

# Office of Research Support and Compliance

Vice President for Research, Scholarship and Creative Endeavors

## Guidelines for the Use of Drugs and Chemicals in Animal Research

The University of Texas at Austin Institutional Animal Care and Use Committee

*These guidelines have been written to assist faculty, staff, and students in performing vertebrate animal procedures in a humane manner and complying with pertinent regulatory requirements. Under some circumstances deviations from these procedures may be indicated but such variances must be approved in advance by the IACUC.*

Biomedical research commonly involves drug or chemical administration to animals as research subjects. In order to promote the humane use of drugs and chemicals, both the NIH and the USDA have issued guidelines for investigators, and the IACUC is charged with ensuring compliance with these guidelines. In general, the use of drugs in animals falls into one of two usage categories: standard veterinary care or experimental use. These guidelines apply to both types of administration. This document provides an overview of requirements that must be met when using drugs and chemicals in laboratory and animal research. It is organized into seven sections:

Section A – Requirements

Section B – Best Practices for Drug Storage, Monitoring Expiration, and Inventory

Section C – Drug Dilutions: Preparation and storage

Section D – Disposal of Substances

Section E – Who to Contact for More Information on Sections A-C

Section F – Administration of Substances to Laboratory Animals

Section G – References

Section H – Acknowledgements

## Section A – Requirements

1. **Current standards for the veterinary care of research animals state that pharmaceutical grade medications should be used for routine medical treatment.** Pharmaceutical grade drugs are tested for purity to reduce the possibility that they are contaminated with toxic compounds that may harm an animal. Examples of routine veterinary procedures that involve the use of drugs include surgery, treatment of infection, administration of pain control, and euthanasia. Drugs used for these procedures (including anesthetics such as ketamine, pentobarbital, or isoflurane; analgesics such as carprofen or buprenorphine; antibiotics; supportive fluids; parenteral nutrients; etc.) used either as part of the IACUC-approved study or a veterinarian-approved treatment plan should be obtained from a veterinary supply house or from a pharmaceutical supplier licensed by the FDA, if it is available from such sources. Typically, drugs obtained this way will be in a form that is packaged, labeled, and licensed for either animal or human clinical use.
2. **Chemicals and compounds administered to research animals for experimental objectives should also be of the highest purity possible.** There are two categories of drugs used for experimental purposes: already in clinical use or not approved for clinical use. If the drug is currently approved for clinical use (either in human or animals), then the investigator should determine whether a formulation of the drug is available that is suitable for the experiment in question. Most drugs are formulated to contain excipients that are safe for clinical use, but may interfere with experimental objectives. If the excipients do not confound the study, then the drug should be obtained from a veterinary supply house or from a pharmaceutical supplier licensed by the FDA. If excipients interfere with the experimental objectives or if the chemical is not approved for clinical use, the investigator is allowed to formulate the drug or chemical

provided that purity and stability of the drug is maintained. In this case, the investigator must submit a protocol that describes and justifies the proposed use of the drug or chemical formulation, and the IACUC must review and approve the protocol before experiments are carried out. For example, the pharmaceutical version of a drug marketed for IV injection may not be formulated with an appropriate concentration or vehicle for administration via an intracerebral cannula or osmotic minipump. Furthermore, controlled scientific studies may require a control group dosed with the vehicle only, and the vehicle may not be readily available. Finally, some studies will use novel chemicals or mixtures of chemicals either synthesized or isolated from natural sources. These chemicals should be of the highest purity attainable (either from commercial sources or from laboratory procedures during their preparation) and formulated in an appropriate manner for a specific route of administration. When new drug or chemical formulations are proposed, the IACUC may consider factors such as the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation, compatibility, and the pharmacokinetics of the chemical or substance to be administered. Investigators are encouraged to contact a veterinarian regarding the preparation of the protocol before submission to the IACUC, but in some instances a pharmacologist or toxicologist consult may be warranted.

3. **Expired drugs must not be administered to research animals without explicit IACUC approval.** If an investigator is going to request IACUC approval to use an expired drug, they must check the “I Do Not Agree” response at the bottom of this guideline in the specific eProtocol in which the request is made, and provide an explanation and justification in the text box provided.
4. **All expired drugs, including anesthetics and analgesics, must be segregated and clearly marked “EXPIRED” on or before their date of expiration.** In order to prevent the use of expired drugs or fluids, each laboratory must establish an inventory procedure to facilitate the identification and discarding of expired drugs.
  - The IACUC recommends laboratories implement the color sticker system (see Section B). However, labs may develop their own inventory tracking systems based on their specific needs.
  - If an investigator is going to request IACUC approval to use a drug inventory system other than the color sticker system, they must check the “I Do Not Agree” response at the bottom of this guideline in the specific eProtocol in which the request is made, and provide an explanation and justification in the text box provided.
5. If an investigator is going to request IACUC approval to use non-pharmaceutical grade compounds, they must list the compounds in the Husbandry section of the eProtocol form and explain why they must be used (i.e. pharmaceutical grade is not available, not concentrated enough, has vehicle or incipient ingredients not compatible with study aims, not equivalent to replicate past studies, exorbitantly priced, etc.). According to the *Guide for the Care and Use of Laboratory Animals* (8<sup>th</sup> Edition, 2011) pg. 31: “Consideration should be given to the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation, compatibility, and pharmacokinetics of the chemical or substance to be administered, as well as animal welfare and scientific issues relating to its use.” The protocol description must explain how investigators will ensure preparation is sterile and accounts for pH and osmolality. Solutions derived from non-sterile components must be filtered (0.22 µm or finer) into sterile, sealed containers. A very viscous product may require a filter with a larger pore size, but this increases the chance of improper sterilization and may require verification of sterility. The protocol must describe why a solution cannot be sterile-filtered if this is not possible for scientific reasons. The pH of solutions must be between pH 4.5 and 8.0. Use of a solution with a pH outside this range must be approved in the animal use protocol.
6. Toxic agents used in animal research (i.e. Tricaine/MS-222, isoflurane, aldehyde fixatives, carcinogens, mutagens, reproductive hazards, cytotoxic drugs, etc.) must be listed in the Are You Using? section of the eProtocol form. Standard Operating Procedures (SOPs) should be implemented for each agent and reviewed regularly to ensure they remain current. The Environmental Health and Safety (EHS) office has developed hazardous chemical SOP templates which may be downloaded here: <https://ehs.utexas.edu/research-labs->

## Section B – Best Practices for Drug Storage, Monitoring Expiration, and Inventory

The following are best practices suggested to facilitate the implementation of these guidelines:

- **The IACUC recommends a color-coded sticker inventory procedure to highlight the expiration date and prevent the use of expired drugs.** In this procedure, each original container is labeled using a colored sticker, which represents the year the item will expire with the month written on the sticker. Figure 1 shows an expiration key that assigns a color to each year. Figure 2 shows a bottle of Isoflurane that has a June 2019 expiration. Therefore, it has a yellow sticker (2019) with the number 6 (June) written on it.





|      |   |
|------|---|
| 2019 |  |
| 2020 |  |
| 2021 |  |
| 2022 |  |

Figure 1: Expiration key



Figure 2: Example

- **Drugs without expiration dates should be dated upon receipt.** The investigator should determine the stability of the drug to come up with a reasonable shelf-life. This is commonly obtained from the manufacturer, and for most stable organic compounds the shelf-life is up to three years. If stability is unknown, the drug should not be used beyond one year. Dates must be tracked and unused drugs must be segregated and clearly marked “EXPIRED” after the labeled shelf-life has expired.
- Consider assigning the inventory responsibilities to one specific individual, with another individual assigned as backup.
- Establish an inventory system that minimizes the amount of drug or medical supplies on hand.
- Perform monthly checks of your inventory and segregate, label, and discard all expired drugs or medical materials. Recurring automatic calendar alerts to remind staff to check drugs and supplies may be useful.
- Place all expired drugs and medical materials in a clearly labeled container that is segregated from non-expired drugs and materials while they await pickup for disposal or return to manufacturer.

## Section C – Drug Dilutions: Preparation and storage

All dilutions of drugs are to be discarded one month after the date of preparation. Drugs and chemicals are more susceptible to degradation and bacterial contamination after dissolution or dilution. The only exception is when drugs are 1) diluted or reconstituted in the primary packaging following label directions and 2) the manufacturer has provided expiry dating guidance for a period longer than one month. The IACUC will consider deviations from this policy if the investigator submits a compelling justification explaining why the stability and purity of the compound is expected to be maintained longer than one month.

- When drugs are diluted, appropriate closed sterile containers designed for needle access must be used, e.g. septum-topped injection vials or additive-free serum (red-topped) tubes
- All containers must be labeled with:
  - Name of the drug(s)/chemical(s) contained within
  - Concentration of the drug/chemical
  - Date of expiration (see above)
- The rubber injection port/cap should be swabbed each time with 70% alcohol prior to insertion of the needle
- A clean, sterile container should be used for each preparation (do not reuse vials)
- Substances must not be used if any of the following are observed:
  - Formation of precipitates, except when expected (e.g., suspension preparations)
  - Discoloration
  - Gross signs of contamination
- Failure to maintain the substance under sterile conditions
- Sustained-release drugs (such as Ethiqva) cannot be diluted (as it alters the kinetics of release and impacts the sustained-release component of the medication)

## Section D – Disposal of Substances

Disposal services for expired controlled substances are available at no cost to the investigator. For proper disposal of controlled substances, go to the EHS website at:

<https://ehs.utexas.edu/programs/labsafety/dea-substances.php>.

For proper disposal of hazardous waste (e.g., chemical, microbiological, animal products, human blood, etc.), go to the EHS website at: <https://ehs.utexas.edu/programs/hazardouswaste/>.

## Section E – Who to Contact For More Information on Sections A-C

For questions about these guidelines or more information about the colored sticker inventory procedure, contact the Office of Research Support (ORS) at (512) 475-8650 or [IACUC@austin.utexas.edu](mailto:IACUC@austin.utexas.edu).

For assistance finding sources for veterinary pharmaceuticals, contact the Animal Resources Center (ARC) at (512) 471-7534 or [arcinfo@austin.utexas.edu](mailto:arcinfo@austin.utexas.edu).

For questions regarding the disposal of pharmaceuticals and chemicals, contact EHS at (512) 471-3511 or [ehs-labstaff@austin.utexas.edu](mailto:ehs-labstaff@austin.utexas.edu). EHS can also provide advice on how to properly dispose of other drugs, some of which may be considered hazardous material or chemical waste.

## Section F – Administration of Substances to Laboratory Animals

This section is intended to provide guidelines about administration of substances to laboratory animals. All procedures must have prior IACUC approval. The route of administration, intervals between substance administration, dose range, and volume to be administered should be carefully chosen and listed in the approved protocol specific to each study. If you have questions or need training in any of these methods, contact the ARC veterinary staff. Researchers should consult with an ARC veterinarian if they plan to use an administration method other than what is described in this guideline.

### Parenteral Administration

These routes administer substances outside of the gastrointestinal tract. Substances administered parenterally should be isotonic (the same concentration of solute as the blood) and close to physiologic pH (6.8 – 7.2). If the pH is outside of physiologic range, administer the substance through a central vessel (such as the jugular or femoral vein) or buffer the solution such that pH is appropriate. All substances given parenterally **must be sterile** and should be delivered aseptically. If the preparation is not a commercially manufactured solution, it must be mixed in a laminar flow hood or biosafety cabinet and filtered through a 0.2 micron filter.

Routes of parenteral administration are listed below.

- a) **Intravenous (IV)** – Administration of substances into venous circulation.
  - i. Substances can be administered as a bolus or as an infusion. Infusions are often administered with specific equipment (precision pumps or microdrip infusion sets).
  - ii. Substances must be free of particulates that may induce foreign body emboli; and minimally irritating to vascular endothelia, to prevent vasculitis and thrombosis, and to erythrocytes, to minimize lysis
  - iii. Site selection for venous access is species-specific. The following are common sites:
    - a. Rodents: lateral tail vein, saphenous vein, or retro-orbital venous sinus (injection volume for retro-orbital injection is limited to 200 µl)
    - b. Rabbits: lateral ear, jugular, or cephalic vein
    - c. Other larger species: jugular, cephalic, femoral, or saphenous vein
  - iv. Consult with ARC veterinary staff for recommendations on refinements to improve animal comfort during repeated IV dosing.
- b) **Intraperitoneal (IP)** – Administration of substances into the abdominal cavity.
  - i. Injections are administered into lower abdominal quadrants. Aspirate before injecting to avoid inadvertent administration into the bladder or gastrointestinal tract.
  - ii. Repeated daily intraperitoneal dosing for up to one month is well-tolerated in rodents. Doses should be administered to alternating sides of the abdomen.
  - iii. Administration of irritating substances may cause ileus (stasis of the gastrointestinal tract) and peritonitis (inflammation of the abdominal cavity).
  - iv. This route is not recommended for rabbits.
- c) **Topical (epicutaneous)** – The application of substances directly to the skin for topical effect.
  - i. Avoid application of caustic or irritating substances unless you have prior approval on an IACUC protocol.

- ii. Apply substances to skin that is unbroken and free of hair.
  - iii. Avoid application of substances to sites that animals can reach during grooming.
- d) **Transdermal (percutaneous)** – The application of substances directly to the skin for systemic effect.
- i. Transdermal dosing is typically accomplished by application of a patch impregnated with the substance of interest.
  - ii. Apply the patch so as to avoid inadvertent ingestion or removal by the animal.
  - iii. Systemic absorption is not immediate. Patches should be applied prior to the time of anticipated need according to manufacturer's instructions.
  - iv. **Do not cut patches** to reduce dose size. If an appropriate dose of patch is not commercially available, consider an alternative route of administration or cover the un-needed portion with surgical tape.
- e) **Subcutaneous (SC)** – Administration of substances into the subcutaneous space.
- i. Tent the skin. Holding the syringe parallel to the animal, direct the needle into the subcutaneous tissue (beneath the skin). Aspirate and inject.
  - ii. When administering large volumes subcutaneously, 2-3 different sites of administration should be used.
  - iii. The rate of absorption from the subcutis may be slower than with other parenteral routes.
  - iv. Subcutaneous infusions can be administered with the use of an oily depot or osmotic mini-pump. Consult veterinary staff for additional information.
- f) **Intradermal (ID)** – Administration of substances into the dermis.
- i. Use a small, sharp needle (25-27G).
  - ii. Tent the skin. Holding the syringe parallel to the animal, direct the needle into the dermis. Aspirate and inject. A “bleb” should be visible if the substance is in the intradermal tissue.
  - iii. Inadvertent subcutaneous administration is common. Consult ARC veterinary staff for assistance or training.
- g) **Intramuscular (IM)** – Administration of substances into the muscle.
- i. IM dosing is best used in larger species with greater muscle mass and is not recommended in rodent species.
  - ii. In smaller animals, use the gluteal or quadriceps muscles.
  - iii. In larger animals, use the gluteal, quadriceps, biceps or epaxial muscles.
  - iv. Take care to avoid the sciatic nerve, which runs along the caudal aspect of the femur. Inadvertent injection into nerves can result in paralysis and localized muscle necrosis.
- h) **Intranasal (IN)** – Administration of substances into the nose.
- i. May (often) require(s) sedation or anesthesia.
  - ii. May be used for local or systemic delivery of substances.
  - iii. Due to high vascularization of nasal mucosa, results in rapid absorption.
  - iv. Always use the smallest volume possible to avoid suffocation.
- i) **Intraosseous (IO)** – Administration of substances into the marrow of a bone.
- i. Generally requires sedation or anesthesia.
  - ii. Substances must be delivered aseptically and be free of particulates.
  - iii. Can be used in some hypovolemic patients with inaccessible or collapsed veins.
  - iv. IO administration of substances requires highly trained personnel. Consult with veterinary staff before attempting this technique.
- j) **Intratracheal (IT)** – Administration of substances within the trachea to deliver substances to the lungs.
- i. Requires sedation or anesthesia.
  - ii. Requires familiarity of intubation of the species being used.

- k) **Intracranial** – Administration of substances into the brain.
- Intracranial injections require anesthesia (and stereotactic equipment when appropriate). Injections can be administered through a surgically implanted cerebral cannula, direct injection, or an osmotic pump catheter.
  - Animals must be heavily sedated or anesthetized for cannula or catheter placement and for direct injections
- l) **Epidural (ED)** – Administration of substances into the epidural space.
- Epidural administration of substances requires highly trained personnel. Consult with veterinary staff before attempting this technique.
  - Animals must be heavily sedated or anesthetized.
- m) **Intrathecal (IT)** – Administration of substances into the subarachnoid space (in the spinal canal but not within the spinal cord).
- Intrathecal administration of substances requires highly trained personnel. Consult with veterinary staff before attempting this technique.
  - Animals must be heavily sedated or anesthetized.
- n) **Inhalation (INH)** – Administration of substances into the lungs.
- Inhalational delivery typically uses vapors or aerosols of nebulized particles in solution.
  - Animals are conscious with this delivery method and are restrained with or without a specialized nose mask to optimize delivery.
  - Complex technique requiring specialized equipment and knowledge. If vapor chambers are used, the investigator must contact ARC and EHS staff prior to use to ensure correct installation for animal and human safety.

**Table 1: Guidelines for Parenteral Administration/ Injection of Substances by Site in Common Laboratory Animals (ideal volumes provided with maximums in parentheses)**

| Species                      | IV bolus<br>/slow IV*<br>ml/kg | IV drip<br>ml/kg/hr<br>** | IP<br>ml/kg  | SC<br>ml/kg** | ID ml/inj | IM<br>mls/site | IN<br>ml/<br>animal | ED***<br>ml/kg |
|------------------------------|--------------------------------|---------------------------|--------------|---------------|-----------|----------------|---------------------|----------------|
| Mouse/<br>Vole/Wild<br>mouse | 1-5 /25                        | 1-4                       | 1-10<br>(20) | 1-5 (20)      | 0.05-0.1  | <0.05<br>(0.1) | 0.03-0.05           | 0.15-0.2       |
| Rat                          | 1-5 /20                        | 1-4                       | 1-10<br>(20) | 1-5 (10)      | 0.05-0.1  | <0.1 (0.2)     | 0.03-0.05           | 0.15-0.2       |
| Guinea Pig                   | <1 /5                          | 2-4                       | 1-10<br>(20) | 1-5 (10)      | 0.05-0.1  | <0.1 (0.2)     | 0.03-0.05           | 0.15-0.2       |
| Hamster/<br>Gerbil           | 1-5 /20                        | 2-4                       | 1-10<br>(20) | 1-5 (10)      | 0.05-0.1  | <0.1 (0.2)     | 0.03-0.05           | 0.15-0.2       |
| Rabbit                       | <1 /10                         | 5-10                      | NR           | 1-2.5 (10)    | 0.05-0.1  | 0.5 (1.0)      | 0.2-0.5             | 0.15-0.2       |
| Ferret                       | 1-5/10                         | 1-5                       | 1-5 (20)     | <2 (5)        | 0.05-0.1  | <0.5 (1.0)     | 0.2-0.5             | 0.15-0.2       |
| Macaque                      | 1-5/10                         | 5-10                      | 1-10         | <1 (2)        | 0.05-0.1  | (2.0)          | 0.2-0.5             | 0.15-0.2       |
| Marmoset                     | 1-5/10                         | 2-10                      | 1-10         | <2 (5)        | 0.05-0.1  | (0.25)         | 0.2-0.5             | NR             |
| Swine                        | 1-5/10                         | 5-10                      | 5-20         | <1 (3)        | 0.05-0.1  | (5.0)          | 0.2-0.5             | 0.15-0.2       |

NR= Not recommended

\* Bolus injections are typically dosed in less than 1 minute. Slow intravenous injections are typically dosed over 3-10 minutes. The maximum recommended injection volume for a dosing solution that is given rapidly IV is 1 ml/kg body weight for most

laboratory animal species. If dosing a larger quantity of solution is necessary, infusion of the dose IV over greater than 5 minutes should be considered and should not exceed 10% of the circulating blood volume over 2 hours. Slow IV injections are given over 10 minutes.

\*\*Higher volumes may be used for fluid resuscitation by veterinary staff.

\*\*For epidural, no more than 6 ml total volume for animals up to 35 kg.

**Table 2: Recommended Locations and Approximate Needle Sizes for Injection by Site in Common Laboratory Animals**

| Species                           | IV   | IM                                       | SQ   | IP   |
|-----------------------------------|--|--|------|------|
| <b>Mouse/<br/>Vole/Wild mouse</b> | Lateral tail<br>25 G                       | NR                                       | 20 G | 25 G |
| <b>Rat</b>                        | Lateral tail<br>23 G                       | NR                                       | 20 G | 21 G |
| <b>Guinea Pig</b>                 | Ear vein, saphenous<br>23 G                | Quadriceps<br>21G                        | 20 G | 21 G |
| <b>Hamster/Gerbil</b>             | 25 G (no peripheral<br>vessel recommended) | NR                                       | 20 G | 21 G |
| <b>Rabbit</b>                     | Marginal Ear<br>21G                        | Quadriceps, epaxial<br>20G               | 20 G | 20 G |
| <b>Ferret</b>                     | Cephalic, jugular<br>23 G                  | Quadriceps, epaxial<br>21G               | 21 G | 21 G |
| <b>Macaque</b>                    | Cephalic, saphenous<br>20 G                | Quadriceps/<br>posterior thigh<br>20G    | 20 G | 20 G |
| <b>Marmoset</b>                   | Tail vein, saphenous<br>25 G               | Quadriceps<br>25 G                       | 22 G | 25 G |
| <b>Swine</b>                      | Marginal Ear vein<br>20G                   | Neck/epaxial,<br>gluteal muscles<br>20 G | 20 G | 20 G |

NR= Not recommended

### Enteral Administration

This route administers substances into the gastrointestinal tract. Routes of enteral administration are listed below.

- a) **Per os (PO)** – Administration of substances by mouth through voluntary consumption.
  - a. Substances are typically mixed with the daily diet, flavored water, or other palatable items to encourage consumption. Care should be taken to maintain an appropriate daily caloric intake and to habituate animals to any novel food items before adding drug.
  - b. Care should be taken to ensure animals consume all agent offered. Laboratory personnel are responsible for ensuring that food and water intake is adequate.
  - c. Food or water containing additives should be clearly labeled and disposed of properly.
- b) **Gavage** – Administration of substances via a tube that is passed through the nose or mouth into the esophagus or stomach.
  - a. Gavage is often used to administer an exact PO dose.
  - b. The anatomy of the pharynx in the rabbit and guinea pig species makes gavaging difficult



- c. Administration of gavage volumes greater than 5 ml/kg may cause distress in species that are unable to vomit such as rodents, rabbits, chinchillas, etc.
  - d. The gavage tube size should be appropriate for the species being dosed. Contact veterinary staff for assistance.
- c) **Rectal** – Administration of substances into the rectum.
- a. This technique is not frequently used in laboratory animals. Substances can be administered via an enema or a suppository.

**Table 3: Guidelines for Enteral Administration of Substances by Site in Common Laboratory Animals**

| Species    | Gavage ml/kg<br>ideal (max) |
|------------|-----------------------------|
| Mouse      | 1-10 (20)                   |
| Rat        | 1-10 (20)                   |
| Guinea Pig | 1-10 (20)                   |
| Hamster    | 1-10 (20)                   |
| Rabbit     | 1-10 (20)                   |
| Ferret     | 1-10 (20)                   |
| Macaque    | 1-10 (15)                   |
| Marmoset   | 1-10 (15)                   |
| Swine      | 1-10 (15)                   |

## Section G – References

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## Section H – Acknowledgements

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| Approval Date | Major Change(s) Approved   |
|---------------|--|
| 01/10/2022    | <ul style="list-style-type: none"><li>• Section A # 3-4 updated regarding the use of expired drugs</li><li>• Section A # 5 updated to include variables concerning the use of non-pharmaceutical grade drugs</li><li>• Section A #6 added to describe requirements for use of toxic agents</li></ul> |
| 7/10/2023     | <ul style="list-style-type: none"><li>• Expanded guidance on drug dilutions and moved this info to a new section.</li></ul>  |