Introduction
The Institutional Animal Care and Use Committee (IACUC) maintains oversight review for federally mandated rules and regulations with regard to animal research, ethics, misconduct and biomedical research for the University of Colorado Denver | Anschutz Medical Campus (CU Denver | Anschutz).

Policy Statement
This policy is intended to provide information on the requirements of the use of Tribromoethanol (TBE) in laboratory animals.

- 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 commonly used in mice, and sometimes rats. Investigators who wish to use TBE as an anesthetic must therefore make their own solutions. Since TBE is no longer available as a pharmaceutical-grade compound, it is subject to the IACUC policy on the use of non-pharmaceutical grade compounds (http://www.ucdenver.edu/academics/research/AboutUs/animal/IACUC/Documents/Non-pharm-grade%20chemicals_compounds.pdf). Specifically, its use must be justified by scientific necessity, the lack of an acceptable pharmaceutical-grade alternative, and 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 for short term procedures in mice and rats (8-20 minutes), in situations where it will be delivered in single-use and in terminal procedures.

Common Pharmaceutical Grade Alternatives*
- Isoflurane**
- ketamine/xylazine or ketamine/medetomidine
- pentobarbital

* For doses, advantages and disadvantages of TBE and its common pharmaceutical grade compounds please see the Common Anesthetics/Analgesics document on the IACUC website. Please note that if there are any differences between another institutions policy, drug doses, etc. and CU Denver | Anschutz Policy, the CU Denver | Anschutz Policy is applicable. Questions on drugs and doses should be referred to an OLAR Veterinarian and any question on Policy should be referred to the IACUC.
**Please note that OLAR maintains several precision vaporizers available for investigator use in the vivarium with appropriate scavenging. There is no charge to use this equipment but the lab must attend training (no charge) and purchase their own isoflurane.**

**IACUC Considerations**

All proposed TBE use must be justified, based on scientific necessity and consideration of pharmaceutical alternatives, within the narrative section of the CU Denver | Anschutz IACUC protocol. 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. Consultation with the veterinary staff is strongly encouraged. Justification of TBE should include details of how TBE is the most appropriate anesthetic agent for the specific circumstances in the protocol, how alternatives may negatively impact outcomes or measurements, and/or how experimental logistics have influenced your choice of anesthetic. Sufficient details concerning the issues of safety, efficacy, and the inadvertent introduction of research complicating variables should be included for the committee’s consideration.

**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 them in 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; or 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 any TBE use requires justification and standardized procedures of preparation, storage, and use, the committee will be more inclined to approve TBE in 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, and Disposal**

Two chemicals are necessary to reproduce a similar drug to Avertin (Tribromoethanol). The first is 2,2,2 tribromoethanol (TBE); the second is amylene hydrate (tertiary amyl alcohol), both obtainable from Aldrich Chemical. There may be other sources as well, but researchers should choose a source 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, 0.9% NaCl, or PBS, stirring continuously, up to a final volume of 200 ml.
- Filter sterilize through a Millipore filter (0.5 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’s often easiest to filter the material through a luer-fitted millipore filter directly into the sterile container.
- Refrigerate the aliquots and protect them from light. The material degrades rapidly in the presence of heat or light. Even refrigerated and wrapped in foil, the material is stable for only about two weeks. If the material degrades, it becomes toxic.
- 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 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.

**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. 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.

**Storage 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 replacing refrigerated TBE at least every 14 days (after mixing). Dispose of outdated TBE using standard EH&S chemical disposal guidelines.

Deviations from the IACUC policy with respect to preparation, dose, storage, and disposal must be outlined and justified in your IACUC protocol.

Per regulatory requirements, failure to comply with this policy may result in notification of your funding agency (e.g. NIH) and regulatory agencies (e.g. USDA) that your research has violated federal and/or local policies regarding the humane use of animals. This notification may affect continuous funding of your animal-related research. Further, depending on the violation, you may be required to take additional training and/or your privilege to conduct animal research at CU Denver | Anschutz might be temporarily suspended or even completely revoked.