Institutional Animal Care and Use Committee		UNTHSC
Title: Avertin Use in Mice		
Document #: 004	**Version #:** 05	
Approved by IACUC Date: January 30, 2025		

A. BACKGROUND INFORMATION

a. Tribromoethanol (Avertin®) in Mice: Tribromoethanol is a popular injectable anesthetic agent used in mice. It was once manufactured specifically for use as an anesthetic under the trade name Avertin®, but this product is no longer available. Investigators who wish to use Tribromoethanol as an anesthetic must make their own solutions.

b. Uses: Tribromoethanol is appropriate for short-term procedures in mice, especially surgical procedures. Avertin ® is best used in situations where it will be given only on a single occasion or for terminal procedures

c. The IACUC must approve the use of Avertin® before it can be used.

B. RESPONSIBILITIES

a. It is the responsibility for the Principal Investigator (PI) at the University of North Texas Health Science Center to follow the procedures set forth below for using Avertin®.

C. PROCEDURES

a. Avertin® use must be described in the protocol and a scientific justification must be provided for using this anesthetic, rather than a pharmaceutical-grade anesthetic (see SOP 011 Use of Non-pharmaceutical Grade Compounds in Animals).

b. Advantages and Disadvantages of Avertin® Use

i. Advantages:

1. Tribromoethanol induces anesthesia rapidly and provides good surgical analgesia for approximately one hour.
2. Since it is given by injection, one is spared the occupational health risks and technical difficulties associated with volatile anesthetics.
3. If used appropriately, Tribromoethanol has a good margin of safety.

ii. Disadvantages:

1. Tribromoethanol is an irritant, especially at high doses, high concentrations, or with repeated use. Adhesions are sometimes seen in the abdominal cavity after IP injections.
2. Tribromoethanol degrades in the presence of heat or light to produce toxic byproducts. Degraded solutions can be both nephrotoxic and hepatotoxic. Administration of degraded Tribromoethanol solutions has been associated with death, often 24 hours after surgery.
3. Tribromoethanol can cause intestinal ileus (stopping of the gut motility and subsequent death of the animal) several weeks after injection. This is more common with Avertin® stored in the presence of light or heat,

stored at higher than recommended doses, or given at higher than recommended concentrations.

4. The effects of Tribromoethanol are also somewhat unpredictable in mice younger than 16 days, or in animals with altered carbohydrate metabolism, such as various mouse strains used for diabetes or obesity models (db/db mice or ob/ob mice).

c. Instructions for Compounding and Use
   i. Chemicals - Two chemicals are necessary to imitate Avertin®:
      1. The first is 2,2,2 Tribromoethanol
      2. The second is amylene hydrate (tertiary amyl alcohol)
      3. Both are obtainable from Aldrich Chemical. There may be other sources as well.
   ii. Compounding – Ingredients:
      1. 2.5 gm 2,2,2 Tribromoethanol
      2. 5 ml 2-methyl-2-butanol (amylene hydrate, tertiary amyl alcohol)
      3. 200 ml distilled water - neutral pH
   iii. Compounding – Instructions:
      1. Dissolve 2.5 grams Tribromoethanol in 5 ml amylene hydrate. This requires heating to approximately 40° Celsius and stirring vigorously.
      2. Add distilled water, stirring continuously, up to a final volume of 200 ml.
      3. Filter sterilize through a Millipore filter (.5 micron).
      4. Aliquot the final solution into appropriate 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 a sterile, red-cap blood collection tube.
      5. 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.
      6. 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 neutral distilled water.)
      7. As prepared above, the solution contains 12.5 mg Tribromoethanol /ml. Do not attempt to make a more concentrated solution - the material is irritating at higher concentrations.
   iv. Dosage – Use:
      1. Mix by stirring or swirling prior to administration.
      2. The material is given by IP injection at a dose of 250 mg/Kg. This amounts to 0.5 ml of the above solution to a 25gm mouse.
      3. Induction requires only 1-2 minutes and the righting reflex returns in approximately 40-90 minutes.

v. Cautions:
    1. 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.
    2. Although some authors report that refrigerated solutions may be kept for months, most authors recommend preparing a new solution every 2 weeks. The IACUC requires replacing refrigerated Avertin® at least every 14 days (after mixing).

D. REFERENCES

a. SOP 011 Use of Non-pharmaceutical Grade Compounds in Animals