

WVU IACUC Policy:

Anesthesia Using Tribromoethanol (Avertin)

Background

Tribromoethanol (TBE) is an injectable anesthetic previously manufactured under the trade name Avertin® but is no longer available as a pharmaceutical-grade drug. The use of non-pharmaceutical grade compounds can present a risk to animal welfare due to concerns over consistency, contamination, preparation, and storage. Tribromoethanol degrades in the presence of heat and light, to produce potentially irritating and toxic contaminants that may be gastrointestinal irritants. Intraperitoneal injection of tribromoethanol can induce inflammation and necrosis of the inner abdominal wall and abdominal organs. Proper compounding and storage are essential to the safe use of this product.

Purpose

To provide guidance on the use of tribromoethanol in animal studies and to provide standardized methods for its preparation and storage.

Associated WVU IACUC Policies & Guidelines

- Veterinary Recommendations for Anesthesia and Analgesia
- Non-Pharmaceutical-Grade Substances Used in Animals

Requirements for Use

1. TBE use **must** be described in the animal care and use protocol and approved by the IACUC before its use. As a non-pharmaceutical-grade drug, scientific justification for its use, instead of other anesthetics (e.g., isoflurane, ketamine), **must** be described in the protocol.
2. TBE is to be administered intraperitoneally (IP).
3. TBE can only be used for a single survival surgery.
4. TBE can only be used in mice and rats.
5. TBE may be used a second time as general anesthesia only for a non-survival surgery. Animal(s) will be euthanized immediately following procedures done under anesthesia.
6. TBE should be prepared and stored in the manner described below.
7. When using TBE, any adverse or unexpected events **must** be immediately reported to veterinary staff.

Considerations

1. TBE degrades in the presence of heat and light to produce toxic byproducts. Both stock and working solutions **must** be maintained in light-protected containers and stored at 4°C or frozen.
2. Storage under the above conditions should be less than one month stored at 4°C or six months stored frozen for stock solution and no more than two weeks for working solution (at 4°C).

3. Do not use if pH is below 7.0.
4. Do not use if yellowed, or crystals are present.
5. Do not administer more than one dose per animal during a survival procedure.
6. All bottles **must** be labeled with:
 - a) Compound name and concentration
 - b) Date of preparation
 - c) Date of expiration

Preparation

1. Ingredients
 - a) 2,2,2 -Tribromoethanol (TBE), 99% purity (Thermo Scientific CAS # 75-80-9)
WARNING: harmful if swallowed, causes skin irritation, causes serious eye irritation, may cause respiratory irritation
 - b) tert-Amyl Alcohol (TAA) (2-methyl-2-butanol) 99% purity (CAS # 75-85-4)
WARNING: flammable, harmful in contact with skin or if inhaled, causes skin irritation, causes serious eye damage, may cause respiratory irritation
 - c) USP-grade Phosphate Buffered Saline
2. Solutions are to be prepared in a chemical fume hood while wearing safety glasses or goggles, nitrile gloves, and a lab coat.
3. Stock Solution
 - a) Triple rinse glassware with TAA. Wrap glassware in aluminum foil for light protection.
 - b) Weigh out 2.5 g of TBE and add to glassware.
 - c) Add 5 ml of TAA.
 - d) Stir (vigorously) in a 37°C water bath until the TBE is completely dissolved. Ensure solution is protected from light. This is a 50% w/v stock solution (500 mg/ml).
 - e) Label the stock solution with the name, preparation date, and expiration date (*6 months from preparation if frozen or one month if refrigerated*).
 - f) The stock solution can be frozen at -20°C or refrigerated at 4°C (protected from light) or diluted immediately to the working solution.
 - g) The stock solution **must** be discarded if any discoloration is noted, even if it is before the expiration date.

Ingredient	50% stock solution
2,2,2 -Tribromoethanol (TBE)	2.5 g
tert-Amyl Alcohol (TAA)	5 ml

4. Working Solution
 - a) Triple rinse glassware with phosphate buffered saline. Wrap glassware in aluminum foil for light protection.
 - b) A working solution of 1.25% or 2.5% can be made according to the following table:

Ingredient	To make working solution	
	1.25% (12.5 mg/ml)	2.5% (25 mg/ml)
TBE 50% stock solution from above	5 ml	10 ml
Phosphate buffered saline	195 ml	190 ml

- c) Stir or vortex for two (2) minutes.
- d) Filter the working solution through a 0.22µm filter into a sterile bottle.
- e) Check pH of the solution. The pH should be between 7.0-7.4.
- f) Aliquot the working solution into sterile containers. Label and mark working solution containers with name, preparation date, and expiration date (*2 weeks from preparation date*). Store at 4°C protected from light.

Use of compound

1. The working solution ***must*** be discarded if any discoloration is noted, even if it is before the expiration date.
2. Warm an aliquot of TBE working solution to 37°C and shake well before use.
3. The pH of the working solution ***must*** be tested before each use (place a drop on litmus paper).
4. Administer recommended dosage intraperitoneally according to WVU IACUC Guidelines: Veterinary Recommendations for Anesthesia and Analgesia.

References

- Lieggi, C.C., Fortman, J.D., Kleps, R.A., Sethi, V., Anderson, J.A., Brown, C.E., Artwohl, J.E. (2005). An evaluation of preparation methods and storage conditions of tribromoethanol. *Contemporary topics in laboratory animal science* 44(1):11-16
- Lieggi, C.C., Artwohl, J.E., Leszczynski, J.K., Rodriguez, N.A., Fickbohm, B.L., Fortman, J.D. (2005). Efficacy and safety of stored and newly prepared Tribromoethanol in ICR mice. *Contemporary topics in laboratory animal science* 44(1):17-22
- Zeller, W., Meier, G., Bürki, K., Panoussis, B. (1997) Adverse effects of tribromoethanol as used in the production of transgenic mice. *Laboratory Animals* 32: 407-413