** The type of secondary container must be compatible with the drug or compound and its intended use. The best type of container for the use is a vial with a septum in the cap (search septum or crimp top vial on a scientific supply website). The sterile drug or compound can be dispensed into the vial and the contents can be removed aseptically with a sterile needle and syringe. The top of the septum should be disinfected with 70% alcohol prior to use. As a second choice, a red capped (untreated) blood collection tube can be used as a secondary container. The use of screw capped tubes should be avoided as it is difficult to remove the contents aseptically.
        1. Container Material:
            a. Does not react with the drug or compound (e.g., glass, polypropylene, or polycarbonate plastic).
            b. Opaque if light sensitive material is to be stored (e.g., covered with foil, brown glass).
            c. Supplies are sterile or able to be autoclaved.
        2. Aseptically Administered: Contents must be removed aseptically – single or multiple draws.
        3. The most common use of secondary containers is for drugs or compounds that are:
            a. Removed multiple times from the same container
            b. Removed and administered aseptically

iii. Labeling:

1. Any drug or compound transferred to a secondary container must be labelled as follows
    a. The name and concentration of each ingredient including the diluent.
    b. Total amount/ volume in the container
    c. The expiration date of the drug or compound
2. For diluted or compounded solutions, secondary containers must also include the following:
    a. The preparation date
    b. The use-by-date
        i. Should not extend past the earliest expiration date of any of the components.
        ii. Should be no longer than 30 days from preparation for compounds or dilutions, unless published or vendor-provided scientific data can demonstrate a duration of efficacy longer than 30 days.
        iii. EXAMPLE: Compounded Ketamine anesthetic cocktails have a use by date of 6 months (or the earliest expiration date of any drug in the compounded solution, if <6 months) on a basis of the publication Taylor, BJ et al. 2009. Beyond-use dating of extemporaneously compounded Ketamine, Acepromazine, and Xylazine; safety, stability, and efficacy over time. JAALAS 48:718-726
    c. For controlled substances, per DEA guidelines, the inventory must reflect all disbursements and the label must include the following:
        i. The total amount/ volume and lot number of each controlled substance.
        ii. The total amount/ volume of the combined drugs
        iii. The concentration of each drug (mg/ml)
        iv. Date of preparation
        v. Date of expiration or use by date, whichever is earliest.
3. Exceptions: Exempt compounds must be prepared and handled using sterile technique, as appropriate. All containers must be identified with a description of the contents. Note that this exception does not apply to veterinary drugs, i.e., anesthetics,

analgesics, or euthanasia drugs. The following compounds are exempt from this guidance:

a. Test compounds that are prepared for single use and will not be stored past this one-time use.
b. Test compounds that are available in small quantities (0.5 ml), such that use of a separate vial poses a risk of losing the contents in the rubber septum.
c. Test compounds that consist of hazardous materials (BSL-2/3, CSL-2/3, Radioisotopes), such that the additional handling needed to place the material into a separate vial increases the risk of accidental exposure.

D. ATTACHMENTS:

a. Substance Administration Table
b. Examples of Needle Re-Use
c. Examples of Secondary Containment

SUBSTANCE ADMINISTRATION TABLE

Species	Subcutaneous (SC)		Intraperitoneal (IP)		Intramuscular (IM)		Intravenous (IV)		Oral Administration (PO)	
	Needle Gauge	Max mL/kg	Needle Gauge	Max mL/kg	Needle Gauge	Max mL/kg	Needle Gauge	Max mL/kg	Gavage Needle Gauge	Max mL/kg
Mouse	27-23	40	27-23	80	30-26	0.1[a]	30-27	5[b] 25[c]	20-18	20
Hamster	27-23	20	27-23	20	27-25	0.1[a]	27-25	5[b] 20[c]	20-18	20
Rat	25-21	10	25-21	20	27-23	0.2[a]	27-25	5[b] 20[c]	18-14	20
Guinea Pig	25-21	10	25-21	20	26-23	0.2[a]	27-25	5[b] 20[c]	18-14	20
Rabbit	25-21	2	25-21	20	25-21	0.5	25-21	2[b] 10[c]	16	10
Pig	23-18	1	23-18	1	23-18	0.25	23-18	2.5[b] 5[c]	TBD	15

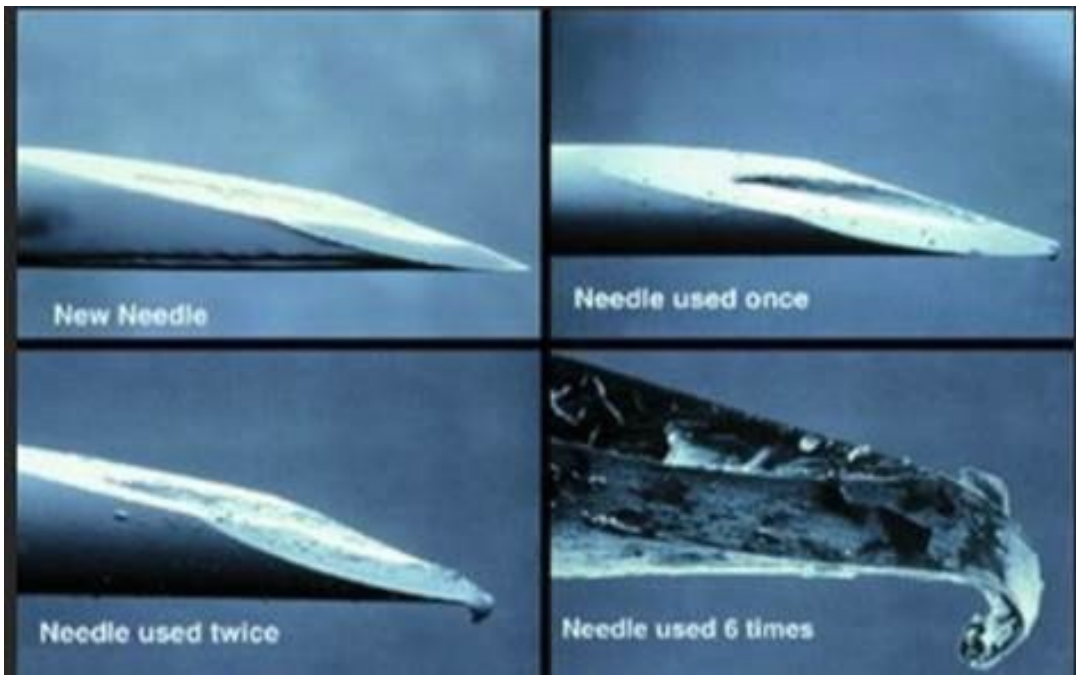
a - Values are mL per site. Based on 25g mouse, 100g hamster, 200g rat, and 200g guinea pig.
b - Bolus
c - Slow infusion
TBD – To be determined

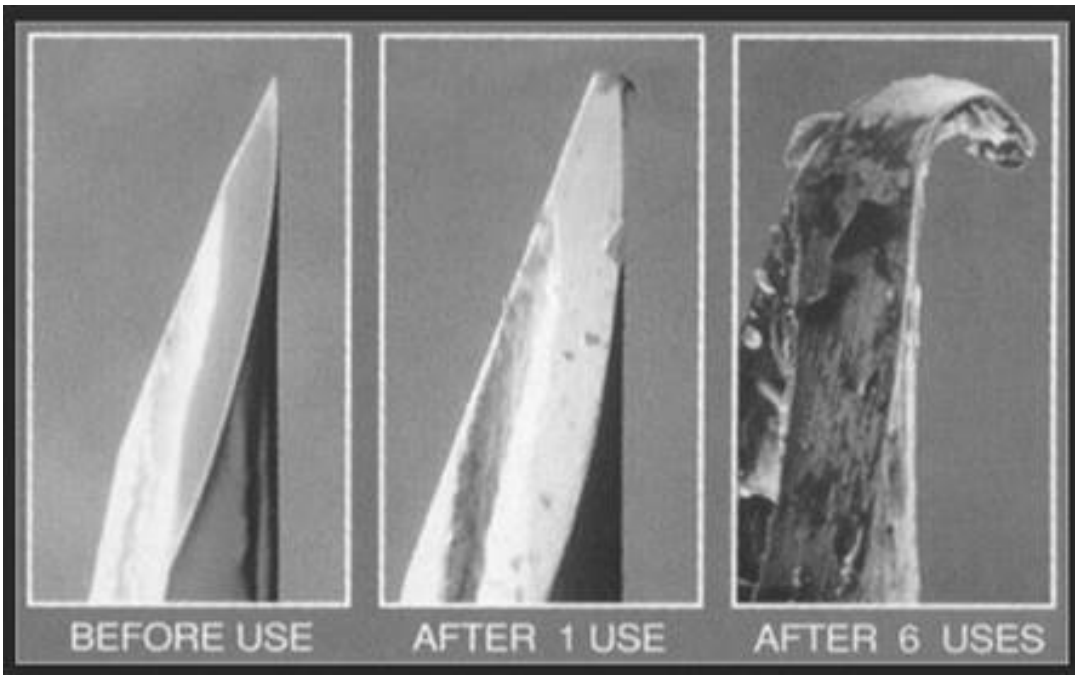
NOTE: If a larger sized needle is required than is recommended, justification for the use of a larger needle must be included in the protocol. Using volumes smaller than the listed maximum is recommended in order to minimize possible pain, distress, or disruption of physiological equilibrium.

REFERENECES:

i. Hau, Jann, Van Hoosier, Jr, Gerald; Handbook of Laboratory Animal Science 2nd Edition, Volume 1.
ii. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, and Vorstenbosch C. A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes. *J. Appl. Toxicol.* 21, 15-23 (2001)
iii. Suckow, MA, et. Al. *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents.* Academic Press, 2012.

EXAMPLES OF NEEDLE REUSE:





Examples of containers

Examples of appropriate vials for liquids These vials can remain sterile when obtaining multiple doses using separate sterile needles			
Examples of vials NOT appropriate for liquids. They cannot remain sterility when obtaining multiple doses.			

Examples of labeling

Examples/templates of appropriate container or bottle labeling:
Ketamine {8.25mg.ml) lot # _____ Acepromazine {0.25mg/ml) Xylazine {0.83mg/ml) Made: ___/___/___ Initials _____ Expires / Use by Date ___/___/_____

Examples/templates of appropriate labeling for bags or transfer containers	
Components In Sterile Vial, mix: Acepromazine Maleate {10mg/ml) 1.2 mg.0.12ml +Ketamine HCl {100mg/ml): 41 mg, 0.41ml + Xylazine HCl {20mg/ml); 4.2 mg, 0.21ml + Sterile Water for injection: 4.26ml Dosage: 0.30ml/25g BW, IP *Ketamine cocktails expire 6 months after made, or the earliest expiration date of any drug in the cocktail if less than 6 months. ** All other cocktails/compounds expire 30 days after preparation date or the earliest expiration date of any drug in the cocktail if less than 1 month.	Comments: Individual dosages for a mouse: 100mg/kg Ketamine {100mg/ml) 20mg/kg Xylazine {20mg/ml) 3mg/kg Acepromazine {10mg/ml) References : Arras M et al, 2001. Optimization of Intraperitoneal (IP) injection anesthesia in mice; drugs, dosages, adverse effects, and anesthesia depth. Comp Med, 51:443-56. Contact information: Name: _____ Phone: _____

VCD (4-Vinylcyclohexene dioxide)

Date: Initials: