

University of Louisville
Institutional Animal Care and Use Committee
Policies and Procedures

Preparation, Storage, and Use of Tribromoethanol (Avertin)

Policy: This policy provides information on the requirements of the use of Tribromoethanol (Avertin, TBE) in laboratory animals. It is based upon the “Guidelines for the Use of Non-Pharmaceutical-Grade Chemicals/Compounds in Laboratory Animals” published by OLAW and the USDA (January 11, 2007). Use will be based upon 1) scientific necessity, 2) non-availability of acceptable veterinary or human pharmaceutical-grade compound(s); and 3) specific review and approval by the UofL IACUC. The UofL IACUC has developed standardized procedures for preparation, evaluation, handling, and storage that must be followed, or any proposed deviations from these procedures must be approved by the IACUC prior to use.

Rationale: Tribromoethanol (TBE) is an injectable anesthetic that is sometimes recommended for manipulations required for the production of genetically engineered mice and rats. While it was previously available as a pharmaceutical grade drug, it is no longer commercially available and investigators who wish to use it must make their own solutions of non-pharmaceutical-grade drug. TBE degrades in the presence of heat or light to produce potentially irritating and toxic byproducts.

Tribromoethanol has been reported to cause peritonitis, abdominal adhesions, intestinal ileus, superficial necrosis of abdominal organs and death in rodents. Concentrations greater than 1.25%, doses exceeding 300 mg/kg IP, and repeated use have a stronger correlations to adverse health effects on animals secondary to irritation/inflammation. Morbidity and mortality have been reported even at doses within the recommended range. TBE may be an appropriate anesthetic for short term procedures in mice and rats (8-20 minutes), in situations where it will be delivered in a single-use and in terminal procedures.

Procedures, Guidelines, and Exceptions:

1. The use of TBE must be justified by scientific necessity, lack of an acceptable pharmaceutical-grade alternative, and only after specific review and approval by the UofL IACUC. Common pharmaceutical grade alternatives to TBE that need to be considered include isoflurane, ketamine/xylazine or ketamine/dexmedetomidine, and pentobarbital.
2. Tribromoethanol is *not* recommended and is *strongly discouraged* for repeated anesthesia.
3. Stock Solution (100%)
 - a. 2, 2, 2-tribromoethanol (Sigma-Aldrich T48402 or equivalent)
 - b. Tertiary-amyl alcohol (Sigma-Aldrich 240486 or equivalent); also known as 2-Methyl-2-butanol or amylene hydrate
 - c. Add 2,2,2-tribromoethanol (TBE) to tertiary-amyl alcohol in a 1g/1ml ratio.
 - d. Stir on magnetic stirrer until the TBE is dissolved (about 12 hours – heating to approx 40-50°C helps dissolve the TBE) WHILE PROTECTED FROM LIGHT.

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- e. TBE stock is light sensitive and hygroscopic. Keep in a dark bottle away from light and tightly sealed.
 - f. Do not leave the bottle open longer than necessary.
 - g. Stock solution may be stored in a non-cycling freezer for up to **6 months** in aliquoted light protected containers. Yellowing of the solution indicates oxidation and the stock must be replaced if discoloration is noted prior to 6 months.
4. Working Solution (1.25%; 12.5 mg/ml)
- a. 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.
 - b. Make 1:80 dilution of stock for a 1.25% working solution (12.5 mg/ml). As an example, add 0.5 ml stock to 39.5 ml diluent.
 - c. Seal container and then wrap in foil to exclude light and stir on magnetic stirrer until dissolved.
 - d. Filter sterilize through 0.2 micron filter and store at 4°C in foil wrapped, sterile vials or kept in a dark, capped sterile bottle.
 - e. Working solution must be mixed **fresh** prior to each use for survival procedures.
 - f. Working solution has a **two week** safe use time limit for non-survival procedures, must be dated with the date it was mixed, and discarded after this date.
 - g. Do not use any working solution that is discolored, and/or has any evidence of a precipitate.
 - h. If the solution **pH is less than 5**, it should be presumed to have degraded and it should be discarded.
5. Dispose unused and/or expired TBE as chemical waste.
6. The IACUC proposal may reference this policy or alternatively must provide details regarding the preparation and storage of the stock and working solutions.
7. Dosages
- a. Mouse: 125-300 mg/kg IP.
 - b. Rat: 300 mg/kg IP or 150 mg/kg if used with dexmedetomidine.

References:

1. *Anesthesia and Analgesia in Laboratory Animals*, 2nd Edition. Eds. Fish, Brown, Danneman and Karas, Academic Press, 2008. pp 43, 260
2. *Formulary for Laboratory Animals*, 3rd Edition. Hawk, Leary and Morris. Blackwell Publishing, 2005.
3. Lieggi, Artwohl, et al. An Evaluation of Preparation Methods and Storage Conditions of Tribromoethanol. *Contemporary Topics in Lab Animal Science*, 2005. 44(1): 11-16.
4. Zeller, Burki, Meier, and Panoussis. Adverse Effects of Tribromoethanol as Used in the Production of Transgenic Mice. *Laboratory Animal Science*. 1998. October, 32(4): 407-413.
5. "Guidelines for the Use of Non-Pharmaceutical-Grade Chemicals/Compounds in Laboratory Animals" published by OLAW and the USDA (January 11, 2007).

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