UNIVERSITY STANDARD

Title

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL STANDARD FOR ADMINISTERED AGENTS

Introduction

PURPOSE
The standards described below provide guidance to all researchers and animal handlers administering chemical agents, whether they be pharmaceutical or non-pharmaceutical grade. The IACUC considers issues related to animal welfare, the scientific goals of the research, and the compound’s chemical, physical and physiological properties.

SCOPE OF APPLICABILITY
All personnel engaged in the hands on administration of agents to laboratory animals. The standards described in this document apply to all chemical agents and compounds, including analgesics, anesthetics, investigational compounds, biologics, cell lines, and fluids administered to animals for research, teaching, or experimental purposes.

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard. Requests for exceptions to this Standard must be reviewed and approved by the IACUC and/or DCM Management.

Standard

I. Pharmaceutical-grade drugs

When selecting drugs, the following order of choice should be applied: (This order of preference from the NIH Office of Animal Care and Use, Animal Research Advisory Committee guideline, “Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals.”).

1. FDA approved veterinary or human pharmaceutical drugs
2. FDA approved veterinary or human pharmaceutical drugs used to compound a needed dosage form; i.e. FDA approved veterinary or human pharmaceutical drugs that have been diluted or mixed with other FDA-approved drugs in order to be delivered at the appropriate dose and/or volume for a given species
3. USP/NF or BP pharmaceutical grade drug used in a needed dosage form; a pharmaceutical grade drug recognized by USP will bear the initials “USP” after the name of the drug
4. Analytical grade bulk chemical used to drug a needed dosage form (requires justification)
5. Other grades and sources of drugs (requires justification).

II. Use of non-pharmaceutical-grade drugs

Investigators are expected to use pharmaceutical-grade drugs whenever available, even in non-survival procedures. The IACUC recognizes that the use of non-pharmaceutical-grade drugs in laboratory animals is often essential to meet the scientific goals of the research. Some of the circumstances in which non-pharmaceutical grade drug would be considered appropriate are listed below. However, if a formulation of the drug is commercially available as a veterinary or medical grade product, the non-pharmaceutical grade product may not be used as an alternative unless adequately justified in the animal protocol. Note that cost savings alone or lack of appropriate licenses are not justification for using non-pharmaceutical grade drugs.

1. experimental goal is to examine the effects produced by the drug
2. lack of availability of a comparable pharmaceutical grade drug
3. used in ongoing studies or required for comparison to previous studies
4. available formulations contain substances that would compromise the scientific validity of the study
5. pharmaceutical-grade drugs are available, but are not compatible with the concentration, formulation, delivery, or vehicle requirements of experimental administration

When determining if non-pharmaceutical grade drugs are acceptable for use in laboratory animals, the IACUC may consider the following factors:

6. purity: quantity of contaminants and unknown substances
7. sterility: injection formulations should be sterile
8. pyrogenicity: injection formulations should be pathogen free
9. plasma pH: injection formulations should be between 6.5 and 8.0
10. osmolality: should be within the normal range of ~300
11. stability: should remain within specifications for a specified period of time
12. storage: should be stored according to manufacturer's recommendations
III. Use of expired pharmaceuticals

According to the USDA, Guide for the Care and Use of Laboratory Animals, and OLAW, the “use of expired pharmaceuticals, biologics, and supplies is not consistent with acceptable veterinary practice or adequate veterinary care. Euthanasia, anesthesia and analgesia agents should not be used beyond their expiration date, even if a procedure is terminal. Other expired materials should not be used unless the manufacturer verifies efficacy beyond the expiration date, or the investigator is able to document to the satisfaction of the IACUC that such use would not negatively impact animal welfare or compromise the validity of the study.” However, if signs of contamination are apparent, the drugs must be discarded even if they have not exceeded the manufacturer’s recommended expiration date.

USDA Policy #3 states: “Research, Teaching, and Testing Acute Terminal Procedures: Expired medical materials except analgesics, sedatives anesthetics, and euthanasia solutions may be used in acute terminal procedures where an animal is anesthetized during the study and euthanized without recovery if such use does not adversely affect the animal’s well-being or compromise the validity of the scientific study. Facilities permitting the use of expired medical materials in acute terminal procedures should have a policy on the use, storage, and disposal of such materials which is in accordance with all relevant institutional, local, state, and federal requirements where applicable; and/or require investigators to describe the intended use in the animal study proposal.”

IV. Compounding of drugs

When drugs are compounded or manipulated, use the following expiration dates:

1. use by the manufacturer’s recommendations for expiration
2. if there are no manufacturer’s recommendations, the drug in the mixture that expires first is the expiration for the whole mixture
3. if there is uncertainty as to the expiration date, consider 180 days the expiration for mixtures: a shorter or longer time frame may be appropriate depending on the nature of the compound and diluents
4. if there is any physical change (color change, sediment) prior to the expiration date, the mixture must be discarded

V. Preparations, solubility and safety of solutions

All solutions administered via injection to laboratory animals should be sterile and free of pathogens. Prior to administration, chemical compounds must be dissolved in a solvent or vehicle, most of which have little or no impact on drug absorption or drug action. The use of single and multiple solvents to establish a stable preparation is an acceptable practice, as almost half of all new chemical entities are not water soluble and some
Chemical entities are only soluble in water at concentrations lower than those required for experimental study. The more common solvents/vehicles used to dilute drugs commonly administered to laboratory animals include water, sterile saline, mixtures of water and polyethylene glycol, 10% Tween 80 or extremely low concentrations of methylcellulose, and phosphate buffered saline. Different oils (e.g., corn, vegetable, peanut) are also utilized when the drug being administered is lipid soluble and the required concentrations cannot be dissolved in water. The use of distilled water as a diluent is discouraged as it can cause pain when administered subcutaneously and hemolysis when administered intravenously.

VI. Transferring drugs from one vial to another or diluting drug concentrations

The following information should be considered when transferring drugs from one vial to another, or when diluting drugs.

1. Needles/syringes, vials/containers and fluids/solvents used for dilution should be sterile.
2. The vial must be labeled with:
   - Drug name
   - Drug concentration
   - Date of expiration
3. All containers used when transferring or diluting drugs should have sealed rubber stoppers.
4. To ensure sterility, if the original container does not have a rubber stopper, the transfer should be done in a laminar flow hood or biosafety cabinet.
5. Best practice when using a vial/bag/container repeatedly is to disinfect the opening with alcohol.
6. Solutions derived from non-sterile components should be filtered into sterile, sealed containers.
7. Discard any substance that contain the following:
   - particulate matter or precipitates
   - turbidity or discoloration
   - mislabeled or unlabeled container
   - damage to the rubber stopper compromising integrity

VII. Administration routes, volumes, needle gauge, and dosages

When administering drugs or other substances to laboratory animals, care should be taken in selecting an appropriate route of administration, dose volume and needle gauge.
Administration Route

When determining the appropriate route of administration, consideration should be given to the drug’s rate of delivery, systemic absorption, distribution, and duration of action. The particular route of drug administration selected should optimize drug delivery, and minimize any short- and long-term discomfort to the animal. Regardless of the route selected, all personnel administering drugs should be well trained.

1. **Intraperitoneal injection:**
   This type of injection is a commonly used method for small rodents, as they do not have readily accessible veins. Intraperitoneal injections are administered into the lower abdominal quadrants where vital organs are absent. It is essential to aspirate before injecting to avoid inadvertent administration into the bladder or gastrointestinal tract. Avoid administering irritating substances that may cause ileus (stasis of the gastrointestinal tract) or peritonitis (inflammation of the abdominal cavity).

2. **Subcutaneous injection:**
   This type of injection can be performed in any area of loose skin, typically along the back or flank. Tenting of the skin between the shoulder blades or over the rump creates an appropriate pocket for the injection. It is essential to aspirate before injecting to avoid inadvertent administration into a blood vessel. Relative to other types of injections, subcutaneous injections allow for larger volumes to be administered, although it is essential to avoid distending the skin. The rate of absorption from a subcutaneous injection may be slower than with other parenteral routes.

3. **Intramuscular injection:** As this type of injection can be painful, it should only be used in instances where other injections routes or methods of administration are deemed inappropriate. This injection method is considered more appropriate in larger species that have greater muscle mass. It is essential to aspirate before injecting to avoid inadvertent administration into a blood vessel. In smaller animals, use of the gluteal or quadriceps muscles is most common, whereas in larger animals the gluteal, quadriceps, or bicep muscles are preferred. When administering into the quadriceps, especially in rodents, it is essential to avoid the sciatic nerve that runs along the caudal aspect of the femur, as inadvertent injection into this or other nerves can result in pain, paralysis and localized muscle necrosis.

4. **Intravenous injection:** Site selection for venous access is species-specific, and
5. **CNS administration:** When it is essential that substances be delivered into the cerebral space or directly into a specific brain site, intracranial injections are the most effective method. These injections require anesthesia, are typically done during a surgical procedure, and can be administered through an implanted cerebral cannula, direct injection, or an osmotic pump catheter. This route is the preferred method when administering an adeno-associated viral vector serotype (along with a promoter that drives gene expression) and requires stereotaxic coordinates to ensure precise delivery of AAV to the desired area with minimal damage to the surrounding tissue. In some cases, intracranial injections can be delivered without the need for surgery, providing the animal is anesthetized and an intradermal needle is utilized that can pierce the cranium and not extend too deeply into the brain. Intra-thecal injections and intra-spinal injections are also included in this category.

6. **Retro-orbital injection:** Delivery of a substance via the venous sinus is an alternative method to tail vein injections in rodents. The IACUC will permit orbital sinus injections when it is scientifically justified, and performed on anesthetized animals. A small gauge needle should be used (27 gauge or smaller). Injections should not exceed 150ul in adult mice or 10ul in neonates. The use of topical proparacaine anesthetic drops in the eye is recommended.

Alternating eyes for each injection is mandatory, and a week must separate each injection in a given eye (unless the final injection is performed as a terminal procedure – animal does not recover from anesthesia). A maximum of two (2) injections per eye is permitted. Orbital sinus injection requires training and must be performed on anesthetized animals only with IACUC approval.
7. **Oral gavage:** The use of stainless steel, ball tipped, or single use, plastic flexible tipped gavage needles are recommended for this route of administration. Before inserting the gavage needle, measure the distance from the tip of the nose to the last rib to ensure an adequate estimate of the distance between the esophagus and the stomach. If the tube is too short, the injected fluid may be aspirated causing pneumonia and death. If the needle is too long, it may perforate the stomach. Improper gavage technique can cause tearing of the esophagus or asphyxiation. Administration of gavage volumes greater than 5 ml/kg may cause distress in species that are unable to vomit, such as rodents and rabbits. If a volume greater than the recommended volume is needed, DCM veterinarians should be contacted. (If DCM veterinarians approve the larger volume, an IACUC protocol exception will be required.)

8. **Footpad injection:** Combination of an intradermal and subcutaneous injection used primarily in models of immunization, inflammation, arthritis, pain and to administer certain types of neurotracer dyes. Footpad injections have the capacity to cause inflammation, tissue necrosis, discomfort, and pain. Scientific justification is required. The use of a 25 gauge or smaller needle in addition to disinfection of the injection site is required. Only one hind foot is allowed to be injected.
**Administration Volume**

Maximum injection volumes vary markedly across routes of administration and species. As a general rule, lower concentrations are preferable. Large volumes administered subcutaneously or intramuscularly can result in pain and necrosis, while intraperitoneal administration of large volumes can impact the drugs absorption rate. Volume is especially critical when a drug is administered intravenously, as higher volumes can cause pulmonary or cardiac abnormalities, as well as death.

<table>
<thead>
<tr>
<th>Route</th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recommended</td>
<td>Maximum (in ~25g mouse)</td>
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<tr>
<td>SC</td>
<td>10 ml/kg</td>
<td>40 ml/kg (1 ml)</td>
</tr>
<tr>
<td>IP</td>
<td>20 ml/kg</td>
<td>40 ml/kg (1.0 ml)</td>
</tr>
<tr>
<td>IM</td>
<td>0.025 ml/site*</td>
<td>0.05 ml total (50 µl) in 2 sites</td>
</tr>
<tr>
<td>Oral (gavage)</td>
<td>≤ 5 ml/kg</td>
<td>≤ 5 ml/kg (125 ul)</td>
</tr>
<tr>
<td>IV (bolus)</td>
<td>5.0 ml/kg</td>
<td>5.0 ml/kg (125 µl)</td>
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<tr>
<td>IV (continuous)</td>
<td>2.0-4.0 ml/kg/hr</td>
<td>4.0 ml/kg/hr (100 µl)</td>
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<tr>
<td>ICV</td>
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<td>4.0 µl</td>
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<tr>
<td>Brain site specific</td>
<td>0.1-5.0 µl</td>
<td>5.0 µl</td>
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<tr>
<td>Intranasal</td>
<td>35-50 µl</td>
<td>50 µl</td>
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<tr>
<td>Retro-orbital</td>
<td>5.0-30 µl</td>
<td>150 µl</td>
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</table>

*Site for IM injection must be quadriceps muscle or equivalent size muscle.
Needle Gauge

<table>
<thead>
<tr>
<th>Species</th>
<th>Injection Site and Needle Gauge</th>
<th>SC</th>
<th>IM</th>
<th>IP</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feline</td>
<td>Scruff, Back 18-22 Gauge</td>
<td></td>
<td></td>
<td>21-25 Gauge</td>
<td>Cephalic Vein 20-25 Gauge</td>
</tr>
<tr>
<td>Canine</td>
<td>Scruff, Back 18-25 Gauge</td>
<td></td>
<td></td>
<td>21-22 Gauge</td>
<td>Cephalic Vein 18-25 Gauge</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>Scruff, Back 20-25 Gauge</td>
<td></td>
<td></td>
<td>22-25 Gauge</td>
<td>Ear Vein or Saphenous Vein 25-27 Gauge</td>
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<tr>
<td>Hamster</td>
<td>Scruff 22-25 Gauge</td>
<td></td>
<td></td>
<td>22-25 Gauge</td>
<td>Femoral Vein or Jugular Vein 25-27 Gauge</td>
</tr>
<tr>
<td>Primate (Small)</td>
<td>Scruff, Back 20-25 Gauge</td>
<td></td>
<td></td>
<td>22-25 Gauge</td>
<td>20-25 Gauge</td>
</tr>
<tr>
<td>Primate (Large)</td>
<td>Scruff, Back 18-25 Gauge</td>
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<td></td>
<td>20-22 Gauge</td>
<td>20-25 Gauge</td>
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<tr>
<td>Rat</td>
<td>Scruff 22-25 Gauge</td>
<td></td>
<td></td>
<td>22-25 Gauge</td>
<td>Lateral Tail Vein 22-25 Gauge</td>
</tr>
</tbody>
</table>

Drug Dosage

Selection of drug dosages is typically made on the basis of the scientific goals of the research. Appropriate selection of dosages, however, should also take into account the investigator's experience with the drug/dose, a thorough literature review, consultation with colleagues and/or pilot studies. When no or limited information is available regarding the drug's potency, potential toxicity can be minimized by initially testing relatively low doses. This is often a critical component of selecting drug dosages, as dosages are not always uniform across animal strains, especially between mutants and wild types, or young and aged animals.
EXCEPTIONS

Requests for exceptions to this Standard must be reviewed and approved by the IACUC and/or DCM Management.

DEFINITIONS

IACUC: Institutional Animal Care and Use Committee

DCM: Division of Comparative Medicine

OACU: Office of Animal Care and Use

University Standard: The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.

Pharmaceutical grade drug (or compound or pharmaceutical or chemical) is a biologic or reagent that is approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been established by the United States Pharmacopeia-National Formulary (USP/NF) or British Pharmacopeia (BP). Pharmaceutical grade drugs have a high level of purity (>99%), are produced in a government approved facility with appropriate documentation, and meet consistent specifications for each batch of product produced.

Compounding refers to any manipulation of a drug beyond that stipulated on the drug label.

Manipulation includes mixing (refers only to anesthetic, analgesic or euthanasia agents), diluting, concentrating, flavoring, or changing a drug’s dosage form.

Related Requirements

UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES

For more general guidance, please refer to the University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes.

Contact Information

PRIMAR Y CONTACT(S)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Contact</th>
<th>Telephone</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>IACUC Protocol and Training</td>
<td>OACU</td>
<td>919-966-5569</td>
<td><a href="mailto:iacuc@med.unc.edu">iacuc@med.unc.edu</a></td>
</tr>
<tr>
<td>Technique or method, protocol consultation prior to submission or amendment</td>
<td>DCM Veterinary Services</td>
<td>919-962-5335</td>
<td></td>
</tr>
</tbody>
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Important Dates

This section must contain: the original effective date for this document or any direct

Effective Date and title of Approver: December 4, 2015, UNC-CH IACUC; Revised September 2018, Added Retro-Orbital (RO) Injection Description and Volumes; revised June 2019: added more specifics to RO bleed description & method, preparation of vial/bag for multiuse containers, updated administration volumes and associated needle gauges, replaced all DLAM with DCM, added acceptable IM muscle location.

Approved by: UNC-CH IACUC

Dr. Roland Tisch
UNC IACUC Chair
06/2019

Policy Title: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL STANDARD FOR ADMINISTERED AGENTS
Effective Date: April 7, 2017
Last Revised: June 2019