Guidance for the Preparation, Storage and Use of Tribromoethanol (TBE) in Mice
Reviewed and approved by UCAR 12/20/23
Background

Tribromoethanol (TBE; also referred to as Avertin) is an injectable anesthetic that is not available as a pharmaceutical grade product. *The Guide* states that pharmaceutical-grade products should be used when available for all animal-related procedures. A number of published reports have drawn attention to potential toxic effects after the intraperitoneal administration of TBE to mice. TBE as a powder or in solution generates toxic degradation products in the presence of heat or light. 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. 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). Acceptable justification relies on known impact on measured outcomes, which is substantiated by data and published reports.

SOP 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
CRITICAL – use ONLY glass containers, pipets etc., never plastic.

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.

4. Aliquot TBE preparation in sterile amber glass bottles. Blow argon gas over the surface of the

liquid before capping. Cap bottles with sterile rubber stoppers and “removable center” crimp seals.

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.

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; testing the dose is recommended. Animals will stay sedated for 20-40min and must be monitored until conscious.

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 5-2653.

References

1. Papaioannou VE and Fox JG. “Efficacy of tribromoethanol anesthesia in mice.” Lab Anim Sci. 1993 43(2):189-92.
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5. Lieggi CC et al. “Efficacy and safety of stored and newly prepared Tribromoethanol in ICR mice.” Contemp Top Lab Anim Sci 2005 44(10): 17-22.
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8. National Research Council. *Guide for the Care and Use of Laboratory Animals: Eighth Edition*. Washington, DC: The National Academies Press, 2011
9. http://grants.nih.gov/grants/olaw/120301_NPG_slides.pdf