

Use of Tribromoethanol (Avertin) in Rodents

Tribromoethanol (Avertin) in Mice: Tribromoethanol is an injectable anesthetic agent used in mice. It was once manufactured specifically for use as an under the trade name Avertin, but this product is no longer available. As a result, investigators who wish to use Tribromoethanol as an anesthetic must make their own solutions from a non-pharmaceutical grade chemical.

1. Use of Tribromethanol for rodent anesthesia is not recommended. There are three main reasons:
 - a. Tribromoethanol is no longer available as a pharmaceutical grade substance. According to ARAC and OLAW guidelines, investigators are expected to use pharmaceutical grade substances when possible.
 - b. There are well documented potential side effects of using Tribromoethanol as an anesthetic including:
 - i. Variability of anesthetic effectiveness
 - ii. Peritonitis
 - iii. Nephrotoxicity and hepatotoxicity
 - iv. Ileus
 - v. The breakdown products are irritating to tissues and can cause abdominal adhesions, peritonitis, ileus, and death.¹
 - c. Ketamine combinations are safer alternatives that provide secure and stable anesthesia.
2. If used for survival procedures, use of Tribromoethanol must be justified in the protocol and approved by the ACUC. This justification should include an explanation of why other anesthetics could not be used. Great care must be taken to ensure that the product is made up fresh each month, is sterile, and is stored properly. Recommendations about mixing and storing are provided below, which should be followed and which should minimize the adverse consequences, when Tribromoethanol is used for brief surgical procedures.
3. Use of Tribromoethanol more than once in the same animal is not acceptable. Repeated injections of Tribromoethanol, regardless of dose interval, greatly increase the risk of significant peritonitis, ileus, and death. Note: Surgically altered mice from JAX Labs and other vendors may have received Tribromoethanol.

Mixing, Storage and Dosage Recommendations

I. Materials

- 2,2,2 tribromoethanol (Aldrich T4, 840.2 or equivalent)
- Tert-amyl alcohol (Aldrich 15, 246-3 or equivalent)

II. Stock solution

Add 5 ml T-amyl alcohol to 5 g tribromoethanol, in a dark bottle to make a 100% stock solution. Shake or stir gently until the solid is dissolved. Stock solution is light sensitive and evaporates rapidly. Do not leave the bottle open longer than is necessary. Label, date and refrigerate in tightly sealed, dark bottle. Yellowing of the solution indicates toxic degradation products and the stock must be replaced. (If the original solution's pH was greater than 5, a drop of Congo Red dye can be added to 5 mls anesthetic stock solution to test for decomposition products, which lower the pH. If

the solution turns purple with the addition of the dye, or if crystallization or any other discoloration is noted, the anesthetic should be discarded.) Otherwise, **unused stock solution should be discarded after 6 months.**

III. Working solution

Mix 0.1 ml stock solution with approximately 7.9 ml normal saline, (or PBS), in a glass vessel, (ie. a graduated cylinder wrapped in foil or a dark bottle). Seal container, heat to improve solubility, and mix well by vortexing until dissolved. Filter sterilize through 0.2 micron filter.. Label, date and refrigerate when not in use. **Unused working solution should be discarded after one month.**

IV. Dosage and Anesthetic Effects

The working solution is administered intraperitoneally at 0.4-0.8 ml/mouse, (approximately 0.2ml/10 grams of body weight). (Inadvertent intravenous injection will cause death within minutes.) It will take about five minutes for the mouse to become fully anesthetized, (evidenced by lack of response to toe or tail pinch). An additional 0.05-0.1ml can be given to effect, allowing sufficient time after administering the additional anesthetic to observe the effect. Note that the effective dosage is dependent upon the weight of the mouse. Older, fatter or lactating mice will need a higher dose for complete anesthesia. The mouse will remain anesthetized for approximately 30-60 minutes.

References:

1. Contemporary Topics, Lieggi et al, Vol 44 No. 1 Jan 2005