COMPOUND PREPARATION AND USE OF SECONDARY CONTAINERS

I-IC-GU-608

A. PURPOSE

To provide guidance on the preparation of compounds to be administered to animals and appropriate secondary containers used for compounding, diluting, or transferring drugs, and compounds to be administered by parenteral injection to animals.

B. GUIDELINES

The USDA, PHS (OLAW), and AAALAC require that pharmaceutical grade compounds be used in animal research, **wherever possible**. The rationale is that pharmaceutical grade compounds meet established standards of purity, which ensures both animal health and wellbeing and the validity of experimental results. Lower grade chemicals contain impurities that have the potential to introduce experimental variability or may have toxic effects on animals.

In research, there may be situations where the use of non-pharmaceutical grade compounds is necessary to achieve study objectives. Even though non-pharmaceutical grade compounds can be used with scientific justification, their preparation, labeling, storage, and use must follow certain guidelines. In addition, the investigator must always use the highest purity compound commercially available. **Note that the following guidelines also apply to dilution of pharmaceutical grade drugs to a working concentration** (e.g., ketamine-xylazine-acepromazine).

DOCUMENTATION:

- Review the protocol or amendment to ensure that an approved compound and concentration/dose will be used
  - **If not**, submit an amendment **before** the work begins.
- Identify the highest grade of all the ingredients used to prepare the compound.
- Prepare an SOP describing the preparation and/or dilution of the compound (see the sample SOP for preparation of Compounds for Use in Animals) to minimize issues of quality control or batch-to-batch variation.

PREPARATION:

- All material to be injected should be sterilized, e.g., through a 0.22µM filter or autoclaved.
- Injectable material that could become contaminated with pyrogens (i.e bacterial endotoxins) should be tested using an *in vitro* assay before use; note that autoclaving or sterile filtering **does not** remove pyrogens.
- Concentrated compounds that must be diluted before administration:
  - The diluent must be USP grade, where possible; note that most diluents can be purchased as USP grade.
  - USP diluents must be used when compounding clinical (veterinary) drugs, such as anesthetic cocktails.
- Assure the diluent is appropriate for the compound:
  - Solubility, salt concentration (osmolality), buffering (pH)
- Assure the diluent minimizes pain and distress, especially for injectable compounds.
- Aseptic technique must be used when handling and preparing sterile compounds.
  - Compounding procedures should be performed inside a laminar flow cabinet.
  - The rubber septum on a multi-use vial must be swabbed with 70% ethanol before introducing the

needle.
  - Compounds must be drawn into a sterile needle and syringe.
- Expiration, stability or shelf-life:
  - Use shelf-life information provided with the compound if available. If information is available in the literature include it on the compounding SOP for IACUC review.
  - The efficacy and stability of sterile, diluted ketamine-xylazine–acepromazine (KXA) was determined to be 180 days when stored in a dark, room temperature environment (JAALAS, 2009, 48:718-726), or the earliest expiration date of any drug in the compounded solution if < 6mo).
  - Expiration dates are based on the earliest expiration date of any drug in the compounded solution, but if no information is available, a general rule is a use-by-date of no more than 30 days.

CONTAINERS:

- Secondary containers must be compatible with the drug or compound and its intended use. Secondary containers are vials, bottle or tubes used when drugs or compounds are moved from their original containers (see below for examples).
  - Transferred
    - Taken out of the primary container and placed into a secondary container (e.g., drugs in glass ampules)
  - Diluted
    - Mixed with a diluent to achieve a working concentration (e.g., antibiotics or analgesics for use in rodents)
  - Compounded
    - Mixed with one or more drugs or diluents (e.g., a mixture of ketamine, xylazine, acepromazine and diluent or test compounds)
- Container Material
  - Does not react with the drug or compound (e.g., glass, polypropylene or polycarbonate plastic)
  - Opaque, if light sensitive material is to be stored (e.g., brown glass, covered with foil)
  - Supplied sterile or able to be autoclaved
- Types of Containers
  - Best: Multi-use vial with a septum in the cap (search septum or crimp top vial on a scientific supply website). Using aseptic technique, dispense the sterile drug or compound into the vial and the contents can be removed aseptically with a sterile needle and syringe. The top of the septum must be disinfected with 70% alcohol prior to use.
  - Second option: a red capped (untreated) blood collection tube can be used as a secondary container. Swab septum with 70% alcohol to disinfect before introducing a needle.
  - NOT appropriate: screw capped tubes should be avoided as the contents cannot be removed aseptically.

LABELING:

Any drug or compound transferred to a secondary container must be labelled as follows (see below for examples):

- The name of each ingredient, including the diluent
- The concentration of the active ingredient(s) in mg/ml
- Date of preparation
- Expiration/Use-by date:
  - Should not extend past the earliest expiration date of any drug in the solution

- Should be no longer than 30 days from preparation for compounds or dilutions, unless published or vendor-provided scientific data can be provided to demonstrate a duration of efficacy longer than 30 days, for example:
  - Compounded ketamine anesthetic cocktails (such as KXA) have a use-by-date of 6 months (or the earliest expiration date of any drug in the compounded solution if < 6 months) on the 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.

For compounds containing Controlled Substances, per DEA guidelines, the label must ALSO contain:

- The label must have:
  - lot number of each controlled substance
  - volume of each drug
  - total amount/volume of the combined drugs
  - initials of the individual disbursing the compound
- Note: the inventory must reflect all disbursements
- For more information on UA oversight of DEA regulated controlled substances, contact RLSS at https://research.arizona.edu/compliance/RLSS/chemical-safety

STORAGE:

- Appropriate storage to ensure stability until the Expire/Use-by-date:
  - Refrigerator or freezer, light-tight container (amber/brown bottle, foil covered), desiccator, etc…
- Double- locked box for controlled substances (see DEA guidelines, contact RLSS)
- Discard Expired/Use-by-date compounds:
  - For >2ml of controlled substances in a syringe, expel into a labeled waste container and store with the other controlled substances
  - For <2ml of controlled substances in a syringe, expel into a labeled waste container (best practice) or discard the syringe directly into a sharps container

ADMINISTRATION:

- Appropriate route of administration for the compound and diluent/vehicle must be followed:
  - E.g., extremely viscous or particulate material may not be suitable for injection
- Aseptic technique must be used when handling and administering sterile compounds
  - The rubber septum on a multi-use vial must be swabbed with 70% ethanol before introducing the needle
  - Compounds must be drawn into a sterile needle and syringe
  - The site of injection should be swabbed with 70% ethanol and allowed to dry
  - Single use only: Do not reuse a needle to withdraw additional compound into a syringe once it has been used to inject an animal
- Appropriately record and discard controlled substances, including residual amounts in the container or syringe
- Controlled substances are dispensed under a DEA license. RLSS and/or the DEA should be contacted regarding the applicable regulations for this activity. See DEA guidelines, below, for labeling requirements.

EXAMPLES OF CONTAINERS

Examples of appropriate vials for liquids These vials can remain sterile when obtaining multiple doses using separate sterile needles.			 Alternative, less feasible choice
Examples of vials <u>NOT</u> appropriate for liquids. They cannot remain sterility when obtaining multiple doses.			

EXAMPLES OF LABELLING:

Examples/templates of appropriate container or bottle labeling for non-controlled substances:
Compound **[name]** (12 mg/ml) + **[diluent name]** (e.g. Sterile Saline) Made: __/__/__ Expires: __/__/__

Examples/templates of appropriate container or bottle labeling for Controlled Substances:
Total Vol: ___ mL ___ml Ketamine (8.25mg/mL) Lot# ___ ___ml Xylazine (0.83mg/mL) ___ml Acepromazine (0.25mg/mL) ___ml 0.9% Saline 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 the 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: _____

PROVISOS:
The following compounds are exempt from the use of septate vials, but these 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 clinical/veterinary drugs, i.e., anesthetics, analgesics, or euthanasia drugs.

- Test compounds that are prepared for single use and will not be stored past this use.
- Test compounds that are available in small quantities (< 0.5ml), such that use of a septate vial poses a risk of losing the contents in the rubber septum.
- 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 septate vial increases the risk of accidental exposure.

DEFINITIONS:
- **Aseptic technique:** Procedures performed with the goal of minimizing contamination by microorganisms.
- **Controlled substance:** A drug or chemical substance whose possession and use are controlled by law, specifically the Drug Enforcement Agency (DEA). Controlled substances commonly used in veterinary medicine include buprenorphine (schedule III), butorphanol (schedule IV), ketamine (schedule III), Telazol (schedule III) and barbiturate drugs such as pentobarbital (schedule II) and phenobarbital (schedule IV). For a complete list please see: http://www.deadiversion.usdoj.gov/schedules/index.html. For more information on UA oversight of DEA regulated controlled substances, contact RLSS at: http://rgw.arizona.edu/compliance/RLSS.
- **Pharmaceutical grade:** The grade of any compound "which is approved by the FDA, or for which a chemical purity standard has been written/established by the US Pharmacopeia/National Formulary (USP/NF)". See: http://www.usp.org/.
- **Pyrogen:** Fever producing substances. The most common source of contaminating pyrogens is the metabolic products of microorganisms, especially bacterial endotoxin or LPS. Bacteria present in unrefrigerated solutions, such as non-sterile saline, can replicate and shed LPS into the solution. Even small amounts of pyrogens administered to animals can have deleterious effects, such as causing inflammation, which may affect experimental outcomes. The LD_{50} for LPS is on the order of 1µg per mouse. Fever and inflammation are seen at significantly lower amounts.
- **Sterile:** The absence of all microorganisms. Acceptable methods for sterilizing solutions include autoclaving (if stable under autoclaving conditions, such as saline) or passage through a 0.22µM sterile filer into a sterile container. Sterility of a solution is only maintained if that solution is handled using aseptic technique. Note that sanitizing or disinfecting something does not render it sterile, e.g., swabbing an injection site.
- **USP grade:** See pharmaceutical grade.

C. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

- I-IC-GU-210 Use of Compounds in Animals