SOP for the Policy on the Use of Non-pharmaceutical-Grade Compounds in Research Animals

See Policy 2010-037

1. **Scope**
   This SOP is a companion to UW-Madison Policy 2010-037, “Policy on the Use of Non-Pharmaceutical-Grade Compounds in Research Animals”, and it applies to all personnel involved in activities that use live vertebrate animals for biomedical research and/or teaching purposes at UW-Madison facilities regardless of the funding source.

2. **Reason for Policy and SOP**
   To remain in compliance with positions held by the Office of Laboratory Animal Welfare (OLAW), the United States Department of Agriculture -Animal Plant Health Inspection Service (USDA-APHIS), and the 8th Edition of The Guide for the Care and Use of Laboratory Animals, this document details what is required for the use of non-pharmaceutical-grade chemicals or compounds in research animals at the University of Wisconsin-Madison. Section 9 of this SOP details specific regulatory requirements. Oral compounds used in agricultural animals are excluded from the policy.

   If applicable, principal investigators may reference this SOP in Animal Care and Use Protocols in lieu of detailed individual descriptions of the preparation and use of novel test compounds or certain protocol-approved non-pharmaceutical-grade compounds (see section 8).

3. **Guidance for identifying and using Pharmaceutical-Grade Compounds**
   Pharmaceutical-grade chemicals/compounds should be used in experimental animals whenever possible. Pharmaceutical-grade compounds include:
   - FDA-approved veterinary or human compounds specifically formulated for clinical application
   - Veterinary or human pharmaceutical compounds listed in the:
     - United States Pharmacopeia (USP)
     - USP-National Formulary (USP-NF)
     - British Pharmacopeia (BP)
     - European Pharmacopeia (EP)
   - Drugs ordered from one of the compounding pharmacies in the Resources section (section 4) of this document.

4. **Resources**
   Licensed pharmaceutical compounding companies may provide pharmaceutical-grade agents to researchers who are able to supply a DEA Researcher License in lieu of a veterinary license. A DEA-222 form is required for the transfer of controlled substances. The following list of companies is supplied as a resource, but other appropriately licensed pharmacies can be utilized (contact an RARC veterinarian for guidance). The Animal Care and Use Committee (ACUC) cannot endorse these pharmaceutical firms, nor can it guarantee that these firms will continue to supply these pharmaceuticals in forms suitable for animal studies.
   - **Zeeh Pharmaceutical Experiment Station**, School of Pharmacy, University of Wisconsin-Madison  [http://pharmacy.wisc.edu/zstation](http://pharmacy.wisc.edu/zstation)
   - **TW Medical**  [http://www.twmedical.com/](http://www.twmedical.com/)
   - **DiamondBack Drugs**  [http://www.diamondbackdrugs.com](http://www.diamondbackdrugs.com).
• PCCA (Professional Compounding Centers of America). http://www.pccarx.com
• Pharmaceutical Grade Tricaine Methanesulfonate (MS222)
  • Finquel: http://www.argent-labs.com/argentwebsite/ms-222.htm
  • Tricaine-S: http://www.wchemical.com/TRICAINE-S-MS-222-P43C7.aspx

5. **How do I justify the use of a non-pharmaceutical-grade compound?**

   When considering the use of non-pharmaceutical grade compounds, UW-Madison researchers can use the following criteria to help guide them in protocol/amendment preparation. Detailed written justification that allows adequate review by the ACUC must be a component of the protocol or amendment.

   **Justification that is typically acceptable:**

   - Pharmaceutical-grade not available from a veterinary or medical supplier
   - Pharmaceutical-grade not available from a veterinary or medical supplier in the needed concentration (e.g., high concentration of penicillin to produce seizures; supersaturated solution of potassium chloride to euthanize pigs)
   - Non-pharmaceutical-grade required in order to produce data that is comparable to previous years’ data
   - Pharmaceutical-grade compounds have known unwanted effects on measured outcomes substantiated by data or published reports.
   - Non-pharmaceutical-grade compounds produce necessary outcomes substantiated by data or published reports that are not replicated by pharmaceutical-grade compounds.
   - Pharmaceutical-grade contains unwanted fillers/diluents/vehicles
   - Pharmaceutical-grade is only available in form not suited for chosen route of administration or the species in which the compound is to be used

   **Inadequate justification:**

   - Cost savings alone
   - Administrative burden of acquiring and maintaining a DEA license
   - Consideration/elimination of only one of multiple pharmaceutical-grade alternatives

   **EXAMPLES OF WHAT A GOOD JUSTIFICATION LOOKS LIKE:**

   A. “Compound XXX is experimental in nature and no pharmaceutical-grade alternative is available. It is not practical or possible to generate a pharmaceutical-grade version of this novel compound.”

   B. “The commercially available pharmaceutical-grade form of YYY is not available in an appropriate concentration to meet the scientific requirements of this study, and it is not practical to alter the concentration to a useable formulation.”

   C. “Though compound ZZZ is commercially available in pharmaceutical grade, a non-pharmaceutical-grade preparation has been used by the laboratory since 199X. The data collected on this protocol are part of a longitudinal study that depends on comparison of new data to results of prior studies. Once comparison of new results to previously collected data is no longer necessary, we will transition to the use of pharmaceutical grade compounds wherever applicable.”
WHAT ABOUT USE IN NON-SURVIVAL PROCEDURES?
Although the possible implications of the use of non-pharmaceutical-grade chemicals/compounds in non-survival studies appears less evident, OLAW has stated that the scientific issues remain the same and professional judgment, as outlined above, must still apply. The ACUC will expect a justification for non-survival use applications and will determine approval on a case-by-case basis. Remember, cost savings alone is not considered adequate justification.

WHAT IF I CAN OBTAIN A COMPOUND THAT IS NO LONGER AVAILABLE IN THE U.S. FROM A SUPPLIER IN ANOTHER COUNTRY?
Compounds from any country of origin must meet the criteria listed in Section 3 of this SOP. Compounds that do not meet the criteria will be considered non-pharmaceutical grade, and will require scientific justification and ACUC approval.

6. **Guidance for use of novel test compounds & compounds with no acceptable pharmaceutical-grade alternative**
The UW-Madison ACUCs acknowledge that many test compounds and experimental agents are used in research and generally classify these agents as non-pharmaceutical grade compounds without an acceptable pharmaceutical grade alternative (and is acceptable practice). However, PI’s should use all available knowledge of the compounds to ensure that the non-pharmaceutical grade agents are prepared under sterile conditions and stored properly.

i. When drugs or chemicals are formulated for injection, they must be prepared in a sterile manner. This requires sterile constituents (e.g. sterile powder, sterile diluents, etc.), a sterile container and a means of keeping the preparation sterile. Injection vials (available from RARC) are preferred as they make it easier to load a syringe and allow removal of solution without exposing the contents to outside contaminants.

ii. Diluents or vehicles should be from the list of acceptable components (below). Exceptions to the approved list must be listed in the protocol in order to be evaluated by the ACUC on a case-by-case basis.

iii. Containers must be labeled with the drug, concentration, and date of preparation, and the date of expiration. Note that sterile injection vials are available through the RARC Pharmacy.

iv. Where possible, prepared solutions must be passed through a syringe filter (0.22 µ or finer) at the time of preparation. This can be done in the process of transfer to an injection vial. If there is any question about the sterility of a stored solution, it must also be filtered at the time of use. If filtering is not possible (e.g., nanoparticles), sterile components should be mixed using sterile technique.

v. Prepare only as much as can be used in a reasonable period of time. Drugs must be stored properly (e.g., freezer, refrigerator, etc.). **Solutions must not be used if they are cloudy, discolored, precipitated, or have become otherwise altered in appearance since initial preparation.**

vi. Expiration date must be in line with Animal User Requirement #5, 30 days after mix date, unless there is published data to the contrary.

vii. Expired drugs must be disposed of properly. If not discarded, expired drug containers must be labeled "expired—awaiting disposal—do not use in animals" and stored separately from drugs in use. Controlled substances cannot be discarded without appropriate approval. All controlled substances must continue to be stored in an
approved secure cabinet or safe.

viii. pH of solutions given by injection should be close to physiologic pH. Use of a solution with a pH outside of a reasonable physiologic pH range must be addressed in the animal use protocol.

ix. Pyrogens, such as endotoxins, may cause fever when injected into an animal. All pharmaceutical drugs are tested for pyrogens. Sterility does not ensure that pyrogens are not present. Filtering does not remove pyrogens. Pyrogen testing is not practical for small lots of prepared drug. Pyrogenicity is a potential experimental variable that researchers should be aware of when using non-pharmaceutical grade drugs.

Acceptable solvents/diluents/vehicles

1. Distilled water
2. PSS (0.9% NaCl), PBS, balanced salt solution (e.g., Hanks)
3. 60% (v/v) propane-1:2-diol (propylene glycol)
4. 0.5% (w/v) carboxymethyl cellulose
5. 10% (v/v) Tween 80 (polyoxyethylene (20) sorbitan mono-oleate)
6. 10% (v/v) ethyl alcohol
7. 50% (v/v) dimethylformamide
8. 50% (v/v) dimethylsulphoxide (DMSO)
9. Cyclodextrins (e.g. 2-hydroxypropyl-beta-cyclodextrin, Trappsol ®)
10. Polyethylene Glycol (PEG)-200 or PEG-300

Others can be approved on a case-by-case basis in an animal care and use protocol.

7. **ACUC-approved standard formulations** *(note that proportions can be adjusted to arrive at desired volumes)*

i. Institution-Approved Recipe for Sodium Pentobarbital for use as an anesthetic agent (survival or non-survival procedures)

**INGREDIENTS**
- 6 Gm sodium pentobarbital
- 10 ml ethanol (95%)
- 40 ml propylene glycol USP
- 0.9% sterile saline

**MIXING**
- 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.
- The pentobarbital concentration in the final solution is 60 mg/ml.

**DOSING**
- Rat, 40-50 mg/kg IP
• Mouse, 40-85 mg/kg IP
• Rabbit, 28 mg/kg IV, IP
• Dog, 30 mg/kg IV to effect
• Cat, 30 mg/kg IV to effect 40-85 mg/kg IP
• Pig, 20-30 mg/kg IV
• Sheep, 20-30 mg/kg IV

NOTES
• Use must be recorded similar to other controlled substances.

ii. UW-Madison ACUC-Approved Recipe for Sodium Pentobarbital for use as a euthanasia agent only

INGREDIENTS
• Sodium pentobarbital
• 0.9% saline

MIXING
• Mix the pentobarbital powder in 0.9% saline to an appropriate concentration (e.g. 100 mg/ml).

DOSING for euthanasia is generally > 100 mg/kg for all species

iii. UW-Madison ACUC-Approved Recipe for MS-222

MS-222 can be used for axolotls, aquatic salamanders, fish and frogs. FINQUEL or Tricaine-S are the best forms of this material on the market and is considered to be pharmaceutical-grade. In the event non-pharmaceutical-grade MS-222 is scientifically justified in an approved protocol, the following formulation is approved by UW-Madison.

INGREDIENTS
• MS-222 powder
• Sodium Bicarbonate
• pH paper

MIXING
1. Dissolve MS-222 in water
   a. Fish (variable by species): 25-300 mg/L (25-100 mg/L = good start point for Zebrafish)
   b. Frogs, salamanders (variable by species): 200-1000 mg/L for anesthesia, 2000 mg/L for euthanasia
2. Adjust the pH to about 7.4 using powdered Sodium Bicarbonate
   a. Most critical at concentrations above 500 mg/L

NOTES
• For surgical purposes, fresh solution should be made for every surgery to minimize contamination and infection.
• For euthanasia, MS-222 should be made fresh weekly.
• Discard old MS-222 down the sink diluted with lots of fresh cold water.
• Avoid exposure to skin; wear gloves while mixing; CAN BE NEUROTOXIC IN HUMANS

iv. UW-Madison ACUC-Approved Recipe for Urethane (generally for use in rats only)

INGREDIENTS
• Urethane powder
• Sterile pyrogen-free distilled water

MIXING
1. Mix the urethane 10-20 gm/100 ml, in sterile, pyrogen-free distilled water. This step must be done in a fume hood!

DOSING
• 1000-1500 mg/kg IP

NOTES
• Urethane is for non-survival procedures only.
• URETHANE IS A KNOWN HUMAN CARCINOGEN

8. Examples of how this SOP can be referenced in animal care and use protocols

A. “Novel test compounds [or other compound(s) with no acceptable pharmaceutical-grade alternative] detailed in this protocol will be prepared, stored and used according to Section 6 of the ‘SOP for the Policy on the use of Non-Pharmaceutical-Grade Compounds in Research Animals’”.

B. “Sodium pentobarbital [or other compound(s) listed in Section 7 of this SOP] will be formulated according to the ACUC-approved recipe detailed in Section 7 of the ‘SOP for the Policy on the use of Non-Pharmaceutical-Grade Compounds in Research Animals’”.

✓ A statement justifying the use of non-pharmaceutical-grade compounds, that allows adequate review by the ACUC, must still be a component of the protocol or amendment even if this SOP is referenced in place of detailed written descriptions of preparation and use of compounds.

9. What do the Regulations say?
Note that the USDA, OLAW and AAALAC have clarified that pharmaceutical-grade policy also applies to compounds used strictly for experimental purposes, not just to medications used to provide clinical veterinary care to research animals.

8th EDITION OF THE GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS
"The use of pharmaceutical grade chemicals and other substances ensures that toxic or unwanted side effects are not introduced into studies conducted with experimental animals. Pharmaceutical grade chemicals should be used, when available, for all animal-related procedures (NIH 2008; USDA 1997b). There may be circumstances when the use of a non-pharmaceutical
grade chemical or substance is necessary to meet the scientific goals of a project or when a veterinary or human pharmaceutical grade product is unavailable. The use of non-pharmaceutical grade chemicals or substances should be described and justified in the animal use protocol and be approved by the ACUC (Wolff et al. 2003). 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 (NIH 2008)."

**OLAW**
OLAW and USDA consider that the use of non-pharmaceutical grade compounds should be based on:
- Scientific necessity
- No availability of an acceptable veterinary or human pharmaceutical-grade compound and
- Specific review and approval by the ACUC.

“Investigators and ACUCs should consider relevant animal welfare and scientific issues including safety, efficacy, and the inadvertent introduction of new variables. Cost savings alone do not adequately justify the use of non-pharmaceutical-grade compounds in animals. Although the potential animal welfare consequences of complications are less evident in non-survival studies, the scientific issues remain the same and the principles and need for professional judgment outlined above still apply.”

**USDA**
“Investigators are expected to use pharmaceutical-grade medications (expanded in 2012 by USDA and OLAW to include all compounds) whenever they are available, even in acute procedures. Non-pharmaceutical-grade chemical compounds should only be used in regulated animals after specific review and approval by the ACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings is not a justification for using non-pharmaceutical-grade compounds in regulated animals.”

10. Related Information

PHS Policy on Humane Care and Use of Laboratory Animals
http://grants.nih.gov/grants/olaw/references/phspol.htm

*The Guide for the Care and Use of Laboratory Animals, 8th ed*
http://www.nap.edu/catalog.php?record_id= 12910

USDA Policy #3: Veterinary Care

OLAW/USDA/AAALAC webinar “Use of Non-Pharmaceutical-Grade Chemicals and Other Substances in Research with Animals”, 2012
http://grants.nih.gov/grants/olaw/educational_resources.htm#