IACUC GUIDELINE
TRIBROMOETHANOL USE IN MICE AND RATS

Office of Regulatory Affairs

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 TBE**[1]. 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[2]

- **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% 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

1. Office of Laboratory Animal Welfare. **May investigators use non-pharmaceutical-grade compounds in animals? (FAQ F4 at:** http://grants.nih.gov/grants/olaw/faqs.htm#useandmgmt_4 **(searched Jan. 25, 2013).**
2. Papaioannou VE, *et. al.* "Efficacy of tribromoethanol anesthesia in mice." *Lab Anim Sci*. 1993 43(2):189-92.
3. Green CJ, *et. al.* "Animal Anesthesia." London: Laboratory Animals Ltd, 1979. p79.
4. Meyer RE, et. al. "A review of tribromoethanol anesthesia for production of genetically engineered mice and rats." *Lab Anim (NY)*. 2005 34(10):47-52.
5. Lieggi CC, *et. al.* "An evaluation of preparation methods and storage conditions of tribromoethanol." *Contemp Top Lab Anim Sci*. 2005 44(1): 11-16.
6. Zeller, W, *et. al.* "Adverse effects of tribromoethanol as used in the production of transgenic mice." *Lab Anim*. 1999 33(2):192-3.
7. Lieggi CC, *et. al.* "Efficacy and safety of stored and newly prepared tribromoethanol in ICR mice." *Contemp Top Lab Anim Sci*. 2005 44(1):17-22.
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), 79-84.