IACUC GUIDELINE  
TRIBROMOETHANOL USE IN MICE AND RATS

This guideline is designed 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 COMPOUNDING2
- Ingredients
  - 2.5 g 2,2,2 tribromoethanol (TBE)

Last updated 5/2013
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- 5 ml 2-methyl-2-butanol (amylene hydrate or tertiary amyl alcohol)
- 200 ml distilled water - neutral pH

**Directions for 1.25% 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 and date** the freshly prepared TBE solution
- As prepared above, the concentration of the solution is 12.5 mg/ml tribromoethanol. **Do not attempt to make a more concentrated solution - the material is irritating and can cause peritonitis and death at higher concentrations.**

**Alternative Compounding:** Freezing concentrated stock solutions at -20°C
- Dissolve 3.0 g TBE in 5 ml 2-m-2-b and dilute to 100 ml with distilled water and aliquot to appropriate volumes.
- Store at -20°C in appropriate, sterile containers. Frozen stock may be stored for up to 3 months. **Please be sure to label the stock bottle from start date of preparation.**
- On day of procedure, thaw and dilute to 12.5 mg/ml with PBS.
- 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 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 from start date of preparation.
- 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**