



## **Guidelines for Use of Tribromoethanol in Rodents**

The expectation is that IACUC Guidelines will be followed as best practice. They allow the Animal Care & Use Program to attain acceptable performance outcomes to meet the intent of the regulations. As such, any planned variation from the guidelines requires prior IACUC approval and must be based on a scientific rationale.

### **Introduction**

Tribromoethanol (TBE) is a popular injectable anesthetic agent used in rodents. It was once manufactured specifically for use as an anesthetic by Winthrop Laboratories under the trade name Avertin<sup>®</sup>, 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grade drug and as such its use must be in accord with these guidelines and the UGA IACUC Policy on the Use of Outdated Drugs and Materials, Non-pharmaceutical Grade Drugs, and Controlled Substances.

When using TBE, researchers must follow these guidelines.

### **Uses**

TBE is appropriate for short term procedures in rodents, including 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.
- 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**

- According to a published review (Lab Animal 34(10):47-52) tribromoethanol has been associated with serious post-anesthetic effects and inconsistent and variable anesthetic effects.
- 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

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heat, stored at higher than recommended doses, or given at higher than recommended concentrations.

- The effects of TBE are also somewhat unpredictable, especially 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). For all uses, the anesthetic depth must be closely monitored.
- As a non-pharmaceutical grade anesthetic, there is the potential for variability, including varying efficacy, across batches, which can carry implications for both animal welfare and the integrity of the science. Care must be taken in preparing the stock and working solutions to ensure consistency across batches.
- Also, as with other non-pharmaceutical grade substances, there is the possibility of pyrogenicity if the TBE is not properly prepared.

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

### **Chemicals**

Two chemicals are necessary to make the anesthetic. The first is 2,2,2 Tribromoethanol; the second is amylene hydrate (tertiary amyl alcohol).

### **Compounding**

There are numerous methods for making TBE solutions. It is expected that users will adhere to the following specific guidelines:

- 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.
- Tertiary amyl alcohol is necessary to make the final 100% TBE stock solution. Record the date received, and use the tertiary amyl alcohol by the manufacturer's expiration date.
- The 100% TBE stock solution must be labeled with the date prepared, and must be assigned an expiration date of 6 months, after which time the stock solution must be discarded.
- Since TBE degrades in the presence of heat or light to produce toxic byproducts that can be nephrotoxic and hepatotoxic, it must be stored refrigerated at 4°C in a light-safe or aluminum-wrapped vial/bottle.
- The working solution for administration (1.25 - 2.5% TBE) must 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 must be discarded. Label each bottle of working solution with concentration and date prepared.
- The working solution must 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 must be prepared, handled and stored to ensure that it remains sterile and does not lose its potency or decompose to form toxic by-products.
- The pH of the working solution must be tested to ensure it is within a physiologically appropriate range for injection (7.35-7.45).

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- TBE is injected intraperitoneally into rodents. Since it can be an irritant, especially at high doses, repeated injections into the peritoneum can result in abdominal adhesions and must be avoided unless approved with scientific justification in the AUP.
- For all rodents anesthetized, the anesthesia record must document morbidity and mortality, to better track any post-procedural complications and any inconsistent or variable anesthetic effects. Routinely, upon necropsy/tissue collection, animals should be assessed for signs of peritonitis or abdominal adhesions. Any unexpected complications must be reported to the IACUC.
- Summary of compound expiration dates:
  - 2,2,2-Tribromoethanol powder: according to manufacturer
  - 100% TBE stock solution stored at 4° C: 6 months from date of preparation
  - 1.25 - 2.5% TBE working solution stored at 4° C: 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. The length of anesthesia is variable; surgical anesthesia remains for approximately 30 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 outlined above.

### **References**

- Guide for the Care and Use of Laboratory Animals (*Guide*), NRC, 2011.
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