The University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) has developed the following policies and procedures to provide a single IACUC-approved source of information for investigators that use tribromoethanol (TBE) to anesthetize mice and rats. Tribromoethanol is an injectable anesthetic agent that was once manufactured as a pharmaceutical grade drug under various trade names such as Avertin®. However, TBE is no longer available in pharmaceutical grade, and investigators that wish to use TBE as an anesthetic must make their own solutions with non-pharmaceutical grade compounds. The use of non-pharmaceutical grade drugs must be scientifically justified in the protocol and approved by the IACUC prior to use.

**Pharmaceutical vs. Non-Pharmaceutical Grade Drugs**

The use of pharmaceutical-grade compounds in laboratory animals ensures that the compounds meet the established documentable standards of purity and composition established in the United States Pharmacopeia National Formulary (USP/NF). The use of non-pharmaceutical grade compounds, which do not meet USP/NF standards, may expose lab animals to higher levels of impurities or impurities that are more toxic compared to those found in pharmaceutical grade compounds.

“.... pharmaceutical-grade 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. However, it is frequently necessary to use investigational compounds, veterinarian- or pharmacy-compounded drugs, and / or Schedule I controlled substances to meet scientific and research goals”. ¹

**Conditional Use of TBE**

The use of TBE is generally discouraged, as several safer, readily available, and pharmaceutical-grade alternatives [i.e., isoflurane] have been shown to be equally effective with fewer side-effects. The IACUC will allow the use of TBE as an anesthetic only with scientific justification and a description of why pharmaceutical grade alternatives cannot be used in a given animal model. Cost or convenience will not be acceptable as reasonable justifications for the use of TBE¹. Additionally, evidence has shown an increased incidence of mortality and morbidity associated with repeated dosing with TBE.², ³ Thus, the IACUC will not approve the use of TBE in repeated survival surgeries in the same animal.

TBE is appropriate only for short-term procedures in mice and rats for situations where it will be given only on a single occasion or in acute terminal procedures. If compounded and dosed properly, TBE can provide a safe, rapid, and stable plane of anesthesia for up to 15-20 minutes with a recovery time of 30-60 minutes. The dose in mice should be within the range of 125-300 mg/kg. At the low end of this range, and for use in rats, it is recommended to combine TBE with a second anesthetic drug for more reliable results.

**Risks of TBE Use**

- TBE degrades in the presence of heat or light to produce potentially irritating and toxic byproducts.⁴,⁵
- TBE is an irritant, especially at high doses, high concentrations, or with repeated use. Adhesions are sometimes seen in the abdominal cavity after IP injections. ³,⁵
- TBE can cause intestinal ileus (slowing of gut motility, can be fatal) several weeks after injection. ⁴,⁷,⁸
- Morbidity and mortality have been reported even at doses within the recommended range.⁴,⁷
INSTRUCTIONS FOR COMPOUNDING

- **Ingredients**
  - 2.5 g 2,2,2 tribromoethanol (TBE)
  - 5 ml 2-methyl-2-butanol (amylene hydrate or tertiary amyl alcohol)
  - 200 ml distilled water - neutral pH

- **Directions for 1.25% Working Solution of TBE**
  - Dissolve 2.5 g TBE in 5 ml of 2-methyl-2-butanol. This requires heating to approximately 40°C (104°F) and vigorous stirring.
  - Add distilled water, stirring continuously, up to a final volume of 200 ml.
  - Filter sterilize through a 0.2 micron filter (e.g. Millipore®).
  - Aliquot the final solution into appropriate, sterile containers. Sterile, red-cap blood collection tubes or sterile conical centrifuge tubes serve as good containers.
  - **Label with preparation and expiration dates should be on** the freshly prepared TBE solution. The expiration date for the working solution should be 2 weeks after preparation.
  - As prepared above, the concentration of the solution is 12.5 mg/ml tribromoethanol. **Do not attempt to make a more concentrated working solution - the material is irritating and can cause peritonitis and death at higher concentrations.**

- **Directions for compounding a 3% Stock Solution of TBE and dilution to make a 1.25% Working Solution**
  - Dissolve 3.0 g TBE in 5 ml 2-methyl-2-butanol and dilute to 100 ml with distilled water and aliquot to appropriate volumes.
  - Freeze and Store at -20°C in appropriate, sterile containers. Frozen stock may be stored for up to 12 months. **Please be sure to label the stock bottle with dates of preparation and expiration.**
  - On day of procedure, thaw and dilute with sterile saline to 12.5 mg/ml (1.25%) with a 1:1.4 ratio (vol/vol) of stock: diluent.
  - Filter sterilize through a 0.2 micron filter (e.g. Millipore®).
  - Discard any remaining thawed TBE solution on the same day of use.

- **Storage**: Diluted TBE must be stored at 4°C (39°F) and protected from light to prevent degradation. Even refrigerated and wrapped in foil, the material will degrade over time. Therefore, **TBE working solution must be made fresh at least every 2 weeks and old solution must be discarded** in order to avoid administering harmful, degraded anesthetic products to mice or rats. **Please be sure to label the bottle with dates of preparation and expiration.**
  - If the solution is less than pH 5, it should be presumed to have degraded. Discard the solution.
  - If the solution develops an unusual discoloration (typically yellow) or forms a precipitate, the solution should also be discarded.

**REFERENCES**


8. 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-