

IACUC Policy for the Preparation, Storage, and Use of Tribromoethanol (Avertin) in Mice

Purpose

Tribromoethanol (Avertin® or TBE) is an anesthetic that is sometimes used in rodents (mice and rats) for short surgical manipulations. In the past, Tribromoethanol was available through Winthrop Laboratories under the trade name Avertin® as a prepared pharmaceutical grade anesthetic, but this product is no longer manufactured. The IACUC recommends inhalation anesthesia or pharmaceutical grade anesthetics when possible but recognizes that the use of tribromoethanol may be scientifically justified. This document provides guidance on the adequate preparation, use and storage of this compound.

Use

The duration of Tribromoethanol anesthesia is relatively short (15 – 30 minutes) but can vary widely according to sex, strain, and body composition of the animals. The effects of Tribromoethanol are also somewhat unpredictable in mice younger than 16 days, or in animals with altered carbohydrate metabolism, such as strains used for diabetes or obesity models (db/db and ob/ob mice).

Tribromoethanol is not recommended for repeated anesthesia. Only one injection may be administered, if for any reason an animal receives a second injection of Tribromoethanol, the procedure must be considered a terminal one and the animal euthanized prior to recovery from anesthesia. The IACUC will however consider requests for two independent doses per animal when adequate scientific justification is provided.

Dosage

Mouse: 125 – 300 mg/kg (0.3 ml – 0.4 ml) IP

Rat: 300 mg/kg IP

Recommended Preparation

It is recommended that Tribromoethanol be mixed using acid washed glass that has been rinsed in distilled water. Rinse with a 10% HCl and rinse with distilled H₂O. Glassware washed in detergents may have a residue that may be toxic.

Stock Solution 1.61 g/ml

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| Materials: | 2,2,2- tribromoethanol (99%) 10 g | Aluminum foil |
| | Tert-amyl alcohol, reagent grade 6.2 ml | Opaque or dark glass bottle |
| | Stir bar | Magnetic stir plate |
| | Nitrile gloves | |

1. Add 6.2 ml tert-amyl alcohol to 10 g Tribromoethanol. The bottle in which the Tribromoethanol arrives is convenient.
2. Always wear Nitrile gloves when handling Tribromoethanol powder or solutions. When measuring powder wear a particulate filter mask and work away from drafts.
3. Add a stir bar and mix on a magnetic stirrer until the Tribromoethanol is completely dissolved. This may take from 12 to 18 hours.
4. Tribromoethanol stock solution is light sensitive and hygroscopic. Store the stock solution in an opaque bottle wrapped in foil at room temperature away from light and tightly sealed. Layering nitrogen gas or Freon from an aerosol duster over the solution is a convenient way to delay degradation of the solution.
5. Stock solution may be kept up to 6 months provided there is no yellowing of the solution or crystal formation.

Working Solution 2.5%

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| Materials: | 50 ml graduated cylinder | 49.2 ml tissue culture grade distilled H ₂ O |
| | Stir bar | Magnetic heated stir plate |
| | Aluminum foil | 0.78 ml Tribromoethanol stock solution |
| | Dark glass bottle | 0.2 micron sterile syringe filter |
| | Parafilm® | Litmus paper |
| | Nitrile gloves | |

1. Ensure all glassware and cylinders are free of detergent residue. Always wear Nitrile gloves when handling Tribromoethanol powder or solutions.
2. Add 48.2 ml pre-warmed tissue culture grade distilled H₂O to the cylinder with a stir bar.
3. Add to the cylinder drop-wise while constantly stirring 0.78 ml Tribromoethanol stock solution. Seal the cylinder with parafilm® and wrap completely in aluminum foil to exclude all light.
4. Stir slowly on low heat overnight to dissolve the stock solution.
5. Filter the working solution through a 0.2 micron sterile syringe filter into a dark glass bottle. All containers should be wrapped in foil. There are reports that tribromoethanol can degrade or dissolve some 0.2 micron filters. It is recommended that a new filter be used after every 25-30 milliliters of solution has passed through.
6. Check the pH of the solution. Do not dip litmus paper into sterile working solution, instead place one drop on the paper using either a sterile pipette or sterile syringe. The pH of the working solution must be in the range of 7.0 – 7.4
7. Working solutions must be pH tested before each use. Any working solution that drops below a pH of 7.0 must be discarded as chemical waste. All working solutions expire 2 weeks from preparation.
8. Refrigerate the working solution except when using. **All bottles must be labeled with the following information:**
 - Compound Name and concentration
 - Date of Preparation
 - Date of Expiration

Summary

1. Stock solution must be stored at room temperature.
2. Working solution must be refrigerated.
3. **DO NOT USE** if working solution pH is below 7.0
4. **DO NOT USE** if working solution is yellowed or crystals are present.
5. **DO NOT** administer more than one dose per animal unless specifically approved by the IACUC.

References

1. Papaioannou, VE and Fox, JG. Efficacy of Tribromoethanol Anesthesia in Mice. *Laboratory Animal Science*, 1993 April, 43(2): 189-192.
2. Lieggi, CC., et.al. Efficacy and Safety of Stored and Newly Prepared Tribromoethanol in ICR Mice. *Contemporary Topics in Laboratory Animal Science*, 2005. 44(1): 17-22.
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4. Meyer, RE and Fish, RE. A Review of Tribromoethanol Anesthesia for Production of Genetically Engineered Mice and Rats. *Lab Animal (NY)*, 2005. 34(10): 47-52.
5. Zeller, W., et al. Adverse Effects of Tribromoethanol as Used in the Production of Transgenic Mice. *Lab Animal*, 1998. 32(4): 407-413.
6. PHS Policy on the Humane Care and Use of Laboratory Animals, Frequently Asked Questions.