Guidance for the Preparation, Storage and Use of Tribromoethanol (TBE) in Mice
Reviewed and approved by UCAR 8-16-17

Background

Tribromoethanol (TBE; also referred to as Avertin) is an injectable anesthetic with a long history of use in laboratory mice and rats. It remains popular as a general anesthetic because deep anesthesia is rapidly induced and lasts for approximately 20 minutes, an ideal time for many procedures. In particular, TBE has advantages as an anesthetic for the administration of materials to the respiratory tract via the intranasal route. TBE suppresses reflexes that could result in administered material being swallowed instead of inhaled into the lung. Furthermore, as an injected anesthetic, TBE has no direct effect on the lung environment or on the administered material. This is particularly important when infectious agents that are susceptible to inactivation (e.g. enveloped viruses) are given intranasally to establish an infection in the lung. TBE is not a controlled substance, contributing to ease of use (purchasing and storage) in a general laboratory setting.

A number of published reports have drawn attention to potential toxic effects after the intraperitoneal administration of TBE to mice. The mechanism appears to relate primarily to the induction of peritoneal inflammation, with intestinal adhesions and gastrointestinal ileus (slowing of gut motility) as possible longer-term consequences. However, the toxic effects of TBE have not been consistently observed in all studies and have even varied from experiment to experiment within studies. It is clear that TBE as a powder or in solution generates toxic degradation products in the presence of heat or light. Thus, the apparently conflicting reports of TBE toxicity may reflect differences in methods of TBE preparation and storage. Another factor may be mouse strain differences in susceptibility to the toxic products. TBE is generally not recommended for repeated anesthesia in a single mouse because of concerns about toxic effects. However, a recent investigation of this issue concluded that TBE appears safe for repeated administration to C57BL/6 mice. Notably, numerous research groups have used TBE for many years without encountering problems from TBE toxicity.

TBE is not available as a pharmaceutical-grade product. This adds to the challenge posed by TBE’s potential to form toxic degradation products. Particular care must be taken to ensure that TBE preparations used for animal anesthesia do not introduce toxic or unwanted side effects into studies and remain safe and effective for the duration of their shelf life. Well-established SOPs for TBE preparation, storage, and use are provided below.

UCAR Considerations

Researchers must provide scientific justification in the UCAR protocol for the use of TBE as an anesthetic (e.g. why commercially available veterinary or human pharmaceutical grade anesthetics cannot be used). Additionally, researchers must justify the use of more than one dose of TBE in an individual animal for survival procedures. Cost savings or convenience are not an adequate justification for the use of non-pharmaceutical grade or compounded drugs in animals (Guide for the Care and Use of Laboratory Animals: Eighth Edition, 2011, p.31).

When considering the use of a non-pharmaceutical grade anesthetic, Investigators can use the following examples to help prepare at protocol modification.

- Inadequate justification, when no additional justification is present:
  - Cost savings;
  - Administration burden of acquiring and maintaining controlled drugs.
- Possible adequate justification, requiring particular attention to the details:
  - Unpublished, anecdotal experience on benefits of TBE for the model or detrimental effects of alternatives;
Experimental logistics or personnel safety, which include 1) access to specialized equipment (fume hoods, vapoizers/scavengers, etc.) 2) interference with measurements or procedures; or 3) reduction in performance standards.

- Justification that is generally acceptable:
  - Detailed concerns about potential detrimental effects on established models or experimental paradigms.

- Justification that is always acceptable:
  - Known impact on measured outcomes, which is substantiated by data and published reports.

**Recommendations for TBE preparation, storage, and use -**

**TBE preparation**

The following method is for the preparation of 500 ml of a 2% working strength solution of TBE. The process can be scaled up for larger volumes.

**Ingredients**

- 2,2,2-tribromoethanol (Aldrich T4,840-2)
- tertiary-amyl alcohol (2-methyl-2-butanol) (JT Baker 9046-01)
- Distilled water

**Directions**

1. Combine 10 g TBE and 10 ml tert-amyl alcohol in a small sealed flask. Stir on a magnetic stirrer (in fume hood) at room temperature until TBE is completely dissolved.

2. Add TBE solution very slowly (drop-wise with constant stirring) to pre-warmed distilled water to a final volume of 500 ml. Continue stirring on a heated magnetic stirrer (lowest heat setting; water should be 22-28°C) until the solution is completely clear. This step can take several hours (or overnight). During this process, keep the container wrapped in foil to exclude light.

3. Allow the clarified solution to come to room temperature and filter sterilize through a 0.2 µm filter (e.g. Millipore filter unit of appropriate size).


5. Label bottles, including production and expiration dates. Store protected from light at 4°C. This working strength TBE preparation can be used for up to 6 months from date of preparation.

**Alternative method**

A concentrated stock solution of 1.0-1.61 g/ml TBE in tert-amyl alcohol can be prepared for longer-term storage. The brown glass bottle in which TBE is supplied is a convenient container for preparing a stock solution. Drop in a stir bar and stir on a magnetic stirrer until the TBE is completely dissolved. This will probably take overnight.
Keep the stock in the dark bottle and tightly capped and store at room temperature. The stock is photosensitive and hygroscopic. Layering dry nitrogen gas or Freon from an aerosol duster over the solution is an excellent strategy to prevent formation of toxic photo-oxidation products. Stock solution is stable for up to 12 months. Yellow discoloration indicates the presence of toxic products.

As required, dilute the TBE stock solution in an appropriate volume of distilled water to produce a working strength TBE solution (see above for preparation and storage details).

**TBE administration**

**Mice:** 250 mg/kg IP. Typically, 0.25-0.30 ml of a 2% working strength TBE solution is appropriate for a 6-8 week-old mouse of approximately 20 g. Dosage may have to be adjusted for some mouse strains.

**Exemptions**

If you adhere to the provisions described above then you are adhering to UCAR guidance. Exemptions to this guidance must be described in the protocol, reviewed and approved by UCAR. If you have any questions about alternative anesthetics, contact DCM at X 5-2563.

**References**