A. Purpose

To provide guidance on the use of tribromoethanol (TBE) in animal studies and to provide standardized methods for its preparation and storage. The NIH Office of Laboratory Animal Welfare (OLAW) has advised IACUCs to critically evaluate the proposed use of TBE and the consideration of alternative methods that avoid or minimize discomfort, distress, and pain.

B. Background

In compliance with federal Animal Welfare Regulations, OLAW, and AAALAC, the IACUC expects that investigators use pharmaceutical grade medications whenever they are available, even in acute-terminal procedures.\(^2\)\(^,\)\(^3\) Non-pharmaceutical grade compounds should only be used after specific review and approval by the IACUC for reasons such as scientific necessity, or non-availability of an acceptable veterinary or human pharmaceutical-grade product (please see WSU IACUC Policy #29 “Use of Non-Pharmaceutical Grade Substances in Laboratory Animals”).

Tribromoethanol (TBE) is an injectable anesthetic previously manufactured under the trade name Avertin\(^{\oplus}\); however, this product is no longer available in pharmaceutical grade.

In addition, TBE can cause several deleterious effects when administered to animals\(^2\)\(^-\)\(^8\):

- TBE degrades in the presence of heat or light to produce the toxic byproducts, dibromoacetaldehyde and hydrobromic acid, which are nephrotoxic and hepatotoxic.
• Administration of degraded TBE solutions has been associated with post-anesthetic illness and death, often within 24 hours of injection.
• Peritonitis, abdominal adhesions, and ileus (reduced gut motility) leading to death of the animal can occur following intraperitoneal (IP) administration of TBE.
• Other side effects include muscle necrosis, hepatic damage, bacterial translocation, sepsis, and serositis of abdominal organs.
• The duration of anesthesia has also been shown to be variable even when the dose is kept constant.\(^8\)

C. Policy

Due to the lack of a pharmaceutical grade drug and the potential for serious side effects, the IACUC does not recommend the use of TBE in animals. Alternate anesthetic agents should be used whenever possible. If TBE remains the anesthetic of choice despite these considerations, the following must be addressed in the protocol or amendment:

• The investigator must **scientifically** justify why the use of TBE is necessary, and why another pharmaceutical grade anesthetic cannot be used. The following will not be considered adequate justification
  o Cost
  o Lack of a controlled substances license
  o Familiarity/historical use of TBE
• The investigator must acknowledge awareness of the potential negative effects of TBE administration.
• The investigator must consult with OCV regarding alternative anesthetic options prior to approval of TBE use.

The IACUC will carefully consider the scientific justification provided. If the use of TBE can be justified on scientific grounds and is approved by the IACUC, **the following conditions must be met as part of that approval:**

• TBE must be prepared, stored, and disposed of in the manner described below.
• The dose must not exceed the range listed below.
• If approved for survival procedures, only a single dose may be administered.
• Any adverse or unexpected events must be immediately reported to the Office of the Campus Veterinarian.
D. Preparation

Stock Solution (1.6 g/ml):

- Ensure that glassware and stirrers are clean by pre-treatment with 10% HCl to remove detergent residue and thoroughly rinsing with reagent grade H2O.
- Prepare the stock solution in a chemical fume hood, wearing nitrile gloves, a lab coat and safety glasses.
- Add 6.2ml tertiary amyl alcohol (2 methyl-2-butanol) to 10g 2,2,2-tribromoethanol (TBE) in a capped container and stir until the TBE is dissolved. This may take several hours to overnight. Ensure the solution is kept away from light.
- Label the bottle (stock solution) with the date of preparation and a use-by date. The stock solution is stable for 6 months when protected from light and heat.
- Aliquot the stock solution into multiple, smaller containers to reduce the risk of contamination due to multiple draws from the same container.
- Store the solution at 4°C, away from light and tightly capped, as the solution is photosensitive and hygroscopic.
- A yellow discoloration indicates the presence of toxic products, and the stock solution must be discarded (even if it is before the use-by date).
- After 6 months, any unused solution must be appropriately discarded.

Working Solution (20 mg/ml):

- Ensure that glassware and stirrers are clean by pre-treatment with 10% HCl to remove detergent residue and thoroughly rinsing with reagent grade H2O.
- Prepare the working solution in a chemical fume hood, wearing nitrile gloves, a lab coat and safety glasses.
- Add 0.5ml stock solution to 39.5ml USP grade saline drop wise with stirring at 40°C to dissolve the TBE. Ensure the solution is kept away from light.
  - The stock solution must be diluted in USP grade diluent, and the composition of the diluent must be suitable for injection into animals (i.e., do not use water).
- Filter the working solution through a 0.22μm sterile filter into a sterile bottle.
- Label the bottle with the concentration, the date of preparation and a use-by date. The working solution is stable for 2 weeks (use-by date).
- Store the solution at 4°C and away from light, as the solution is photosensitive. Warm to 40°C to dissolve precipitates, if necessary.
- If the solution changes in color from clear to yellow, the solution must be appropriately discarded.
- Dispose of any solution that is past 2-week expiration date.

**Dose:**

- **Mice:** Use at range of 125-250mg/kg IP; approximately 5 minutes to induce anesthesia, 15-30 minutes of anesthesia, and 90 minutes to complete recovery.
- **Rats:** Use at maximum of 300mg/kg IP; approximately 5 minutes to induce anesthesia, 15-30 minutes of anesthesia, and 90 minutes to complete recovery.

**E. References**

- AAALAC FAQs Non-Pharmaceutical-Grade Compounds
  [https://www.aaalac.org/accreditation-program/faqs/](https://www.aaalac.org/accreditation-program/faqs/)