Institutional Animal Care and Use Committee Guidance 211

TITLE: Preparation of Compounds for Use in Animals

PURPOSE: To provide guidance on the preparation of compounds to be administered to animals.

REVIEW/REVISIONS: The IACUC will review and revise this guidance as needed.

DATE OF LAST REVIEW: 10/16/2014

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.

However due to the nature of research, there will be many 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).

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 commences
- 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:
- Should the prepared compound be sterilized, and if so, how?
  - As a general rule, material to be injected should be sterilized, e.g., through a 0.22μM filter or autoclaved
- Should the prepared compound be tested for pyrogenicity?
  - Injectable material that could become contaminated with pyrogens should be tested using an in vitro assay before use; note that autoclaving or sterile filtering does not remove pyrogens
- Is the compound prepared as a concentrate that must be diluted before administration?
  - If so, the diluent must be USP grade, where possible; note that most diluents can be purchased as USP grade
- Is the diluent appropriate for the compound?
  - Solubility, salt concentration (osmolality), buffering (pH)
- Does the diluent minimize pain and distress, especially for injectable compounds?
- What is the stability or shelf-life of a compound or mixture?
  - Was shelf-life information provided with the compound or is there information available in the literature?
  - The efficacy and stability of sterile, diluted ketamine-acepromazine-xylazine was determined to be 180 days when stored in a dark, room temperature environment (JAALAS, 2009, 48:718-726)
  - If no information is available, a general rule of thumb is a use-by-date of no more than 30 days
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Labeling:

- The label must include:
  - The name and concentration of all the compounds in the mixture (including the diluent)
  - The vendor/source of the active ingredient(s), especially for analgesics, anesthetics and euthanasia drugs
  - The preparation date
  - A use-by-date, which should not extend past the earliest expiration date of any of the components

Storage:

- Is the compound stored appropriately to ensure stability until the use-by-date?
  - E.g., in a refrigerator or freezer, in a light-tight container or a desiccator, double locked for controlled substances, etc...
- Are compounds that have reached their use-by-date discarded appropriately?
  - 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

Use:

- Are the compound and diluent/vehicle appropriate for the route of administration?
  - 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
  - Where possible, do not reuse a needle to withdraw additional compound into a syringe once it has been used to inject an animal or animals
- For controlled substances, are residual amounts in the container or syringe discarded appropriately?
  - See above
- If controlled substances are dispensed under a DEA license there are additional considerations. RLSS/the DEA should be contacted regarding the applicable regulations for this activity. At a minimum, all controlled drug containers should be labeled with the concentration of each drug, the total amount of drug, and the expiration date.

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.

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.

For additional information, see IACUC Policy 210 Use of Drugs and Compounds in Animal Studies (http://rgw.arizona.edu/compliance/IACUC/policies-procedures-and-guidelines)