Denver | Anschutz

IACUC
Institutional Animal Care and Use Committee

Subject:	**Use of Tribromoethanol (TBE) in Laboratory Animals**
Source	Institutional Animal Care and Use Committee (IACUC)
Effective Date	9/14/2026
Replaces:	10/09/2023
Applies to:	Personnel involved in Research or Teaching involving animals.
Reference:	Animal Welfare Act; PHS Policy on Humane Care & Use of Laboratory Animals. Guide for the Care & Use of Laboratory Animals

Introduction

The Institutional Animal Care and Use Committee (IACUC) provides oversight of the institution's animal care and use program, ensuring compliance with federally mandated regulations, ethical standards, and institutional policies governing the humane use of animals in research and teaching at the University of Colorado Denver | Anschutz. The IACUC evaluates and reviews all activities related to the care and use of animals and determines if the proposed activities are in accordance with regulations.

Guidance Statement

Tribromoethanol (TBE) is a non-pharmaceutical-grade chemical compound.

- Based upon the "Guidelines for the Use of Non-Pharmaceutical-Grade Chemicals/Compounds in Laboratory Animals" published by OLAW and the USDA (January 11, 2007) and subsequent supplements, the use of non-pharmaceutical grade chemical compounds, such as Tribromoethanol (TBE), in experimental animals under certain circumstances has been, and will continue to be, a necessary and acceptable component of biomedical research. Use of such compounds at CU Denver | Anschutz will be based upon:
  - scientific necessity;
  - non-availability of acceptable veterinary or human pharmaceutical-grade compound(s); and
  - specific review and approval by the CU Denver | Anschutz IACUC.

Background on Tribromoethanol

Tribromoethanol (TBE) is an injectable anesthetic agent used in mice, and sometimes rats. Since TBE is no longer available as a pharmaceutical-grade compound, investigators must prepare their own solutions, and TBE is subject to the IACUC Policy on the Use of Non-pharmaceutical Grade Compounds. Specifically, its use must be justified by scientific necessity, and the lack of an acceptable pharmaceutical-grade alternative, only after specific review and approval by the CU Denver | Anschutz IACUC (see considerations for IACUC review and approval below). The IACUC has developed standardized procedures for preparation, evaluation, handling, and storage that also must be followed; or any proposed deviations from these procedures must be approved by the IACUC prior to use.

Uses*

TBE may be an appropriate anesthetic when justified, in situations where it will be delivered for single-use and in terminal procedures.

Common Pharmaceutical Grade Alternatives*

- Isoflurane**
- Ketamine/Xylazine or Ketamine/Medetomidine
- Pentobarbital-based Euthanasia Solutions

* For doses, advantages and disadvantages of TBE and its common pharmaceutical grade alternatives please see the CU Denver Veterinary Formulary on the IACUC website. Questions on drugs and doses should be referred to an OLAR Veterinarian, and any questions on Policy should be referred to the IACUC.

IACUC Considerations

The IACUC expects researchers to provide sufficient detail and consider the advantages and disadvantages of TBE, as well as all common alternatives, when seeking the most appropriate anesthetic agent(s) for their procedures.

All proposed use of TBE must be justified, based on scientific necessity, and consideration of pharmaceutical alternatives. This justification should be included in both the Description of Procedures section and in the Justification of the use of a Non-pharmaceutical grade compound section within the submenu for the TBE entry in the non-experimental drug section of the CU Denver | Anschutz IACUC protocol.

Justification of TBE should include the following details:

- Why is TBE the most appropriate anesthetic agent for the specific circumstances outlined in the protocol.
- How alternative options may negatively impact outcomes or measurements.
- How experimental logistics have influenced your choice of anesthetic, if applicable.

Sufficient details concerning the issues of safety, efficacy, and the inadvertent introduction of research complicating variables should be included for the committee's consideration.
Consultation with the veterinary staff is strongly encouraged.

General Guidelines for TBE Use in Rats and Mice. When considering the use of TBE and its pharmaceutical-grade alternatives, CU Denver | Anschutz faculty can use the following examples to help with protocol/amendment preparation.

- Inadequate justification when no additional justification is present:
  - Cost savings.
  - Administrative burden of acquiring and maintaining a DEA license.
  - Consideration/elimination of only one pharmaceutical-grade alternative.
- Possible adequate justification, requiring particular attention to 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, vaporizers/scavengers, etc.)
    2) interference with measurements or procedures
    3) reduction in performance standards
    4) security of regulated drugs (in rare circumstances).
- Justification that is generally acceptable:
  - Detailed concerns about potential detrimental effects on established models or experimental paradigms.
  - Back-up anesthetic, used in emergencies in case pharmaceutical-grade alternatives are not available (may require greater post-approval monitoring).
- Justification that is always acceptable:
  - Known impact on measured outcomes, which is substantiated by data or published reports.

Additional Considerations While TBE use requires justification and standardized procedures for preparation, storage, and use, the committee will be more inclined to approve TBE for terminal procedures but less so with single-use survival procedures. Even greater concern and scrutiny can be expected from protocols that propose multiple procedures with TBE, use in pre-weaned animals (<16 days), and use in animals with impairments in carbohydrate metabolism (obesity, diabetes, etc.). TBE use in these situations presents additional animal welfare and efficacy complications that must be weighed against the advantages of using this anesthetic in the proposed research.

IACUC Approved Procedures Preparation, Storage, Use, Occupational Safety and Disposal

Two chemicals are necessary to synthesize a drug similar to Avertin (Tribromoethanol). The first is 2,2,2 tribromoethanol (TBE); the second is amylene hydrate (tertiary amyl alcohol). Researchers should choose a source/vendor with a good reputation for the quality and purity of their products.

- **Ingredients**:
  - 2.5 gm 2,2,2-tribromoethanol
  - 5 ml 2-methyl-2-butanol (amylene hydrate, tertiary amyl alcohol)
  - 200 mL distilled water or 0.9% NaCl or PBS - neutral pH

- **Instructions**:
  - Dissolve 2.5 grams tribromoethanol in 5 mL amylene hydrate. This requires heating to approximately 40° Celsius and stirring vigorously.
  - Add distilled water or 0.9% NaCl or PBS, stirring continuously, up to a final volume of 200 mL.
  - Filter sterilize through a Millipore filter (0.22-micron).
  - Aliquot the final solution into appropriate sterile containers - empty, sterile, red-cap blood collection tubes make a good receptacle, as do brown injection bottles with appropriate caps. It is often easiest to filter the material through a-Luer-fitted Millipore filter directly into the sterile container.
  - Tribromoethanol degrades to dibromoacetaldehyde and hydrobromic acid. If the pH of the solution is less than 5, it should be presumed to have degraded. Test the solution by adding one drop of Congo Red to 5 mL of the solution. If a purple color results, the solution has degraded and should be discarded. (Note: this method is only useful if the original pH of the solution is greater than 5 - hence the recommendation for pH-neutral diluent). An alternative is to use pH strips.
  - As prepared above, the solution contains 12.5 mg TBE/mL. Do not attempt to make a more concentrated solution - the material is irritating at higher concentrations.
  - Store the aliquots strictly at 4°C in dark, foil-wrapped containers, and discard after 14 days or if the pH drops below a safe threshold. If the material degrades, it becomes toxic.

Dosage - Use

Mix by stirring or swirling prior to administration. The material is given by IP injection at a dose of 250 mg/Kg. This amounts to 0.5 mL of the above solution for a 25gm mouse. Do not give animals a higher dose, as it can be irritating at higher doses. Induction requires approximately 4-5 minutes, anesthetic duration is approximately 18-20 minutes, and recovery is approximately 25-30 minutes. If needed, supplemental doses are 10-25% volume of the original dose.

Occupational Safety and Disposal

Do not administer non-sterile solutions, outdated solutions, more concentrated solutions, or higher doses than recommended above. Store the solution under refrigeration and in the dark. Containers should be wrapped in foil. Although some authors report that refrigerated solutions may be kept for months, the CU Denver | Anschutz IACUC requires that refrigerated TBE be replaced at least every 14 days (after mixing).

Handling chemicals like 2,2,2-tribromoethanol and amylene hydrate requires appropriate Personal Protective Equipment (PPE), including gloves, lab coats, and eye protection. Handling should occur in a well-ventilated area or a fume hood to prevent inhalation of vapors. Dispose of outdated TBE using standard EH&S chemical disposal guidelines.

Compliance and Accountability

In accordance with regulatory requirements, failure to comply with this policy will result in a review by the IACUC. Depending on the severity of the violation, you may be required to complete additional training, and your privileges to conduct animal research at CU Denver | Anschutz may be suspended or completely revoked.

If your research privileges are suspended, your funding agency (e.g., NIH) and relevant regulatory agencies (e.g., USDA) may be informed about your violation of federal and/or local policies regarding the humane use of animals. This notification will have a direct impact on your ongoing funding for animal-related research.