The University of Houston
IACUC Guidance

Title: Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals.

Scope:

This guidance applies to all personnel involved in biomedical research using laboratory animals at the University of Houston.

Reason for Guidance:

This guidance directly supplements the following IACUC Policy:

- Pharmaceutical-Grade Compounds in Research

The guidance provides specific information regarding the use of non-pharmaceutical grade chemicals or compounds in laboratory animals at the University of Houston: it is intended to guide both the Investigator proposing to use such agents as well as the IACUC in reviewing the use of these agents in animal care and use protocols.

These documents are provided to assure compliance with the Office of Laboratory Animal Welfare (OLAW) guidance, the United Sates 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.

Agent specific considerations and IACUC approved formulations may be found starting on page 8.

Guidance for test compounds and compounds with no acceptable pharmaceutical grade alternative:

Test compounds and experimental agents are used in research and are generally classified as non-pharmaceutical grade compounds without an acceptable pharmaceutical grade alternative. This is an acceptable practice if the proper precautions are taken and documentation provided."

- 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), a sterile container and a means of keeping the preparation sterile.

Injection vials are preferred as they make it easier to load a syringe and allow removal of solution without exposing the contents to outside contaminants.

- **Diluents or vehicles** must be specified in the animal use protocol. Use of solvents will be evaluated on a case-by-case basis. Use of such solvents may limit amounts, concentration and routes of administration. See list of acceptable solvents below.
- Containers must be **labeled** with the drug, concentration, and date of preparation. In general, any solution made up should be discarded in 30 days unless efficacy and sterility can be assured beyond that time. Compounding pharmacies or a Pharm D may have a good suggestion on how long an individual reconstituted agent may last if kept sterile. If you keep a reconstituted agent beyond 30 days, please address the issue in your SOP provided in your protocol.
- Where possible, prepared solutions must be passed through a syringe **filter** (0.22 µm 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.
- Prepare only as much as can be used within one month unless there is documentation that the drug remains effective and sterile for a longer period. Drug solutions prepared and stored properly in a suitable injection vial can be stored for a time frame that is in-line with a similar commercial product as long as this has been verified by a compounding pharmacist or other knowledgeable pharmacist. Drugs must be stored properly (e.g. freezer, refrigerator, etc.) as indicated. Solutions must not be used if they are cloudy, discolored, precipitated, etc.
- **Expired drugs** must be disposed of properly. If not discarded, expired drug containers must be labeled “expired” and stored separate from drugs in use. **Controlled substances** cannot be discarded without appropriate paperwork. All controlled substances must continue to be stored in an approved secure cabinet or safe.
- **pH** of solutions must be between pH 4.5 and 8.0. Use of a solution with a pH outside this range must be addressed in the animal protocol.
- **Pyrogens**, such as **endotoxins,** may cause **fever** when injected into an animal. All pharmaceutical drugs are tested for pyrogens. Sterility does not assure 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:

- Distilled water
- PSS (0.9% NaCl), PBS, balanced salt solution (e.g., Hanks)
- 60% (v/v) propane-1:2-diol (propylene glycol)
- 0.5% (w/v) carboxymethyl cellulose
- 10% (v/v) Tween 80 (polyoxyethylene (20) sorbitan mono-oleate)
- 10% (v/v) ethyl alcohol*
- 50% (v/v) dimethylformamide
- 50% (v/v) dimethylsulphoxide (DMSO). Please note that the concentration of the final solution of DMSO delivered to the animal must not exceed 25% (or 0.3ml/kg of the full strength solution). Hanslick, Jennifer L. et al. "Dimethyl Sulfoxide (DMSO) Produces Widespread Apoptosis in the Developing Central Nervous System." Neurobiol. Dis. 2009 April; 34(1):1-10.
- 9. Cyclodextrins5 (e.g. 2-hydroxypropyl-beta-cyclodextrin, Trappsol ®)

*Exceptions can be approved on a protocol by protocol basis.

Guidance for compounds for which acceptable pharmaceutical grade alternatives exist:

Although pharmaceutical grade chemicals and compounds should be used in experimental animals whenever possible, the use of non-pharmaceutical-grade chemicals and compounds in experimental animals is an acceptable practice under certain circumstances. For uses of anesthesia and analgesia, the animal's welfare must come first.

The IACUC realizes that many test compounds and experimental agents are used in research and generally classifies these agents as non-pharmaceutical grade compounds without an acceptable pharmaceutical grade alternative, and it is an acceptable practice. However, PI's should use all available knowledge of the compounds to ensure that the aforementioned preparation, evaluation, storage, use, and disposal standards are maintained.

When selecting anesthesia and analgesic compounds, the UH IACUC will allow you to select one or more than one of the following choices on your protocol form:

- FDA approved veterinary or human pharmaceutical compounds;

- FDA approved veterinary or human pharmaceutical compounds used to compound a needed dosage form;
- Order the drug via one of the compounding pharmacies in the Resources section of this document;
- Use one of the IACUC's recipes that are included in this document. Note that these recipes have been approved by a pharmacist, and are specifically formulated to be more pure than drugs offered by veterinary and pharmaceutical suppliers.
- Justify the use of non-pharmaceutical grade drugs (see discussion below).

Justification of non-pharmaceutical grade compounds:

When considering the use of non-pharmaceutical grade compounds, UH investigators can use the following decision criteria to help them in protocol/addendum preparation.

- Justification that is always acceptable:
  - Known impact on measured outcomes, which is substantiated by data or published reports.
    - not available from a veterinary or medical supplier
    - 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)
    - needed in order to produce data that is comparable to previous years' data
    - more pure in a reagent grade version than a pharmaceutical grade version
    - pharmaceutical grade contains unwanted fillers
    - pharmaceutical grade only available in form not suited for chosen route of administration
- Justification that is generally acceptable:
  - Detailed concerns about potential detrimental effects on established models or experimental paradigms.
  - Back-up anesthetic, used in emergencies in case pharmaceutical-grade alternatives are not available (may require greater post-approval monitoring).
- Possible adequate justification, requiring particular attention to details:
  - Unpublished, anecdotal experience on benefits of the model or detrimental effects of alternatives;
  - Experimental logistics or personnel safety, which include
    - access to specialized equipment (fume hoods, vaporizers/scavengers, etc.)
    - interference with measurements or procedures;

        - reduction in performance standards;
- Inadequate justification, when no additional justification is present:
    - Cost savings
    - Administrative burden of acquiring and maintaining a DEA license
    - Consideration of only one pharmaceutical-grade alternative

Where possible the description should include the chemical grade of the agent(s) being used, source of the reagents, as well as a description of the appropriateness of the agent, its formulation and vehicle. Formulations and vehicles may need to be adjusted depending on the route and site of administration, as well as the species under consideration.

Regulatory Language:

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 nonpharmaceutical 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 IACUC (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 website:

"OLAW and USDA agree that pharmaceutical-grade[1] chemicals and other substances, when available, must be used to avoid toxicity or side effects that may threaten the health and welfare of vertebrate animals and / or interfere with the interpretation of research results[2]. However, it is frequently necessary to use investigational compounds, veterinarian- or pharmacy-compounded[3] drugs, and / or Schedule I[4] controlled substances to meet scientific and research goals.

The IACUC is responsible for evaluating the potential adverse consequences of such agents when used for research. In making its evaluation, the IACUC may consider factors including, for example:

- grade,

- purity,
- sterility,
- acid-base balance,
- pyrogenicity,
- osmolality,
- stability,
- site and route of administration,
- compatibility of components,
- side effects and adverse reactions,
- storage, and
- pharmacokinetics.

The IACUC may use a variety of administrative methods to review and approve the use of such non-pharmaceutical-grade agents. For example, the IACUC may establish acceptable scientific criteria for use of these agents within the institution, rather than on a case-by-case basis. Investigators and IACUCs should consider relevant animal welfare and scientific issues including safety, efficacy, availability of pharmaceutical-grade compounds, and the inadvertent introduction of new variables. Cost savings alone are not an adequate justification for the use of non-pharmaceutical-grade or compounded drugs in animals.

Although the potential animal welfare consequences of complications are less evident in non-survival studies, the scientific issues remain the same. The principles and need for professional judgment outlined above apply to non-survival studies.

From the OLAW website, seminar in March 2012 regarding chemical grade pentobarbital:

"Chemical /analytical-grade pentobarbital: Agents for sedation, analgesia, or anesthesia should be veterinary or human pharmaceutical-grade compounds, when available, unless the use of a non-pharmaceutical chemical or formulation is scientifically necessary, appropriately justified and approved by the IACUC. The use of a non-pharmaceutical-grade euthanasia agent must meet the same standards. If no equivalent veterinary or human drug is available for experimental use, then the highest-grade equivalent chemical reagent should be used and formulated aseptically and with a non-toxic vehicle as appropriate for the route of administration. Recent exorbitant cost increases of pentobarbital have placed it logistically into the unavailable category. Pentobarbital from a reagent or analytical-grade powder, properly prepared by a pharmacist or other knowledgeable individual (e.g., chemist, veterinarian, researcher), with assurance of appropriate storage and handling, and approval by the IACUC is acceptable. IACUC approval can be institution-wide for the drug prepared in this fashion for all approved users.

Diluted Fatal-Plus for perfusions: OLAW and USDA received inquiries as to whether investigators may use diluted Fatal-Plus as an anesthetic. The use of Fatal-Plus for anesthesia is specifically prohibited by FDA in the instructions included on the label of

the product. The extra-label use of a euthanasia product for its pentobarbital content is unacceptable and violates the PHS Policy and Animal Welfare Act and Regulations. Such proposed use may not be approved by an IACUC or used by investigators at Assured institutions or used on regulated species. We note that some euthanasia procedures include perfusion of the animal prior to death. FDA approved euthanasia solutions may be used in those procedures in combination with the perfusion agent to perform perfusion and euthanasia as a single procedure."

USDA:

"Investigators are expected to use pharmaceutical-grade medications 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 IACUC 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."

Footnotes:

[1] A pharmaceutical grade compound is a drug, biologic, or reagent that is approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been established by the United States Pharmacopeia-National Formulary (USP-NF), or British Pharmacopeia (BP). According to guidance from the FDA, "pharmaceutical secondary standards" are acceptable for use in clinical animal studies if obtained from a reputable source and comply with compendia standards.

[2] A listing of pharmaceutical-grade drugs and biologics is available through the FDA database. The Orange Book is the reference for FDA-approved human drugs. The Green Book is the reference for FDA-approved veterinary drugs.

[3] Veterinary compounding is the customized manipulation of an approved drug by a veterinarian, or by a pharmacist upon the prescription of a veterinarian, to meet the needs of a research study. IACUCs considering the use of veterinary compounding for research purposes are advised to consult: http://www.avma.org/issues/drugs/compounding/veterinary_compounding_brochure.asp for more information about federal regulations.

[4] United States Department of Justice Drug Enforcement Agency controlled substances Schedule I and II-IV drugs may be used in biomedical research according to the standards of the Code of Federal Regulations 1301.13.

Special considerations for the use of tribromoethanol (Avertin®)

Background:

Tribromoethanol (TBE) is a popular injectable anesthetic agent used in mice. It was once manufactured for use as an anesthetic by Winthrop Laboratories under the trade name Avertin®, but this pharmaceutical grade product is no longer available and it must be prepared in the laboratory using chemical-grade ingredients that have no guarantee of purity or effectiveness. Multiple reports in the scientific literature indicate that tribromoethanol is associated with significant side effects including peritonitis, ileus, and death, particularly when repeated doses are administered. In addition, the sleep time associated with Avertin® has been shown to be variable even when the dose is kept constant.

The IACUC understands that for some labs where the use of TBE anesthesia has been a long-standing practice, investigators may be concerned that a different anesthesia may adversely affect their research model or operating procedures. However, after consideration of the current regulatory environment and published literature, the IACUC strongly discourages the use of TBE/Avertin®.

When appropriately prepared and stored, tribromoethanol can be a safe and effective anesthesia for short (15-30 minute) surgical procedures in mice and rats.[1] Anesthesia may be unpredictable in young animals (<16 days), genetically modified lines with altered carbohydrate metabolism (db/db, ob/ob), or strains that may be predisposed to hyperglycemia (C57Bl/6J).[2]

Tribromoethanol solutions exposed to heat and/or light produce hepatotoxic and nephrotoxic by-products associated with abdominal irritation, intestinal ileus, and death. The incidence of adverse effects increases with the concentration administered and with repeated dosing.[3]

The IACUC strongly discourages the use of TBE but will review and may approve its use in mice when scientifically justified. To avoid increased potential for adverse effects, tribromoethanol should be restricted to a single survival anesthesia or to terminal procedures. Tribromoethanol administration must be described and justified in an approved IACUC protocol before use in experimental animals.

Inhaled Isoflurane and ketamine/xylazine are suitable USP grade alternatives to TBE/Avertin® anesthesia. Investigators must describe why USP grade alternatives cannot be used and provide a detailed justification that describes why TBE/Avertin® is the most appropriate anesthetic agent. This may include protocol specific requirements,

a description of how the USP grade alternatives may negatively influence scientific outcomes/measurements, or experimental logistics.

IACUC Committee members and Principal Investigators may use the following examples to review and/or prepare justifications for TBE/Avertin use.

- **Inadequate justifications:**
  - Cost savings
  - Administrative burden of acquiring and maintaining a DEA license
  - Consideration/elimination of only one alternative

- **Potentially adequate justifications:**
  - Scientific need to replicate methods from previous studies because current studies will directly compare results. This is more likely to be approved if the completed studies were performed recently (<2 years) rather than historically (10-20 years ago).
  - Potential/predicted influence of anesthesia agent on scientific outcome measures or performance standards. This is more likely to be approved if a pilot study is included to investigate the potential suitability of a USP grade alternative.

- **Adequate justifications:**
  - Known/Published impact on measured outcomes

Issues that need to be addressed before tribromoethanol can be considered for approval with adequate justification are:

- TBE is for use in mice only
- Solution must be prepared using sterile technique and filter-sterilized with a 0.2 micron filter before injection. It also must be stored and used under sterile conditions.
- Store in a dark or foil-covered bottle to prevent breakdown by light
- Store stock and working stock solutions at 4° C
- Do not use if solution is discolored or precipitates
- Check pH before each use and use only when pH is greater than 5.0
- Discard working solution after 1 week and stock solution after 1 month
- Label all containers with the name and concentration of drug, date prepared, and initials of the person making the solution
- Tribromoethanol can be used ONLY one time in any individual animal for a survival procedure
- Tribromoethanol cannot be used in mice that are known manifest adverse reactions to TBE

- An analgesic must be given after anesthesia and before starting any painful procedure such as surgery because TBE does not produce adequate procedure analgesia

Preparation and Storage:

Preparation methods should be described in your approved protocol.
Tribromoethanol [2,2,2-tribromoethyl alcochol] and amylene hydrate [tert-amyl-alcohol] should be purchased from reputable suppliers and be of high grade and purity [ie. Sigma, Aldrich Chemical or similar].
Containers must be labeled with the name, concentration, preparation or expiration date and the initials of the individual who prepared the solution.
Solutions must be sterile filtered before administration, stored at 4°C and protected from light (foil wrapped or opaque container). Working stock TBE solutions expire one (1) week from preparation date. **Expired anesthesia agents may never be administered to animals.**

Preparation of Stock Solution [1g/ml]:

Mix 10 g of 2,2,2-tribromoethyl alcohol with 10 ml of tert-amyl alcohol in a foil wrapped or dark/opaque bottle. Dissolve by heating to approximately 40ºC for 3 hrs and stirring vigorously overnight at room temperature. Heating the solution to temperatures above 40ºC is not recommended. Stock solutions should be appropriately labeled [1g/ml TBE, initials of individual preparing, expiration date], protected from light and stored at 4ºC. Stock solutions expire 1 month after preparation. Solutions that are discolored, have a precipitate, or a pH<5 should be discarded.

Preparation of Working Stock [1.25% solution, 12.5mg/ml]
Dilute the Stock solution by mixing 1 ml of stock in 80 mls of diluent. Diluents should be sterile, endotoxin-free and physiologically compatible. Investigators may choose PBS, water, saline for injection or other appropriate diluent. Filter sterilize through a 0.2 micron filter and aliquot in sterile containers. Protect from light (foil wrap or dark container) and store at 4ºC. Working stock solutions should be appropriately labeled [1.25% TBE, initials of individual preparing, expiration date] and will expire 1 week after preparation. Solutions that are discolored, have a precipitate, or a pH<5 should be discarded. Higher concentrations are not recommended.
Administration: 250mg/kg – 0.2ml of the above 1.25% working stock would be administered for every 10 grams of body weight.

Failure to follow these instructions may reduce the potency and efficacy of the drug, and/or result in tissue necrosis at the injection site, peritonitis, or other adverse events.

A Standard Operating Procedure for preparation and storage of TBE must be included in the animal protocol.

****Please Note:** Investigators may want to seriously consider avoiding the use of TBE anesthetics. An editorial published by Cardiovascular Research (2012) 93(1):1–3 includes the information that "6% of the total articles received in the past year for evaluation in Cardiovascular Research were rejected for ethical reasons. One of the most frequent causes of rejection on ethical grounds is the improper choice of anesthetic drugs for major surgical procedures." OLAW has been informed that the use of tribromoethanol was a factor in rejection of a study.[4]

References

1. Papaioannou, VE and Gox, JG. Efficacy of Tribromoethanol Anesthesia in Mice. Laboratory Animal Science, 1993. April 43(2):189-192.
2. Gargiulo, S., et al. Mice Anesthesia, Analgesia, and Care, Part I: Anesthetic Consideration in Preclinical Research. ILAR J. 2012, 53(1):E55-69.
3. Meyer, RE and Fish, RE. A review of tribromoethanol anesthesia for production of genetically engineered mice and rats. Lab Animal. 2005, 34(10):47-52.
4. Brown, P., Clarke, C., Collins, J. OLAW webinar, broadcast March 1, 2012. Use of Non-Pharmaceutical Grade Chemicals and Other Substances in Research Animals. http://grants.nih.gov/grants/olaw/120301_seminar_transcript.pdf
5. 8th Edition, NRC Guide for the Care and Use of Laboratory Animals (2011)
6. USDA Animal Care and Resource Guide, Policy #3, Veterinary Care, March 25, 2011.
7. Lieggi, CC, et. al. "An evaluation of the preparation methods and storage conditions of tribromoethanol." *Contemp Top Lab Anim Sci.* 2005 44(1):11-16.
8. Lieggi, CC, et. al. "Efficacy of stored and newly prepared tribromoethanol in ICR mice." *Contemp Top Lab Anim Sci.* 2005 44(1):17-22
9. Zeller, W, et.al. "Adverse effects of tribromoethanol as used in the production of transgenic mice." *Lab Anim.* 1998 33(2): 192-193.
10. Tarin, D, et.al. "Surgical anesthesia of mice: evaluation of tribromoethanol, ether, halothane and methoxyflurane and development of a reliable technique." *Lab Anim.* 1972 6(1), 79-84.

IACUC Approved Formulations

• UH IACUC Approved Recipe for Inactin (Thiopental)

This recipe provides a very stable long duration anesthesia and should only be used for terminal experiments. Thiopental and Inactin are metabolized quite slowly and have the reputation of being short duration anesthetics. The IV injection route and dosage used does cause rapid induction and a rapid recovery because the drugs are very lipid soluble and enter tissues from the blood stream. The dosages listed overwhelm the body reservoirs and allow very long (8-10 hour) anesthesia with excellent cardiovascular reflexes and adequate respiratory reflexes. The animals do lose much of their thermoregulation and need to be heated with a heating pad at 37C.

INGREDIENTS
• 100 mg sterile powder
• 4 ml sterile saline

Please see the section on **Guidance for test compounds and compounds with no acceptable pharmaceutical grade alternative** above for guidance on filtration and preparation.

ROUTES OF INJECTION
• 0.8 ml per 100 grams distributed over 4 subcutaneous sites.
• 100 mg/kg IP
• 100 mg/kg IV
• Intramuscular injection is to be avoided and is very painful.
CAUTION
• Inactin and thiopental are extremely basic and pH of 10 or higher is likely.
• Neither drug lasts more than a day after being put in solution. New solutions must be made up daily.

• UH IACUC Approved Recipe for Sodium Pentobarbital

INGREDIENTS
• 6 Gm sodium pentobarbital
• 10 ml ethanol (95%)
• 40 ml propylene glycol USP
• qs to 100 ml with 0.9% saline

1. Dissolve the pentobarbital powder in the ethanol.
2. Add 25 ml of saline (but only after the pentobarb is completely dissolved), mix thoroughly.
3. Add 40 ml propylene glycol, mix.

4. Bring to final volume (100 ml) with 0.9% saline.

The pentobarbital concentration in the final solution is 60 mg/ml. Use a dose of 50 mg/kg i.p. in rats.

NOTES

1. Stock solutions must be protected from light and maintained at 4°C no longer than 6months.
2. Stock solutions must be passed through a sterile 0.2 micron filter prior to being stored.
3. Stock solutions must be prepared and stored in sterile tubes.
4. Please see the section on **Guidance for test compounds and compounds with no acceptable pharmaceutical grade alternative** above for guidance on filtration and preparation.
5. Working solutions can be prepared and maintained similar to stock solutions, but can be stored at room temperature for up to 30 days.
6. Transfer of solutions must utilize sterile supplies and techniques (e.g. sterile needles and syringes).
7. All containers must be labeled with material name, concentration, date prepared, storage requirements, expiration date, and the initials of the person making the solution.
8. Use must be recorded in accordance with other controlled substances record keeping requirements.
9. Standard procedures for monitoring plane of anesthesia apply and supplemental dosing is to be given as needed.

• UH IACUC Approved Recipe for MS-222

MS-222 can be used for axolotls, aquatic salamanders, and fish. FINQUEL is the best form of this material on the market.

INGREDIENTS

- MS-222 powder
- Artificial pondwater mixture
- Sodium Bicarbonate
- pH paper

MIXING

1. Dissolve MS-222 in artificial pondwater (20% Holtfreter's Salts).
2. Adjust the pH to about 7.4 using only powdered Sodium Bicarbonate.
3. Use a 5 pad pH paper (pH 2-14 from Fisher) to monitor the pH.

NOTE

- MS-222 should be made fresh weekly.
- For surgical purposes, fresh solution should be made for every surgery to minimize contamination and infection.
- Discard old MS-222 down the sink diluted with lots of fresh cold water.
- The concentration used for anesthesia is 0.2-0.5% depending on the animal size.
- Use 10% strength of anesthetizing solution (most frequently 0.025% (wt/vol) MS 222) as an analgesic to reduce pain and surgery stress for 20 minutes right after the surgery.
- MS-222 does cause GI response, i.e., some animals (especially the big ones) might vomit if feeding occurs within the last 24 hours

• UH IACUC Approved Recipe for Urethane

INGREDIENTS

- Urethane (800 mg/kg)
- PBS
- alpha-chloralose (at least 99% pure, 80 mg/kg)

MIXING

1. Mix the urethane (800 mg/kg) in solution with PBS.
2. Add the alpha-chloralose (at least 99% pure, 80 mg/kg).
3. Warm up the solution while continuously stirring it to allow the chloralose to dissolve.
4. Allow solution to cool.
5. Please see the section on **Guidance for test compounds and compounds with no acceptable pharmaceutical grade alternative** above for guidance on filtration and preparation.

DOSING

- Inject 55 mg/kg IP
- You may want to give a female a smaller dose to start (~70% of male dose), then 20 minutes later give 10% more of the dose and check level of anesthesia. Repeat this step until there is no response to tail pinching and blinking reflex is gone.

REDOSING

- The anesthetic should last for at least 3h without needing supplementation.
- Supplement with the same urethane-chloralose solution (re-warm it and stir for a short time before using it) , so the concentration is the same, with no more than 0.2 cc (~10-20% of the initial dosage) at the 4th hour after first injection and every hour afterward or as needed.

NOTES

Urethane is for non-survival procedures only. You must apply to the Chemical Safety officer for approval for this agent in your lab due to human safety issues as it is a potent carcinogen. A Standard Operating Procedure must be approved by Chemical Safety and included in the animal protocol.

Resources:

- **MedVet International** (800-544-7521) http://www.shopmedvet.com/ Has an in-house veterinarian who reviews all veterinary product orders. The veterinarian may permit the purchase of these veterinary products (controlled or non-controlled) with a DEA Researcher License, particularly if the recipient is a research institution.
- **Miller Vet supply (**1-800-880-1920) http://www.millervetsupply.com Rep name: Cole.
- **Southern Anesthesia** (http://sasvet.com/) (800-456-0757) Currently, researchers holding a DEA Researcher License may purchase veterinary drugs.
- **TW Medical** (888-787-4483) http://www.twmedical.com/ Has merged with Animal Health International.
- **Butler Schein** (888-224-3204 ext 5406) 614-553-6882 FAX, Clay Barber Sales Support Specialist: cbarber@ButlerSchein.com
- **Webster/Patterson Vet Supply** http://www.pattersonvet.com/ UH rep Simona at 877-366-7387, ext 5528

- **Diamond Back Drugs** http://www.diamondbackdrugs.com/. Requires a written prescription.
- Currently, pharmaceutical grade powdered **Sodium Pentobarbital** can be ordered from PCCA (**Professional Compounding Centers of America**). Contact at www.pccarx.com or 1-800-331-2498. A copy of the DEA-222 license will need to be mailed to PCCA for regulatory purposes.
- Local compounding pharmacy: **BCP Veterinary Pharmacy** http://www.bcpvetpharm.com/, 713-771-1144
- Pharmaceutical Grade **Tricaine Methanesulfonate (MS-222**)
    - **Finquel**: http://www.argent-labs.com/argentwebsite/ms-222.htm
    - **Tricaine-S**: http://www.wchemical.com/TRICAINE-S-MS-222-P43C7.aspx

References:

Indiana University "Policy Regarding the Use of Non-Pharmaceutical-Grade Chemicals/Compounds in Laboratory Animals"
Arizona State University "Use of Drugs and Compounds in Animal Studies"
Emory University "IACUC Policy for Non-Pharmaceutical Grade Drugs"
http://www.iacuc.pitt.edu/druglist.pdf
National Institutes of Health "Guidelines for the Use of Non-Pharmaceutical Compounds in Laboratory Animals"
Rutgers, the State University of New Jersey "Custom Formulated Compounds for Use in Animals"
Texas A & M University "Guidelines for Use of Non-Pharmaceutical-Grade Agents or Mixtures of Pharmaceuticals (Cocktails)"
University of Colorado Denver "Use of Non-Pharmaceutical-Grade Chemicals/Compounds"
University of Colorado Denver "Use of Tribromo Ethanol (TBE) in Laboratory Animals"
University of Illinois "Policy on Use of Expired Drugs and Materials and Non-Pharmaceutical Grade Compounds in Animals"
University of Kentucky "The Use of Non-Pharmaceutical-Grade Chemicals/Compounds in Laboratory Animals"
University of Wisconsin Madison "SOP for the Policy on the Use of Non-Pharmaceutical-Grade Compounds in Research Animals"
Washington College "Standard Operating Procedure no. 6 "Preparation of Sterile Non-Pharmaceutical Grade Compounds"
Wayne State University "Use of Non-Pharmaceutical Grade Drugs"