Notre Dame Institutional Animal Care and Use Committee
Guidelines 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

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

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 hydroscopic. 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%

Materials:
- 50 ml graduated cylinder
- Stir bar
- Aluminum foil
- Dark glass bottle
- Parafilm®
- Nitrile gloves
- 49.2 ml tissue culture grade distilled H2O
- Magnetic heated stir plate
- 0.78 ml Tribromoethanol stock solution
- 0.2 micron sterile syringe filter
- Litmus paper

1. Add 49.2 ml of tissue culture grade distilled H2O.
2. Add the Tribromoethanol stock solution and stir until the Tribromoethanol is completely dissolved.
3. Add 0.78 ml of Tribromoethanol stock solution and stir until the Tribromoethanol is completely dissolved.
4. Add 0.2 micron sterile syringe filter and stir until the Tribromoethanol is completely dissolved.
5. Add Litmus paper and stir until the Tribromoethanol is completely dissolved.
6. Store in a dark bottle wrapped in foil at room temperature away from light and tightly sealed.
7. Layering nitrogen gas or Freon from an aerosol duster over the solution is a convenient way to delay degradation of the solution.
8. Working solution may be kept up to 6 months provided there is no yellowing of the solution or crystal formation.
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
6. PHS Policy on the Humane Care and Use of Laboratory Animals, Frequently Asked Questions.

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