USE OF PHARMACEUTICAL & NON-PHARMACEUTICAL-GRADE SUBSTANCES IN ANIMALS

PERFORMANCE STANDARD: All compounds/substances/chemicals introduced into animals should be pharmaceutical grade (as defined in this policy), unless specifically exempted by the IACUC.

BACKGROUND/PURPOSE: The Guide to the Care and Use of Laboratory Animals, 8th Edition states that pharmaceutical grade chemicals should be used, when available, for all animal-related procedures. The use of lower grade substances/compounds with undefined or higher levels of impurities or poorly formulated non-commercial preparations can introduce unwanted experimental variables or toxic effects. The use of pharmaceutical grade chemicals may help ensure that the substances administered meet established documentable standards of purity and composition, which may also prevent adverse effects on animals or research outcomes. Although pharmaceutical grade substances should be used in experimental animals whenever possible, the use of non-pharmaceutical-grade substances in experimental animals is an acceptable practice under certain circumstances. For example, new investigational compounds are available only as new ungraded compounds.

The NIH Office of Laboratory Animal Welfare (OLAW) and the United States Department of Agriculture (USDA) have determined that the use of non-pharmaceutical-grade substances should be based on:

(1) Scientific necessity,
(2) Non-availability of an acceptable veterinary or human pharmaceutical-grade compound, and
(3) Specific review and approval by the IACUC.

Other specific regulatory considerations include:

A. Cost savings alone is not considered an adequate justification for the use of non-pharmaceutical-grade substances.

B. While the possible implications of the use of non-pharmaceutical grade substances in non-survival studies appears less evident, the scientific issues remain the same and professional judgment, as outlined above, must still apply.

C. The consideration for non-pharmaceutical substance use pertains to all components, both active and inactive, contained in the preparation to be administered. Therefore, vehicles or diluents are as important a consideration as the active compound in the preparation.
DEFINITIONS:

- **Pharmaceutic**: An agent or substance listed in the [FDA Green Book](https://www.accessdata.fda.gov/ scripts/cder/greenbook/index.cfm) (Approved Animal Drug Products) or agents or substance listed in [The Index of Legally Marketed Unapproved New Animal Drugs for Minor Species](https://www.fda.gov/animalveterinary/drugs/).  

- **Pharmaceutical grade compound**: A substance which is approved by the FDA (Green Book), or for which a chemical purity standard has been written/established by USP/NF, BP, Ph. Eur.  
  ◇ **FDA Green Book**: The Generic Animal Drug and Patent Restoration Act requires that each sponsor of an approved animal drug submit to the FDA certain information regarding patents held for the animal drug or its method of use. The Act requires that this information, as well as a list of all animal drug products approved for safety and effectiveness, be made available to the public.  
  ◇ **USP (United States Pharmacopeia)**: A substance which has been approved by the United States Pharmacopeia Convention through evaluation of the participant’s quality systems using an audit of each manufacturing site for compliance with good manufacturing practices (i.e., [ICH Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients](https://www.usp.org/sites/default/files/9781633420772.pdf)). The National Formulary (NF) is the official publication of the USP Convention, and contains two separate compendia – the USP and the NF.  
    ➢ The United States Pharmacopeia (USP) contains legally recognized standards of identity, strength, quality, purity, packaging, and labeling for drug substances, dosage forms, and other therapeutic products, including nutrional and dietary supplements.  
    ➢ The National Formulary (NF) includes standards for excipients, botanicals, and other similar products.  
  ◇ **BP (British Pharmacopoeia)**: Produced by the British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare Products Regulatory Agency, the British Pharmacopoeia (BP) is the official, authoritative collection of standards for United Kingdom (UK) medicinal substances for human and veterinary use. Now used in over 100 countries, the BP remains an essential reference for all individuals and organizations working within pharmaceutical research and development, manufacture and testing around the globe.  
  ◇ **Ph. Eur. (European Pharmacopoeia)**: The European Pharmacopoeia defines requirements for the qualitative and quantitative composition of medicines, the tests to be carried out on medicines and on substances and materials used in their production. It covers active substances, excipients and preparations of chemical, animal, human or herbal origin, homoeopathic preparations and homoeopathic stocks, antibiotics, as well as dosage forms and containers. The European Pharmacopoeia and its requirements are legally binding in the member states of the European Pharmacopoeia Convention and the European Union.
Non-pharmaceutical Grades: There are several other grades of substances that may be offered for sale. High grade products are not necessarily biologically appropriate products. Scientific justification is required for use of any grade other than pharmaceutic grade. Examples of product grading includes:

◊ **Analytical grade:** Generally applied to bulk chemicals, these products have a purity level of 99.9%. These may be used in animals with a certificate of analysis that documents the contaminants are ≤ 0.1% (for example: analytic grade ethanol may have up to 5% water or 5% benzene. Water is acceptable for animals; benzene is not). Specialized names such as Bio-tech grade, DNA grade, HPLC grade, Tissue Culture grade may have differing meanings. For example: Cell culture grade commonly includes substances such as cell culture media, laboratory preparations, biological extracts, selective and sterile reagents for several applications. Biotech grade (sometimes called Biochemistry grade) uses highly pure reagents suitable for biochemical research and analysis. The critical parameters involved are absence of inhibitors such as traces of heavy metals as well as biochemical function tests for enzymes, coenzymes and enzyme substrates. Each requires a certificate of analysis for consideration in live animal use.

◊ **Technical grade:** These products are suitable for general industrial or non-critical tasks in the laboratory such as rinsing, dissolving or are used as raw materials in production tasks. Technical grade products may be acceptable for live animal activities under certain circumstances, but require a certificate of analysis to assure they are free of toxins or other significant substances. See analytic grade discussion.

◊ **Reagent grade:** Describes chemical substances of sufficient purity for use in chemical analysis, chemical reactions or physical testing.

◊ **Lab grade:** A chemical grade of relatively high quality with exact levels of impurities unknown; usually pure enough for educational applications, but not pure enough to be offered for food, drug, medicinal, or research use.

◊ **Food grade:** Products meet the strength specifications and maximum impurity limit indicated in the Food Chemicals Codex (FCC). Food grade is acceptable when used as a food or carrier product for orally consumed substances. Food grade is not acceptable for injection in a live animal.

- **Non-availability:** Refers to whether a product is commercially available from an active US vendor. It includes formulations supplied as tablet, capsule, injectable, etc.

- **New investigational substance (or drug):** Supplied by its manufacturer for testing in an experimental setting only. These substances generally do not have chemical purity standards established and are considered non-pharmaceutical grade compound. See NOTE.
This policy establishes the institution’s position for the use of non-pharmaceutical grade substances in live animal activities. The policy is consistent with the guidance from the NIH/ILAR Guide for the Care and Use of Animals, the corresponding Position Statement from AAALAC, International, and the NIH/Office of Laboratory Animal Welfare’s Position Statement.

**POLICY:** The Duke Animal Care & Use Program accepts substances as Pharmaceutical Grade as defined above. All other substances are considered non-pharmaceutical grade.

1. Scientific justification is required for use of any grade other than pharmaceutic grade for clinical or research use.

   - **Clinical Use** - When available, pharmaceutical-grade compounds must be used for the clinical treatment of animals and to prevent or reduce/eliminate animal pain or distress.

   - **Research Use** - When available and suitable, pharmaceutical-grade compounds must be used to accomplish the scientific aims of the study. However, non-pharmaceutical-grade preparations may be used with IACUC approval. The IACUC should consider the following factors:
     o Use must be compliant with applicable national or regional regulatory guidelines and requirements and the requirements of relevant funding agencies;
     o A scientific justification is provided in the approved IACUC protocol;
     o The pharmaceutical-grade compound is not available in the appropriate concentration or formulation or the appropriate vehicle control is unavailable;
     o The compound is required to generate data that are part of an ongoing study or that are comparable to previous work;
     o The chemical properties of the compound are appropriate for the study and the route of administration (e.g., the purity, grade, stability in and out of solution, solution vehicle properties, pH, osmolality, and/or compatibility of the solvent and other components of final preparation). In some cases the reagent-grade of the compound may be as or more pure than the pharmaceutical-grade; and
     o The method of preparation, labeling (i.e., preparation and use-by dates), administration and storage of formulations should be appropriately considered with the aim of maintaining their stability and quality (i.e., to prevent inadvertent co-administration of infectious agents or contaminants).
2. **COMMON SUBSTANCES:** For a majority of common substances used in animal research, pharmaceutical grade (FDA, USP, NF, BP grade, etc.) substances are available and should be used. Examples include:

- Saline
- DMSO
- Corn oil
- Tamoxifen
- Tetracycline
- Analgesics (e.g., buprenorphine)
- Anesthetics (e.g., ketamine)
- Euthanasia reagents (e.g., Euthasol)

3. **SELECTING SUBSTANCES:** The preferred prioritization for substances if pharmaceutical grade is not available is:
   1. Analytical grade
   2. Technical grade
   3. Bio-tech grade, DNA grade, HPLC grade, Tissue Culture grade
   4. Food grade: Not acceptable for injection

**NOTE:** Investigational substances (or drugs) are not pharmaceutic grade, but may be used as a focal component of the proposed research when a certificate of analysis (or a description of the procedure for purification of the non-pharmaceutical substance <e.g. HPLC or MS>) addresses toxins or significantly harmful agents for live animals.

4. **PROPOSING A NON-PHARMACEUTICAL GRADE SUBSTANCE:** The use of non-pharmaceutical-grade compounds in animals shall be clearly delineated and justified in the protocol document and/or covered by this policy.

   1. **Investigator Obligations:** The investigator shall consider animal welfare and scientific issues related to the use of the substances, including potential for contamination, safety, efficacy, and the inadvertent introduction of confounding research variables. The investigator shall provide the IACUC justification for the use of a non-pharmaceutical grade substance (as discussed elsewhere in this policy).

   2. **IACUC Obligations:** The IACUC shall review the justification for use of a non-pharmaceutical grade product and shall also consider the grade/purity being proposed, the formulation of the final product, and issues such as sterility, pyrogenicity, stability, pH, osmolality, site/route of administration, pharmacokinetics, physiological compatibility, and quality control.

**NOTE:** The IACUC may use a variety of methods to review and approve the use of such agents. For example, the IACUC may establish
acceptable scientific criteria that apply institution-wide as well as on a case-by-case basis.

5. CONDITIONS WHERE NON-PHARMACEUTICAL GRADE PRODUCTS MAY BE APPROVED: The IACUC may review & approve the use of non-pharmaceutical-grade substances in the following situations:

1. If no pharmaceutical grade drug is available for use, then other grades may be considered, and if scientifically justified, may be approved by the IACUC. The formulation should be formulated aseptically and with a non-toxic vehicle as appropriate for the route of administration. If the vehicle or diluent is not pharmaceutical grade, this also must be justified.

2. Although an equivalent veterinary or human drug is available for experimental use, a different grade substance is required to replicate methods from previous studies because results are directly compared to those of previous studies.

6. COMPOUNDING AGENTS: It is often necessary or desirable to dilute or mix agents prior to administration into a research animal. The following provides guidance as to when a dilution or mixture can change the nature of pharmaceutical grade compound to a non-pharmaceutical grade compound.

- When diluting a pharmaceutical grade substance with a pharmaceutical grade diluent such as sterile water, buffered saline or similar solution, the resulting mixture may be considered pharmaceutical grade.
- When diluting a pharmaceutical grade substance with a second pharmaceutical grade substance (e.g., ketamine with xylazine), immediately prior to use, the mixture is considered pharmaceutical grade product.
- When simply diluting a pharmaceutical grade compound with a non-pharmaceutical grade diluent such as olive oil, the resulting mixture would be considered non-pharmaceutical grade.
- When diluting or mixing a pharmaceutical grade compound with another compound or solution containing a non-pharmaceutical grade compound or compounds, even if the non-pharmaceutical grade compound was prepared in a pharmaceutical grade diluent, the resulting mixture is considered non-pharmaceutical grade.
- When diluting or mixing a pharmaceutical grade compound with another compound or solution containing a pharmaceutical grade compound or compounds, the resulting mixture is considered non-pharmaceutical grade, depending upon the composition of the compounds and the potential interaction they may have.
NOTE: Substance combinations outlined in the Duke Animal Care and Use Program guidelines do not require scientific justification when used as described in the IACUC approved protocol. See labeling and expiration items below.

NOTE: If dilution/compounding affects the potency or stability of the compound, this needs to be taken into consideration. See labeling and expiration items below.

NOTE: Administration of a pharmaceutical grade compound via a route for which the compound was not manufactured for (example: crushing and dissolving pharmaceutical grade tablets for intravenous injection), renders the product non-pharmaceutical grade and this preparation MUST be justified and approved by the IACUC.

For more information, see the web page ‘Guidelines for Alternates to Non-Pharmaceutical Agents.’

7. Discard Date: All chemicals used on or in animals must have a discard date clearly labeled on the container. If an expiration date is not indicated by the manufacture or if the chemical is compounded/adulterated and the discard date is not detailed in the approved IACUC protocol, follow these guidelines:

- Whenever possible, items should be compounded for the project the day of use and discarded immediately after use.

- Liquid, non-compounded products with a manufacturer-supplied expiration date: Use the manufacturer expiration date.

- Sterile diluents without an manufacturer expiration date: When investigators wish to access sterile diluents multiple times (for example, to obtain small volumes for administration and drug mixing), the investigators can do so only if they do not add any chemical to the fluid, they access the fluid(s) aseptically and they store the fluid(s) as recommended by the manufacturer. Under these conditions, the investigator can use the sterile fluid(s) for up to 30 days after initial opening, unless continued sterility is documented via accepted methods (see references).

Upon the first use, the container must be marked with the 30 day expiration date, for example: ‘DISCARD AFTER <insert the date 30 days in the future>.

- When investigators wish to access on multiple occasions any sterile fluid to which they have added drugs or substances (for example, to obtain small volumes for administration or drug mixing), the investigators can do so only if they access the solution aseptically and they store the solution as described by the manufacturer. Under these conditions, the
investigators can use the sterile solutions for **up to 7 days** after initial opening, unless continued sterility and appropriate activity of the diluted drug has been documented and approved by the IACUC. Upon the first use, the container must be marked with the 7 day expiration date, for example: ‘DISCARD AFTER $<$insert the date 7 days in the future$>$’

- Use dates for compounded sterile preparations may vary (<7 or >30 days) depending upon known manufacturer or published stability, compatibility, or sterility data.

**NOTE:** Dilution of any medication will shorten its stability. If proper sterile technique is followed for dilution or fluid preparation, the discard date should be set according to manufacturer preparation data or no greater than 7 days after dilution if manufacturer or published data is not available. Upon the first use, the container must be marked with the expiration date, for example: ‘DISCARD AFTER $<$insert the date 7 days in the future$>$’

**NOTE:** All sterile fluids that will be administered to an animal should be labeled with ‘DISCARD AFTER …’ on the container once opened (if USP/NF, or BP grade), diluted, or compounded. Disposition of the compound should follow the guidelines above unless there is a provided manufacturer or published stability, compatibility, or sterility data is documented or otherwise approved on your IACUC protocol.

**NOTE:** Purchasing small volume containers of fluids (e.g., 10 ml bottle; 250 ml bag) may be advantageous, cost-effective, and prevent disposal of unused fluids / diluents.

**APPENDIX List**
1. Special campus-wide exemptions for use of non-pharmaceutical grade products:
   a. Avertin
   b. Pentobarbital
2. Suggestions for Pharmaceutical Calculations
3. Suggestions for Combining 2 or More Medications
4. Suggestions for Sterile Preparation of Solutions for Parenteral Administration
5. Special Notes Regarding Pharmacetic / Non-Pharmaceutic Use
6. Quick Facts
APPENDIX 1a
AVERTIN

AVERTIN: Avertin is the trade name for the injectable anesthetic 2,2,2-tribromoethanol. Avertin was once manufactured as a pharmaceutical-grade drug, but is no longer available.

1. **Use:** Avertin (or Tribromoethanol) is appropriate for short term procedures in mice, especially surgical procedures. It is best used in situations where it will be given only on a single occasion or for non-survival procedures.

2. **Compliant use of tribromoethanol in research activities:** The preparation and use of this anesthetic must be:
   - Scientifically necessary,
   - Appropriately justified, and
   - Approved by the IACUC. In making its decision the IACUC must consider the side effects, stability, storage requirements and other considerations associated with the preparation of this agent (the PI must provide this information for IACUC consideration).

3. **Advantages of Avertin:**
   - Tribromoethanol induces anesthesia rapidly and provides good surgical analgesia for approximately one hour.
   - Since it is given by injection, one is spared the occupational health risks and technical difficulties associated with volatile anesthetics.
   - If used appropriately, Tribromoethanol has a good margin of safety.

4. **Disadvantages of Avertin:**
   - Tribromoethanol is an irritant, especially at high doses, high concentrations, or with repeated use. Adhesions are sometimes seen in the abdominal cavity after IP injections.
   - Tribromoethanol degrades in the presence of heat or light to produce toxic byproducts. Degraded solutions can be both nephrotoxic and hepatotoxic. Administration of degraded Tribromoethanol solutions has been associated with death, often 24 hours after surgery.
   - Tribromoethanol can cause intestinal ileus (stopping of the gut motility and subsequent death of the animal) several weeks after injection. There are multiple reports in the literature of physiologic harm to animals including ileus. This is more common with Avertin stored in the presence of light or heat, stored at higher than recommended doses, or given at higher than recommended concentrations.
   - The effects of Tribromoethanol are also somewhat unpredictable in mice younger than 16 days, or in animals with altered carbohydrate metabolism, such as various mouse strains used for diabetes or obesity models (db/db mice or ob/ob mice).
• **Compounding Avertin:** Two chemicals are necessary to generate Avertin. The first is 2,2,2 Tribromoethanol; the second is amylene hydrate (tertiary amyl alcohol), both obtainable from Aldrich Chemical. There may be other sources as well. Avertin may be compounded according to the following recipe:

**Ingredients:**
- 2.5 gm 2,2,2 Tribromoethanol
- 5 ml 2-methyl-2-butanol (amylene hydrate, tertiary amyl alcohol)
- 200 ml distilled water - neutral pH

**Recipe Instructions:**
- Dissolve 2.5 grams Tribromoethanol in 5 ml amylene hydrate. This requires heating to approximately 40° Celsius and stirring vigorously.
- Add distilled water, stirring continuously, up to a final volume of 200 ml.
- Filter sterilize through a Millipore filter (.5 micron).
- Aliquot the final solution into appropriate containers - empty, sterile, red-cap blood collection tubes make a good receptacle, as do brown injection bottles with appropriate caps. It's often easiest to filter the material through a luer-fitted millipore filter directly into a sterile, red-cap blood collection tube.
- Refrigerate the aliquots and protect them from light. The material degrades rapidly in the presence of heat or light. Even refrigerated and wrapped in foil, the material is stable for only about two weeks. If the material degrades, it becomes toxic.

5. **Storage:**
- Store the solution under refrigeration and in the dark. Containers should be wrapped in foil.
- Although some authors report that refrigerated solutions may be kept for months, most authors recommend preparing a new solution every 2 weeks. The Duke DLAR requires replacing refrigerated Avertin at least every 14 days (after mixing).

6. **Dosing:**
- Mix by stirring or swirling prior to administration.
- The material is given by IP injection at a dose of 250 mg/Kg. This amounts to 0.5 ml of the above solution to a 25gm mouse. Induction requires only 1-2 minutes and the righting reflex returns in approximately 40-90 minutes.

**NOTES:**
1. Tribromoethanol degrades to dibromoacetaldehyde and hydrobromic acid. If the pH of the solution is less than 5, Avertin should be presumed to have degraded. Solutions maybe be tested by adding one drop of Congo Red to 5 ml of solution. If a purple
color results, the solution has decreased to or below pH 5 and should be discarded. (Note: this method is only useful if the original pH of the solution is greater than 5 - hence the recommendation for neutral distilled water.)

2. As prepared above, the solution contains 12.5 mg Tribromoethanol /ml. Do not attempt to make a more concentrated solution – Avertin is irritating at higher concentrations.

3. Do not administer non-sterile solutions, outdated solutions, more concentrated solutions, or higher doses than recommended above.
APPENDIX 1b
PENTOBARBITAL

PENTOBARBITAL: Pentobarbital has a long history of effective use, especially in rodents or small mammal species. In recent years, the medication has fallen out of favor with human healthcare use and consequently, most producers have ceased producing or selling pentobarbital as an injectable pharmaceutical grade product (currently there is only one company producing Nembutal). Current costs for pharmaceutical grade pentobarbital exceeds $1000.00 per 50 ml bottle. Availability has also declined as one provider exercises a monopoly on the sales and distribution of this agent. While most researchers are able to convert to alternative medications, for certain applications and certain studies, scientific necessity requires continued use of this barbiturate.

The NIH has stated that ‘The exorbitant cost of this product has placed it logistically into the unavailable category. Regulatory guidance on this matter specifically allows for use of non-pharmaceutical-grade compounds due to non-availability and with IACUC approval. Therefore, the IACUC will consider and may approve requests to use a non-pharmaceutical grade of pentobarbital under the following circumstances.

The preparation and use of this anesthetic must be:

- Scientifically necessary,
- Appropriately justified,
- Prepared from a reagent or analytical-grade powder; properly prepared by your pharmacist or other knowledgeable individual (e.g., chemist, veterinarian, researcher), with assurance of appropriate storage and handling, and
- Approval by the IACUC. In making its decision the IACUC must consider the side effects, stability, storage requirements and other considerations associated with the preparation of this agent (the PI must provide this information for IACUC consideration).

1. **Recipe for Sodium Pentobarbital**

   a. **INGREDIENTS**

   - 6 grams sodium pentobarbital
   - 10 ml ethanol (95%) USP
   - 40 ml propylene glycol USP
   - 0.9% saline USP

   b. **STEPS:**

   - Dissolve the pentobarbital powder in the ethanol.
   - Add 25 ml of saline (but only after the pentobarbital is completely dissolved), mix thoroughly.
   - Add 40 ml propylene glycol, mix.
   - Bring to final volume (100 ml) with 0.9% saline.
NOTES:

1. The pentobarbital concentration in the final solution is 60 mg/ml.
2. Stock solutions must be protected from light and maintained at 4°C for no longer than 6 months.
3. Stock solutions must be passed through a sterile 0.2 micron filter prior to being stored.
4. Stock solutions must be prepared and stored in sterile containers.
5. Please see the IACUC Policy on Use of Non-Pharmaceutical-Grade Chemicals and Compounds for guidance on filtration and preparation.
6. Working solutions can be prepared and maintained similar to stock solutions, but can be stored at room temperature for up to 30 days.
7. Transfer of solutions must utilize sterile supplies and techniques.
8. All containers must be labeled with material name, concentration, date prepared, storage requirements, expiration date, and the initials of the person making the solution.
9. Use must be recorded similar to other controlled substances.
10. Standard procedures for monitoring plane of anesthesia apply and supplemental dosing is to be given as needed.
11. Prior to removal or transfer of any medication from a vial, swab rubber port of the vial with alcohol and allow the port to dry completely.
12. **Labeling** – If a drug is removed from its original packaging a label **MUST** be placed on the new container. Please list the name of the drug, the concentration of the drug and the beyond use date.
13. Commercial instruments are available for measuring pH and osmolality.
14. Do not administer non-sterile solutions, outdated solutions, more concentrated solutions, or higher doses than recommended above.
APPENDIX 2
Suggested Pharmaceutical Calculations

Volume of undiluted drug needed X Undiluted drug concentration =
Final total volume X Desired final drug concentration

Diluting Pharmaceutical Grade Parenteral Medications

- After volume of undiluted drug needed is calculated, a pharmaceutical grade
  sterile diluent (e.g., sterile normal saline) should be added to reach the final
  volume desired.

  EXAMPLE:
  Ketamine undiluted concentration = 100mg/mL
  Ketamine desired final concentration = 10mg/mL
  Final volume desired = 10mL.

  Plug into formula:
  (volume of undiluted ketamine needed) X (100mg/mL) = (10mL) X (10mg/mL).
  Volume of undiluted ketamine needed = 1mL.
  Mix the 1 ml of undiluted ketamine with 9 mL of pharmaceutical grade sterile
  saline in a sterile vial to obtain 10mL of 10mg/mL ketamine.

IV Fluid Preparations

- After volume of undiluted drug needed is calculated, this volume should be
  removed from the fluid bag prior to adding the additive. Thus the final volume will
  be the starting volume of the fluid bag.

  EXAMPLE:
  Dextrose starting concentration = 50% (0.5g/mL);
  Dextrose desired final concentration = 5% solution in LRS (0.05g/mL);
  Final volume desired = 1000mL of a 5% dextrose solution.

  Plug into formula:
  (volume of 50% dextrose needed) X (0.5g/mL) = (1000mL) X (0.05g/mL).
  Volume of 50% dextrose needed = 100mL of 50% dextrose.
  Remove 100mL of LRS from 1000mL bag, then add 100mL 50% dextrose.

  NOTE: This method will give you a generalized concentration of medication in
  suspension, do not use if precise drug dosing is needed.

Molarity calculator: http://www.tocris.com/molaritycalculator.php#.VSaE91TD9aQ

Dilution Calculator: http://www.tocris.com/dilutionCalculator.php#.VSaFaVED9aQ
APPENDIX 3
Suggested Methodology for Combining Two or More Medications

(volume of drug A needed) \( \times \) (starting concentration of drug A) = (final total volume of mixture desired) \( \times \) (desired final concentration of drug A) + (volume of drug B needed) \( \times \) (starting concentration of drug B) = (final total volume of mixture desired) \( \times \) (desired final concentration of drug B)

- Use the formula above to determine the volume of drug A, B, C, etc needed to reach the desired final concentration of drug A, B, C, etc. The volume calculated for each drug should be added, then a sterile diluent should be added to reach the final volume desired. Only pharmaceutical grade sterile diluents should be used (e.g., sterile normal saline).

NOTE: Some medications cannot be mixed, please make sure that drugs are compatible prior to mixing.

EXAMPLE:
1. Ketamine (drug A):
   - Ketamine starting concentration = 100mg/mL;
   - Ketamine desired final concentration = 8.8mg/mL;
   - Final total volume of mixture desired = 25mL.
   - Plug into formula:
     \[ (\text{volume of drug A needed}) \times (100\text{mg/mL}) = (8.8\text{mg/mL}) \times (25\text{mL}). \]
   - Volume of Ketamine needed for mixture = 2.2mL.

2. Xylazine (drug B):
   - Xylazine starting concentration = 100mg/mL;
   - Xylazine desired final concentration = 1.0mg/mL;
   - Final total volume of mixture desired = 25mL.
   - Plug into formula:
     \[ (\text{volume of drug A needed}) \times (100\text{mg/mL}) = (1.0\text{mg/mL}) \times (25\text{mL}). \]
   - Volume of Xylazine needed for mixture = 0.25mL

3. For Ketamine 8.8mg/mL and Xylazine 1.0mg/mL (Total Volume = 25 ml):
   - Mix 2.2mL of 100mg/mL Ketamine + 0.25mL 100mg/mL Xylazine + 22.55mL of sterile pharmaceutical grade diluent.

0.2mL of this Ketamine-Xylazine mixture would deliver a dose 1.76mg of Ketamine and 0.2mg of Xylazine.
APPENDIX 4
Suggestions for Sterile Preparation of Solutions for Parenteral Administration

1. All preparation should be performed under a clean laminar flow hood. Some powders may require a fume-hood or a biosafety cabinet (BSC) due to potential human risk and exposure. Contact OESO for more information.
2. Only pharmaceutical grade sterile diluents should be used (e.g., sterile normal saline or phosphate buffered saline).
3. The top of vial or injection port of fluid bags should be swabbed with 70% isopropyl alcohol and allowed to dry prior to puncture with needle.
4. Combine the measured amount of powder and sterile diluent necessary to achieve desired concentration, as directed by manufacturer, USP monograph, or experiment, and mix.
5. All solutions intended for parenteral use must be sterile and pH balanced. The following is a guideline:
   - Filtering - filter through a 0.2µ filter
   - pH Testing - the pH should be between 6.8-7.2. If the compound is intended to be administered intravenously through a central vein (jugular, femoral, etc) the pH range can be 3-9.
   - Osmolality Testing – the final solution should be isotonic, with an osmolarity around 300 mOsm.
   - Culture (optional) – For further confirmation of sterility, the final solution can be cultured for bacterial growth.
   - Endotoxin testing can be performed by commercial laboratories or with test assay materials.
6. If compounding medications are NOT in the specified range or if particulates are visible in the final solution, they should NOT be injected.
7. The final product should be steriley transferred to a sterile vial or syringe within a laminar flow hood.
APPENDIX 5
Special Notes Regarding Pharmaceutical / Non-Pharmaceutical Use

1. Compounded doses should be calculated according to the animal's weight prior to administration.
2. Compounded doses MUST be listed in an approved IACUC protocol.
3. Swab the rubber port of the vial with alcohol and allow to completely dry prior to removal or transfer of any medication from a vial.
4. If a drug is removed from its original packaging, a label MUST be placed on the new container. The label must include the drug / substance name, the concentration of the drug, and the discard date.
5. Light may cause significant degradation in some substances and is critical in certain cases (e.g. Avertin). In all cases, protecting substances from light is advised.
6. Crushing pharmaceutic grade tablets is prohibited for parenteral administration, as tablets frequently have additives for absorptive or protective purposes that may be toxic when given parentally.
APPENDIX 6
Quick Facts
Regarding Pharmaceutical/Non-Pharmaceutical Grade Substances

• LABEL RECOMMENDATIONS
  o Name of compound
  o Concentration
  o Date of compounding
  o Expiration date or discard after date NOTE: The “Expiration date” and “Discard after” date are not the same. See Guidelines below.

<table>
<thead>
<tr>
<th>LABEL EXAMPLE</th>
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<tbody>
<tr>
<td><strong>Compound:</strong> ____________________</td>
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<tr>
<td><strong>Concentration:</strong> ____________________</td>
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<tr>
<td><strong>Compound date:</strong> ____________________</td>
</tr>
<tr>
<td><strong>Discard after:</strong> ____________________</td>
</tr>
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</table>

• DISCARD/EXPIRATION: All chemicals must have expiration OR discard after date.
  o PHARMACEUTICAL GRADE COMPOUNDS:
    ▪ Expiration date indicated by the manufacturer. Aliquots must have a label.
  o STERILE FLUIDS OR DILUENTS (un-adulterated without manufacture expiration date):
    ▪ 30 days after initial opening
  o NON-PHARMACEUTICAL GRADE or ADULTERATED DILUENTS:
    ▪ Use the day of the project then discard (recommended)
    ▪ 7 days after initial opening/adulteration/compounding, if mixed in lab
    ▪ As indicated, if mixed by a compounding pharmacy

• OTHER NOTES:
  o Compounds stored in an animal use area (e.g., fluid bags, expired compounds) that will NOT be used in animals should be marked “Not for Animal Use.”
  o Discard dates indicated are the default with the expectation that the fluid has been accessed aseptically and has been stored as recommended by the manufacturer or protocol.
  o Expiration/discard dates may vary depending on known manufacturer or published stability, compatibility, or sterility data.
  o Use of a compound after the policy described discard date must be described and approved in your IACUC protocol

REFERENCES

- U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Animal Care, Policy 3-Veterinary Care, April 14, 1997.
- OLAW transcript on Use of Non-Pharmaceutical-Grade Chemicals and Other Substances in Research with Animals - March 1, 2012: http://grants.nih.gov/grants/olaw/120301_seminar_transcript.pdf