



**INDIANA UNIVERSITY**  
**Office of Research Compliance (ORC)**  
**Institutional Animal Care and Use Committee (IACUC)**

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**Use of Non-Pharmaceutical Grade Chemicals  
and Compounds in Laboratory Animals**

**FULL POLICY CONTENTS**

<b>Scope</b>	<b>Responsible University Office:</b>
<b>Reason for Policy</b>	Office of Research Compliance IACUC Office(s)
<b>What do the Regulations Say?</b>	<b>Responsible University Administrator:</b>
<b>Definitions</b>	Vice President for Research
<b>Guidance</b>	<b>Policy Contacts:</b>
<b>Resources</b>	Indianapolis: somiacuc@iupui.edu
<b>Approved Recipes</b>	
<b>Sanctions</b>	
<b>Related Information</b>	

**Effective:** 1.2014  
**Last Updated:** 4.2013

## Scope

This policy applies to all personnel involved in activities involving the care and/or use of live vertebrate animals in IU SOM facilities regardless of the funding source.

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## Reason for Policy

To remain compliant with the guidance provided by the Office of Laboratory Animal Welfare (OLAW), the United States Department of Agriculture – Animal Plant Health Inspection Service (USDA-APHIS), and the 8<sup>th</sup> Edition of the Guide for the Care and Use of Laboratory Animals, this document details what is required in regards to the use of non-pharmaceutical-grade chemicals or compounds in laboratory animals at the IU School of Medicine. Non-parenteral agents used in agricultural animals are excluded from this policy.

While the animal's welfare must be considered when using all non-pharmaceutical grade agents, it is most important that agents used for anesthesia and analgesia are properly prepared, reconstituted, stored, etc.

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## What do the Regulations say?

### 8<sup>TH</sup> 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 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

“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 IACUC.

Investigators and IACUCs 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 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.”

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## Definitions

### Pharmaceutical grade compound:

OLAW: 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).

USDA: Pharmaceutical-grade compound is any active or inactive drug, biologic, reagent, et cetera, which is approved by the FDA for which a chemical purity standard has been written or established by any recognized pharmacopeia, which is a book or a compendia, such as the US Pharmacopeia [USP], the National Formulary [NF], the [British Pharmacopoeia](#) [BP], the Pharmacopoeia of the Council of Europe [EP]. Note the USP and the NF have combined their standards into one compendia... [<http://www.usp.org/usp-nf>].

**Analytical Standards:** “Certificate of Analysis” a document that goes with each product run. This certificate lists the formula for the ingredients as well as the amount of each raw material/ingredient. The product name and lot number are listed to avoid confusion with other batches. The Certificate of Analysis also may contain results of tests for contaminants.

**Analytical Grade:** ~99% purity; Certificate of Analysis *usually available*; appropriate preparation is imperative.

**Reagent ACS:** This designates the highest quality commercial chemical. The “ACS” means the American Chemical Society. A Certificate of Analysis is available upon request.

**Reagent Grade:** The highest quality commercial chemical; however, ACS has not set specifications for materials. A Certificate of Analysis is *usually not available*

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## Guidance for Obtaining Compounds for which Pharmaceutical Grade Alternatives Exist

Although pharmaceutical grade chemicals/compounds should be used in experimental animals whenever possible, the use of non-pharmaceutical-grade chemicals/compounds in experimental animals is an acceptable practice under certain circumstances.

However, PI's should use appropriate knowledge of the compounds available to ensure that the aforementioned preparation, evaluation, storage, use, and disposal standards are maintained (see section below).

When selecting anesthesia and analgesic compounds, the IU SOM IACUC recommends that you select one of the following methods for obtaining pharmaceutical grade agents:

- Use an FDA approved veterinary or human pharmaceutical compounds;
  - Use an FDA approved veterinary or human pharmaceutical compound to compound a needed dosage form;
  - Order the drug via LARC's Distribution Center, who offers common anesthesia and analgesia drugs in common concentrations
  - 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 agents (see discussion below).
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## How do I find out if a compound exists in Pharmaceutical Grade?

We recommend a simple Google search with the terms **pharmaceutical grade** and your agent. This may yield you the best results with the least amount of effort.

You may determine what is available by consulting 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.

FDA database

<http://www.fda.gov/Drugs/InformationOnDrugs/default.htm>

Orange Book:

<http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm>

Green Book:

<http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/ucm042847.htm>

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## How do I know when I need to justify the use of a non-pharmaceutical grade compound, and how do I justify it?

When considering the use of non-pharmaceutical grade compounds, IUSOM faculty can use the following decision criteria to help them justify the use of a non-pharmaceutical grade agent in protocol/amendment preparation.

- Justification that is always acceptable:
  - Known impact on measured experimental 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)
  - required 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 or non-toxic vehicles
  - pharmaceutical grade only available in form not suited for chosen route of administration
  - no pharmaceutical grade available
  - the agent is necessary to replicate methods from previous studies because results are directly compared to those of replicated studies.
  - in the case of sodium pentobarbital, OLAW has decided that the exorbitant cost increases has placed it logistically into the unavailable category.

- 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; or
    - security of regulated drugs (in rare circumstances).
- Inadequate justification, when no additional justification is present:
  - Cost savings
  - Administrative burden of acquiring and maintaining a DEA license
  - Consideration/elimination of only one pharmaceutical-grade alternative

Where possible the description should include the chemical grade of the agent(s) being used (see definitions below), 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.

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## What does a good justification look like?

- “The XXX protein used will be synthesized and purified in several variations in our lab. This will be carried out using automated chromatography equipment to ensure the tightest, most reproducible column-based purification possible. However, given the nature of the process, it is not practically possible to generate pharmaceutical-quality protein reagent in a basic research lab, as the equipment, training, and facilities required are specialized.”
  - “The compound I inject, YYY, I use in 10 microliters/dose. The commercially available concentration of YYY is not an appropriate concentration.”
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## What about use in non-survival procedures?

A euthanasia solution (such as Euthasol or Fatal Plus) may not be used as an anesthetic (alone) for survival or non-survival procedures. OLAW in concert with USDA agree that a procedure may be performed as a part of euthanasia. And this would be limited to terminal perfusion or exsanguination. In both cases, death is an immediate outcome of the procedure.

However, it is acceptable to use non-sterile euthanasia solution (such as Euthasol or Fatal Plus) for euthanasia as well as non-pharmaceutical-grade pentobarbital as long as it is scientifically justified and administered appropriately.

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## What if I can obtain a compound that is no longer available in the U.S. from a supplier in another country?

The compound will be considered non-pharmaceutical grade. You will be required to scientifically justify its use in the animal protocol, and to follow the documentation and other practices in this document.

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## Guidance for Reconstituting or Diluting Compounds and Drugs

The IU SOM IACUC acknowledges that many test compounds and drugs are used in research and generally classifies these agents as non-pharmaceutical grade compounds **without** an acceptable pharmaceutical grade alternative. As such, their use **is acceptable practice**. However, PI's should use appropriate information of the compounds available to ensure that the non-pharmaceutical grade agents are prepared under sterile conditions and stored properly.

### When Preparing Compounds and Drugs:

1. When 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** (available from LARC) are preferred as they make it easier to load a syringe and allow removal of solution without exposing the contents to outside contaminants.
2. **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.
3. Containers must be **labeled** with the agent, concentration, and date of preparation. Note that sterile injection vials are available through LARC's Distribution Center.
4. Where possible, prepared solutions must be passed through a syringe **filter** (0.22 um 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.
5. Prepare only as much as can be used in a reasonable period of time. Agents 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. Agents must be stored properly (e.g., freezer, refrigerator, etc.). Solutions must not be used if they are cloudy, discolored, precipitated, etc.
6. **Expired agents** must be disposed of properly. If not discarded, expired containers must be labeled "expired" and stored separate from agents 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.
7. **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 use protocol.
8. **Pyrogens**, such as **endotoxins**, may cause **fever** when injected into an animal. All pharmaceutical agents 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 agent. Pyrogenicity is a potential experimental variable that researchers should be aware of when using non-pharmaceutical grade agents.

### Acceptable solvents

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<sup>5</sup> (e.g. 2-hydroxypropyl-beta-cyclodextrin, Trappsol ®)

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

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## Resources

The following companies may provide veterinary grade agents to researchers who are able to supply a DEA Researcher License in lieu of a veterinary license. The IACUC cannot endorse these pharmaceutical firms, nor can it guarantee that these firms will continue to supply these pharmaceuticals in forms suitable for animal studies.

- **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.

- **Southern Anesthesia** (<http://sasvet.com/>), ask for Cooper (800-456-0757 ext 265)  
Currently, researchers holding a DEA Researcher License may purchase veterinary drugs.
- **TW Medical** (888-787-4483) <http://www.twmedical.com/>
- 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](http://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
- 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>

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## IU IACUC Approved Recipe for Inactin (Thiobarbital) (ethyl-(1-methyl-propyl))

This recipe provides a very stable long duration anesthesia and should only be used for terminal experiments. Inactin is metabolized quite slowly and has the reputation of being a long duration anesthetic. 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 Novel Test Compounds & Compounds with No Acceptable Pharmaceutical Grade Alternative 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

- Note that the lethal dose is close to the effective dose.
- Inactin is extremely basic and pH of 10 or higher is likely. This should be buffered.
- This drug does not last more than a day after being put in solution. New solutions must be made up daily.

### References:

Fish, Brown, Danneman and Karas (Eds) (2008) Burlington, MA *Anesthesia and Analgesia in Laboratory Animals*, Academic Press.

Suckow, Weisbroth, Franklin (Eds) (2006) Burlington, MA *The Laboratory Rat*. Academic Press.

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## IU IACUC Approved Recipe for Thiopental

Thiopental is an ultrashort acting barbiturate. It induces a rapid anesthetic effect within 60 seconds of administration. It is most useful as an induction agent for short procedures or for procedures that will be maintained with inhalant anesthesia. It is usually administered IV.

For longer procedures, doses of 30-100 mg/kg of 2.5% will result in 4 hours of anesthesia.

For euthanasia, use a dose of 100 mg/kg.

#### CAUTION

- Thiopental is extremely basic and pH of 10 or higher is likely. It is not recommended to use this drug IP to animals in survival procedures.
- This drug does not last more than a day after being put in solution. New solutions must be made up daily.

#### References:

Fish, Brown, Danneman and Karas (Eds) (2008) Burlington, MA *Anesthesia and Analgesia in Laboratory Animals*, Academic Press.

Suckow, Weisbroth, Franklin (Eds) (2006) Burlington, MA *The Laboratory Rat*. Academic Press.

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## IU 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. We used 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 6 months.
  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 Novel Test Compounds & Compounds with No Acceptable Pharmaceutical Grade Alternative 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 similar to other controlled substances.
  9. Standard procedures for monitoring plane of anesthesia apply and supplemental dosing is to be given as needed.
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## IU IACUC Approved Recipe for MS-222

MS222 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.
- MS222 does cause GI response, i.e., some animals (especially the big ones) might vomit if feeding occurs within the last 24 hours.

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## IU IACUC Approved Recipe for Urethane

Urethane will produce an extended period of anesthesia with minimal physiological changes in laboratory animals. The use of urethane anesthesia for non-survival studies, clinical veterinary medicine, and other applications is limited; however, due to adverse post-operative health effects observed in animals and suspected health risks to humans. Urethane has been classified as a mutagen (Lewis, 2004) and as a group 2B carcinogen by the International Agency for Research on Cancer (IARC). It is readily absorbed through the skin, targets multiple organs, suppresses bone marrow, readily crosses the placenta, induces fetal tumor formation (in utero), and initiates preneoplastic changes in the skin (Field and Lang 1988). These potentially severe side effects make urethane exposure a significant health threat to laboratory staff, animal handlers and other personnel who may be accidentally exposed.

NOTE: When handling urethane in the crystalline or powdered form and when mixing urethane into aqueous solutions always use a fume hood, wear a lab coat, protective eye-wear, and chemical resistant gloves. Urethane should only be heated if mixing takes place in a fume hood. Containers of urethane should never be opened outside of a fume hood. Once mixed into an aqueous solution, urethane should then be transferred into a sealed bottle to prevent volatilization and potential employee exposure. Due to the teratogenic potential pregnant women should avoid working with urethane. Urethane should be limited in use to non-recovery procedures due to its long term carcinogenic effects in laboratory animals.

#### PERSONAL PROTECTIVE EQUIPMENT

- Lab coat
- Nitrile gloves
- ANSI Z-87 approved safety glasses
- ANSI Z-87 approved chemical safety goggles if a splash hazard exists
- Appropriate laboratory attire.

#### Engineering Controls

- Certified fume hood

#### 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 Novel Test Compounds & Compounds with No Acceptable Pharmaceutical Grade Alternative 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.

#### STORAGE AND DISPOSAL

Do not administer non-sterile solutions, outdated solutions, more concentrated solutions, or higher doses than recommended above. Store the solution in a sealed container and according to the EHS Chemical Hygiene Plan. Urethane solutions are very stable and can be stored up to 6 months in sealed containers. Dispose of outdated urethane solutions using standard EHS chemical disposal guidelines.

*Deviations from the IACUC policy with respect to preparation, dose, storage, and disposal must be outlined and justified in your IACUC protocol.*

For more information on the preparation and storage of tribromoethanol, please refer to:

- Field, K. J. and Lang, C. M. (1988) Hazards of urethane (ethyl carbamate): a review of the literature. *Laboratory Animals* 22, 255-262
- Henshaw PS and Meyer HC (1944) Minimal number of anesthetic treatments with urethane required to induce pulmonary tumors. *Journal of the National Cancer Institute* 4, 523-525.
- Kay A. M. and Trainin N. (1966) Urethane carcinogenesis and induction. *Cancer Research* 26, 2206-2212.
- Tannenbaum A., Vesselinovitch S. D., Maltoni C. and Mitchell D. S. (1962) Multipotential carcinogenicity of urethane in the Sprague-dawley rat. *Cancer Research* 22, 1362-1371.
- Klien M. (1954) Induction of lung adenomas following exposure of pregnant, newborn, and immature male mice to urethane. *Cancer Research* 14, 438-440.
- Vesselinovitch S. D., Mihailovich N., Rao K. and Itze L. (1971) Perinatal carcinogenesis by urethane. *Cancer Research* 31, 2143-2147.
- Lewis Sr., R. J. (2004), *Sax's Dangerous Properties of Industrial Materials*.

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## IU IACUC Approved Recipe for Avertin/TBE

Note for Avertin users:

“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.”

Two chemicals are necessary to reproduce a similar drug to Avertin (Tribromoethanol). The first is 2,2,2 tribromoethanol (TBE); the second is amylene hydrate (aka tertiary amyl alcohol or 2-methyl-2-butanol), both obtainable from Aldrich Chemical. There may be other sources as well, but researchers should choose a source with a good reputation for the quality and purity of their products.

NOTE: Oxidation products of tribromoethanol are toxic, causing kidney and/or liver failure. These breakdown products produce a yellow color when the compound is dissolved – do not use a stock solution that is yellow.

#### INGREDIENTS

- 2.5 gm 2,2,2-tribromoethanol
- 5 ml 2-methyl-2-butanol (amylene hydrate, tertiary amyl alcohol)
- 200 ml distilled water or 0.9% NaCl or PBS - neutral pH

#### PERSONAL PROTECTIVE EQUIPMENT

- Lab coat
- Nitrile gloves
- ANSI Z-87 approved safety glasses
- ANSI Z-87 approved chemical safety goggles if a splash hazard exists
- Appropriate laboratory attire.

#### INSTRUCTIONS

1. Dissolve 2.5 grams tribromoethanol in 5 ml amylene hydrate. This requires heating to approximately 40° Celsius and stirring vigorously.
2. Add distilled water, 0.9% NaCl, or PBS, stirring continuously, up to a final volume of 200 ml.
3. Filter sterilize through a Millipore filter (0.5 micron).
4. Aliquot the final solution into appropriate sterile 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 the sterile container.
5. 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 eight weeks. If the material degrades, it becomes toxic.
6. Tribromoethanol degrades to dibromoacetaldehyde and hydrobromic acid. If the pH of the solution is less than 5, it should be presumed to have degraded. Test the solution by adding one drop of Congo Red to 5 ml of solution. If a purple color results, the solution has degraded 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 pH-neutral diluent). An alternative is to use pH strips.
7. As prepared above, the solution contains 12.5 mg TBE/ml. Do not attempt to make a more concentrated solution - the material is irritating at higher concentrations.

#### DOSAGE - USE

Mix by stirring or swirling prior to administration. Give by IP injection at a dose of 250 mg/Kg. This amounts to 0.5 ml of the above solution for a 25gm mouse. Induction requires approximately 4-5 minutes, anesthetic duration is approximately 18-20 minutes, and recovery is approximately 25-30 minutes. If needed, supplemental doses are 10-25% volume of the original dose.

#### STORAGE AND DISPOSAL

Do not administer non-sterile solutions, outdated solutions, more concentrated solutions, or higher doses than recommended above. 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, the IU IACUC requires replacing refrigerated TBE at least every eight weeks (after mixing). Dispose of outdated TBE using standard EHS chemical disposal guidelines.

*Deviations from the IACUC policy with respect to preparation, dose, storage, and disposal must be outlined and justified in your IACUC protocol.*

For more information on the preparation and storage of tribromoethanol, please refer to: Lieggi CC, Fortman JD, Kleps RA, Sethi V, Anderson JA, Brown CE, Artwohl JE. 2005 "An evaluation of preparation methods and storage conditions of tribromoethanol." Contemporary Topics **44**: 11-16.

#### Sources:

- Alfa Aesar
  - Aldrich Chemical - Aldrich technical services keeps records on complaints from customers, including reports on the color of the tribromoethanol solution from each batch sold.
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## Sanctions

Personnel that are found to be in violation of this policy may be subject to sanctions relating to their participation in activities involving the use of live vertebrate animals.

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## 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 Edition

[http://www.nap.edu/catalog.php?record\\_id=12910](http://www.nap.edu/catalog.php?record_id=12910)

USDA Policy #3: Veterinary Care

[http://www.aphis.usda.gov/animal\\_welfare/policy.php?policy=3](http://www.aphis.usda.gov/animal_welfare/policy.php?policy=3)

Rutgers, The State University of New Jersey, Animal Care and Facilities Committee Policy: **CUSTOM FORMULATED COMPOUNDS FOR USE IN ANIMALS**

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## References

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"

OLAW Webinar, March 1, 2012. Use of Non-Pharmaceutical-Grade Chemicals and Other Substances in Research with Animals

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