

## Guidelines for Use of Avertin

**Tribromoethanol (Avertin) in Mice:** Tribromoethanol is an injectable anesthetic agent used in mice. It was once manufactured specifically for use as an anesthetic by Winthrop Laboratories 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 from a non-pharmaceutical grade chemical. **Please note that this anesthetic cannot be used unless you have described it on your protocol, provided a scientific justification for using this anesthetic rather than a pharmaceutical-grade anesthetic, and received IACUC approval.**

**Uses:** Tribromoethanol is appropriate for short procedures in mice, especially surgical procedures. It's best used in situations where it will be given only on a single occasion. A repeat anesthetic episode can be associated with an increase in morbidity and mortality.

### Disadvantages of Avertin:

- 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.
- 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.
- 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.
- 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).

**Chemicals:** Two chemicals are necessary to imitate Avertin. The first is 2,2,2 Tribromoethanol; the second is amylene hydrate (tertiary amyl alcohol), both obtainable from Aldrich Chemical. There may be other sources as well.

## Compounding:

- Ingredients:
  - 2.5 gm 2,2,2 Tribromoethanol
  - 5 ml 2-methyl-2-butanol (amylene hydrate, tertiary amyl alcohol)
  - 200 ml distilled water - 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, stirring continuously, up to a final volume of 200 ml.
  - Filter through a Millipore filter (.5 micron)
  - 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.
  - 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. Many labs make a fresh batch for each use and even with this added precaution there can still be problems.
  - Tribromoethanol degrades to dibromoacetaldehyde and hydrobromic acid. If the pH of the solution is less than 5, it should be presumed to have degraded.
  - 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.
  - Label the vial with your initials, date prepared and expiration date (in 14 days). Cover with foil and refrigerate.

**Dosage - Use:** Mix by 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 to a 25gm mouse. Induction requires only 1-2 minutes and the righting reflex returns in approximately 40-90 minutes. Surgical anesthesia lasts for 15-45 minutes with a sleep time of 60-120 minutes.

**Cautions:** 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. Replace refrigerated Avertin at least every 14 days.