

**UGA IACUC Guideline for
Use of Tribromoethanol in Mice**
***Approved by the UGA IACUC
Effective June 20, 2013***

Tribromoethanol (TBE) is a popular injectable anesthetic agent used in mice. It was once manufactured specifically for use as an anesthetic by Winthrop Laboratories under the trade name Avertin®, but this product is no longer available. Currently, it is only available as a non-pharmaceutical-grade powder that must be aseptically reconstituted for injection. When used properly, it has a good margin of safety and it is still popular for certain research applications.

TBE is considered a non-pharmaceutical grade drug and as such its use must be in accord with these guidelines and the UGA IACUC Policy on the Use of Outdated Drugs and Materials, Non-pharmaceutical Grade Drugs, and Controlled Substances. Keeping this in mind that you will still be required to provide information regarding scientific necessity and must take into account the following when proposing the use of this agent in rodents:

- According to a recent review (Lab Animal 34(10):47-52) tribromoethanol has been associated with serious post-anesthetic effects and inconsistent and variable anesthetic effects.
- Have these effects been observed by your laboratory?
- Have alternative anesthetics for this procedure been considered? If so, why has TBE been chosen over these alternatives?
- Contact your attending veterinarian for consultation on the use of anesthetics.

Uses: TBE is appropriate for short term procedures in mice, especially surgical procedures. It is best used in situations where it will be given only on a single occasion.

Advantages of TBE: TBE induces anesthesia rapidly and provides good surgical analgesia for approximately one hour. Since it is given by injection, one is spared the occupational health risks and technical difficulties associated with volatile anesthetics. If used appropriately, TBE has a good margin of safety.

Disadvantages of TBE:

- 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.
- TBE degrades in the presence of heat or light to produce toxic byproducts. Degraded solutions can be both nephrotoxic and hepatotoxic. Administration of degraded TBE solutions has been associated with death, often 24 hours after surgery.
- TBE can cause intestinal ileus (stopping of the gut motility and subsequent death of the animal) several weeks after injection. This is more common with TBE stored in the presence of light or heat, stored at higher than recommended doses, or given at higher than recommended concentrations.

- The effects of TBE are also somewhat unpredictable in mice younger than 16 days, or in animals with altered carbohydrate metabolism, such as various mouse strains used for diabetes or obesity models (db/db mice or ob/ob mice).

Should the use of TBE be a scientific necessity, it is recommended that your laboratory follow these guidelines on the accepted methods of preparation, storage, and use of TBE:

Chemicals: Two chemicals are necessary to replicate Avertin. The first is 2,2,2 Tribromoethanol; the second is amylene hydrate (tertiary amyl alcohol).

Compounding: There are numerous methods for making TBE solutions. Following are specific recommendations:

1. 2,2,2-Tribromoethanol can be purchased as a powder from various chemical suppliers (e.g., Aldrich, Fischer Scientific, etc.). Record the date received on the bulk chemical label and use by the manufacturer's expiration date. Tribromoethanol powder has a shelf life of up to 5 years, but can vary based on manufacturer.
2. Tertiary amyl alcohol is necessary to make the final 100% Avertin stock solution. Record the date received, and use the tertiary amyl alcohol by the manufacturer's expiration date.
3. The 100% Avertin stock solution should be labeled with the date prepared, and should be assigned an expiration date of 6 months, after which time the stock solution should be discarded.
4. Since TBE degrades in the presence of heat or light to produce toxic byproducts that can be nephrotoxic and hepatotoxic, it should be stored refrigerated at 4°C in a light-safe or aluminum-wrapped vial/bottle.
5. The working solution for administration (1.25 - 2.5% TBE) should be prepared using a sterile diluent (e.g. sterile saline) and prepared fresh for each administration, or stored at 4°C in a light-safe or aluminum-wrapped vial/bottle with an expiration date of 2 weeks, after which time the working solution should be discarded. Label each bottle of working solution with concentration and date prepared.
6. The working solution should be filter (0.2 µm) sterilized prior to administration. Empty, sterile, red-cap blood collection tubes make a good receptacle, as do brown injection bottles with appropriate caps. It is often easiest to filter the material through a luer-fitted millipore filter directly into a sterile, red-cap blood collection tube. The working solution should be prepared, handled and stored to ensure that it remains sterile and does not lose its potency or decompose to form toxic by-products.
7. TBE, when injected intraperitoneally into mice, will provide approximately 30 min of surgical plane anesthesia. Since it can be an irritant, especially at high doses, repeated injections into the peritoneum can result in abdominal adhesions and should be avoided.
8. A morbidity and mortality log should be maintained for all mice anesthetized to better document any post-procedural complications and any inconsistent or variable anesthetic effects.

9. Summary of compound expiration dates:

- 2,2,2-Tribromoethanol powder = up to 5 years
- 100% TBE stock solution = 6 months from date of preparation
- 1.25 - 2.5% TBE working solution = 2 weeks from date of preparation

Dosage - Use: Mix by stirring or swirling prior to administration. The material is given by IP injection at a dose of 250 mg/Kg. Induction requires only 1-2 minutes and the righting reflex returns in approximately 40-90 minutes.

Cautions: Do not administer non-sterile solutions, outdated solutions, more concentrated solutions, or higher doses than recommended above. Store the solution under refrigerated at 4°C in a light-safe or aluminum-wrapped vial/bottle. Replace refrigerated TBE stock solutions at least every 14 days (after mixing).

References:

- Guide for the Care and Use of Laboratory Animals (*Guide*), NRC, 2010.
- A review of tribromoethanol anesthesia for production of genetically engineered mice and rats. *Lab Anim (NY)*. 2005 Nov; 34(10):47-52.
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