Appendix: Tribromoethanol (Avertin) Anesthesia in Mice

Tribromoethanol is an injectable anesthetic agent once manufactured specifically for anesthetic use under the trade name Avertin. The manufactured product is no longer available. As a result, investigators who wish to use Tribromoethanol as an anesthetic must make their own solutions from non-pharmaceutical grade chemicals. This fact, coupled with the agent's potential for side effects and the abundance of alternative anesthetics currently available, has led to the recommendation that Tribromoethanol not be routinely used for rodent anesthesia. **If used, it must be justified within an Animal Study Proposal (ASP) and approved by the IC Animal Care and Use Committee (ACUC).**

Tribromoethanol is not generally recommended for rodent anesthesia for the following reasons:

1. 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.
2. There are well documented potential side effects of using Tribromoethanol as an anesthetic including:
    a. Variability of anesthetic effectiveness dependent upon the procedure being conducted
    b. Potential for peritonitis and ileus
    c. Potential for nephrotoxicity and hepatotoxicity
    d. The breakdown products are irritating to tissues and can cause abdominal adhesions, peritonitis, ileus, and death.[1]
    e. Unknown mechanism of action
3. Ketamine combinations are usually[2] safer alternatives that provide secure and stable anesthesia along with an enhanced analgesic component, which tribromoethanol does not provide.

If used for survival or non-survival procedures, use of Tribromoethanol must be justified in the ASP and approved by the IC ACUC. This justification should include an explanation of why other, more appropriate anesthetics cannot be used. The ASP must reference this document or describe the deviation for the preparation, storage, and use of tribromoethanol solution. It is preferred that a working solution of tribromoethanol is prepared fresh for each use, but storage for up to one month is permitted given appropriate storage conditions. Preparation must consider steps to maintain sterility of the final product for injection. Recommendations for mixing and storing are provided below; if these tips are followed, they should minimize adverse consequences, when Tribromoethanol is used for brief surgical procedures.

Use of Tribromoethanol for more than one procedure in the same animal may lead to a higher incidence of side effects. Repeated injections of Tribromoethanol, greatly increase the risk of significant peritonitis, ileus, and death.

Mixing, Storage and Dosage Recommendations:

Only properly trained and qualified individuals should prepare drugs or perform anesthesia. When diluting and mixing chemical compounds, as opposed to purchasing pharmaceuticals prepared under strict and sterile manufacturing practices, careful attention must be paid to precise measuring and aseptic procedures. This will prevent an adverse outcome when the compound is used in animals such as inadequate anesthesia, overdose, toxicity, or infection.

Preparation of Tribromoethanol:

1. A concentrated (100%) **stock solution** of Tribromoethanol is prepared by mixing 5g of 2,2,2-tribromoethanol with 5ml of tertiary amyl alcohol (Sigma, Aldrich). Only use Tribromoethanol powder until the expiration date set by the manufacturer. Shake or stir gently until the solid is dissolved. The stock solution is light sensitive and evaporates rapidly. Do not leave the bottle open longer than is necessary. Label, date, and refrigerate (4°C) in tightly sealed, dark amber or foil covered bottle. Yellowing of the solution indicates toxic degradation products and the stock must be replaced. Solutions with any crystal formation or precipitate must be discarded. ***Unused stock solution refrigerated at 4°C should be discarded after 6 months.*** Alternatively, the stock solution can be made and aliquoted into 1ml snap cap tubes. The tubes containing the stock solution are then stored, wrapped in foil (to protect it from the light,) under an inert gas (nitrogen or argon) at -70°C. ***Stock solution stored frozen at -70°C under inert gas is stable indefinitely.*** Whether refrigerated or frozen, each tube must be labeled with the contents, preparation date, name of the preparer and, if refrigerated, the date of expiration. Stock solutions should be prepared in a fume hood while wearing gloves.
2. A **working solution** of Tribromoethanol is prepared by diluting 0.1ml of the stock solution to 1.25 % in 7.9 ml isotonic saline (PBS), sterilize by filtration (0.2-micron filter) into a sterile injection vial. Label, date, and refrigerate when not in use. NOTE: When diluting the stock alcohol mixture with some commercially available complex phosphate buffered saline solutions, precipitation of the tribromoethyl alcohol may occur. This is due to the presence of calcium and/or magnesium in the PBS. To avoid this, check that the PBS you use is simple sodium phosphate buffered saline (0.9 %) and does not contain calcium and/or magnesium or use the following Tris buffered saline solution (0.9 % sodium chloride, 1mM Tris, pH 7.4, 0.25mM EDTA). If there is crystallization or a change in color of the Tribromoethanol solution, it MUST NOT be used and should be disposed of properly. ***Working solutions are kept in the dark at 4°C and utilized within thirty (30) days of preparation.***

Use of Tribromoethanol:

1. The working solution is administered intraperitoneally at 0.4-0.8ml/mouse, (approximately 0.2ml/10 grams of body weight). Note that inadvertent intravenous injection will cause death within minutes. It will take about five minutes for the mouse to become fully anesthetized. 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 animals, those with more body fat, or lactating animals will need a higher dose for complete anesthesia.
2. Verify anesthesia by confirming a reduced respiratory rate and lack of response to gentle pinching of the footpad (look for a lack of a withdrawal reflex).
3. Perform required surgery within five to ten minutes. The mouse will remain anesthetized for approximately 30-60 minutes. Supplemental dosing is not recommended.
4. After surgery, the mouse should be placed under a heating lamp or on a heated surface to prevent hypothermia. The animal should be observed post- surgically until it returns to consciousness.
5. Monitor the animal daily for several days for alertness, movement, and feeding. If any adverse effects of the surgery are observed, contact the facility or IC veterinarian for assistance or terminate the experiment by the method of euthanasia indicated in the ACUC-approved ASP.

Stability of Tribromoethanol:

1. Tribromoethanol may be transformed into toxic byproducts when exposed to light and temperatures above 4°C. Tribromoethanol properly formulated, filtered, and stored below 4°C in aliquots should assure the suitability of the anesthetic for routine use in mice.
2. Working solutions are kept in the dark at 4°C and utilized within thirty (30) days of preparation. Working solutions must be labeled with the expiration date of the solution.
3. When a bad stock of this compound is used, Tribromoethanol may induce lethality and/or peritoneal damage. If animals do not recover rapidly from the surgery or appear listless or die from non-experimentally induced procedures after several days to a week, then the tribromoethyl alcohol is suspect. In this case, order a fresh bottle and make a new stock of Tribromoethanol.

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