



UNIVERSITY STANDARD

Title

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL STANDARD ON PREPARATION, STORAGE AND USE OF TRIBROMOETHANOL (AVERTIN)

Introduction

PURPOSE

To provide uniform guidance to researchers in the preparation, storage and use of Tribromoethanol.

SCOPE OF APPLICABILITY

All laboratory personnel engaged in the preparation, storage and use of Tribromoethanol (Avertin).

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard. Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

Standard

Tribromoethanol (TBE), commonly referred to by the brand name Avertin, has been used as an anesthetic agent for mice that provides rapid induction and recovery for single use, short duration (approximately 15-20 minutes) surgical procedures. Some investigators choose TBE over other anesthetics because it is inexpensive, easy to use, not a controlled substance, and because they believe it produces a good quality of anesthesia with minimal side effects and a wide margin of safety. However, there are conflicting reports in the literature as to variable effectiveness, and some adverse effects, associated with the use of TBE as an intraperitoneal anesthetic in mice. Reported adverse effects include peritoneal inflammation and superficial necrosis of abdominal organs, which could affect experiments if those organs (e.g. liver) were the organ of study. Regulatory agencies have subsequently recommended that alternative options for rodent anesthesia be considered, where possible, in place of TBE. These alternatives would include pharmaceutical grade injectable and/or inhalation options that the DCM veterinary staff would be happy to discuss with investigators.



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Preparation

- Components
 - 2,2,2-tribromoethanol (Sigma-Aldrich T48402 or equivalent)
 - tertiary-amyl alcohol (Sigma-Aldrich 240486 or equivalent); also known as 2-Methyl-2-butanol or amylene hydrate
 - Diluent, usually tissue-culture grade distilled water or injection quality sterile saline
 - Polypropylene is recommended for pipetting the tertiary-amyl alcohol, not polystyrene. Polystyrene can dissolve in the solvent and then precipitate out in the TBE working solution, possibly causing adverse effects.
- Stock solution (100%)
 - Add 2,2,2-tribromoethanol (TBE) to tertiary-amyl alcohol in a 1g/1ml ratio.
 - Dissolve by heating to 40-50°C and stirring WHILE PROTECTED FROM LIGHT.
- Working solution
 - Dilute stock solution with warm diluent [physiologic saline (0.9% NaCl) or distilled water (tissue culture or mouse embryo culture grade)] WHILE PROTECTED FROM LIGHT.
 - Make 1:80 dilution of stock for a 1.25% working solution (12.5 mg/ml)
 - Example: add 0.5 ml stock to 39.5 mls diluent.
 - Make 1:40 dilution for a 2.5% working
 - Filter through 0.20 micron filter
- Ready to use working solution
 - Heat 200 ml embryo quality water (Sigma W1503) to ~ 50°C, set aside
 - Heat 5 ml tertiary-amyl alcohol to ~ 50°C, add 2.5g TBE, swirl to mix/dissolve
 - Add pre-heated water, making sure that final solution doesn't go above 35-40°C
 - Add stir bar to mix well WHILE PROTECTED FROM LIGHT



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- Filter through 0.20 micron filter
- Place in autoclaved amber bottle or glass bottle wrapped in aluminum foil
- Label bottle (as 1.25% Tribromoethanol) and store in refrigerator @ 2-8°C

Storage and Expiration Period

- All storage containers should be labelled as TBE with dates of preparation and expiration
- TBE working solution must be stored at 2-8°C in light protected containers.
 - When stored at this temperature, the solution may be used for up to 3 months.
 - Stored TBE working solution should be discarded if it becomes discolored (dark yellow or brown)
- Frozen TBE stock solution can be stored in a non-cycling freezer for up to 1 year.
- TBE stock solution should be aliquoted into light protected containers. Frozen aliquots can be stored in a non-cycling freezer for up to 1 year.
- Stock and working solutions should be accessed in a way that maintains their sterility.

Dosages

- 125-250 mg/kg
- 0.02 ml per gram body weight (1.25% solution)
 - Example: 25g mouse gets 0.5 ml, 30g mouse gets 0.6 ml
- NOTE: 1.25% TBE working solution is usually sufficient for most surgical procedures. However, as with all anesthetics, sex and strain may have an effect on efficacy.

Higher concentrations (2-2.5%) and/or dosages (up to 300 mg/kg) have been used for non-survival, or more invasive procedures, but carry a higher risk of adverse reactions.



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Animal Use Protocol

- The animal use protocol can simply reference this SOP or alternatively must provide details regarding the preparation of the working solution and proper storage of the stock solution in the animal use protocol.
- Tribromoethanol (TBE) is only available as a non-pharmaceutical grade compound so its use requires completing Section 11.3 in the IACUC application.

References

1. Weiss, J. and Zimmermann, F., Tribromoethanol (Avertin) as an anesthetic in mice. *Lab. Animal*, 1999. 33(2): 192-3.
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3. Zeller, WM; Burki, G; and Panoussis, B. Adverse Effects of Tribromoethanol as Used in the Production of Transgenic Mice. *Laboratory Animal Science*, 1998. October, 32(4): 407-413
4. Lieggi, C.C., et al., An evaluation of preparation methods and storage conditions of tribromoethanol. *Contemp Top Lab Anim Sci*, 2005. 44(1): 11-6
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6. Meyer, R.E. and R.E. Fish, A review of tribromoethanol anesthesia for production of genetically engineered mice and rats. *Lab Anim (NY)*, 2005. 34(10): 47-52.
7. Papaioannou, VE and Gox, JG. Efficacy of Tribromoethanol Anesthesia in Mice. *Laboratory Animal Science*, 1993. April, 43(2): 189-192.
8. Kohn, DF; Wixson, SK; White, WJ; and Benson, GJ. *Anesthesia and Analgesia in Laboratory Animals*, 1997.
9. PHS Policy on the Human Care and Use of Laboratory Animals, Frequently Asked Questions
10. Koizumi, T., H. Maeda, and K. Hioki, Sleep-time variation for ethanol and the hypnotic drugs tribromoethanol, urethane, pentobarbital, and propofol within outbred ICR mice. *Exp Anim*, 2002. 51(2): p119-24. National Human Genome Research Institute Guideline 03.2, http://www.theodora.com/rodent_laboratory/guideline_03_2.html



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EXCEPTIONS:

Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

Definitions

IACUC: Institutional Animal Care and Use Committee

DCM: Division of Comparative Medicine (formerly DLAM)

University Standard: The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.

TBE: Tribromoethanol

Contact Information

Subject	Contact	Telephone	Email
Use or alternative uses for TBE	Department of Comparative Medicine	919-843-3407	
Amendments or Exceptions	OACU	919-966-5569	iacuc@med.unc.edu

Important Dates

- Effective Date and title of Approver 8/4/2017; UNC IACUC
- Revision and Review Dates, Change notes, title of Reviewer or Approver: 08/04/2017; UNC IACUC

Approved by: UNC IACUC

Dr. Mitchell Picker
UNC IACUC Chair

8/2017