Use of Non-pharmaceutical Grade Compounds in Live Vertebrate Animals

Standard Procedures for Anesthesia Using Tribromoethanol ("Avertin")

Division of Comparative Medicine

Background on Compound

Tribromoethanol is a non-pharmaceutical-grade compound whose use is allowed as an exception to the *ASC Policy on the Use of Pharmaceutical Grade Compounds*. This agent was once commercially available under the brand name "Avertin" in Europe but is no longer in production[1]. The utility of the agent as an anesthetic for mice led people to devise methods for compounding the agent from reagent-grade chemicals [2,3]. There have been some reports of problems associated with TBE use.[4] It seems likely that the majority of these problems have been associated with flaws in the preparation, use, and storage of the agent. Therefore, adherence to the procedures listed below is essential.

The ASC recognizes that alternatives to TBE use are available and are suitable for many applications. However, the long history of effective and reliable use of TBE in mice is deemed sufficient to support its continued use in an appropriate fashion[5,6].

Requirements for Use of Compound

1. TBE use must be described in an ASC protocol and **reviewed and approved** before its use.
2. TBE use is allowed **only in mice**;
3. TBE is to be administered **intraperitoneally (IP)**;
4. TBE must be **prepared, used, stored, and disposed of** as described in DCM Guidelines that follow;
5. TBE can be used for a survival procedure **one time**; repeated use is associated with a greater incidence of peritonitis;
6. TBE can be used for **general anesthesia prior to euthanasia** of mice.

The use of TBE other than as described above requires review of the non-standard use by the ASC prior to implementation.

Preparation of Compound

1. Ingredients:
    - 2,2,2 tribromoethanol (CAS # 75-80-9); beige to white crystalline powder;
    - 2-methyl-2-butanol (CAS # 75-85-4; aka, *tert*-Amyl alcohol); clear, colorless liquid with a strong odor of peppermint or camphor
    - phosphate-buffered saline (PBS).

2. TBE should be prepared in a chemical fume hood by one or two people who are fully trained in the preparation of TBE.
3. Protective laboratory gloves, lab coat, and eye protection are to be worn at all times when preparing TBE.
4. Weigh out appropriate amount (see below) of 2,2,2-tribromoethanol crystal. Empty into a 50 ml sterile conical tube wrapped with aluminum foil.
5. Add appropriate amount (see below) of 2-methyl-2-butanol to the crystal. Swirl it to mix. Warm the tube in a 37°C water bath until the crystal is dissolved (approximately 20 minutes). Make sure all crystals are dissolved. This is the **stock solution** (50% w/v solution or 500 mg/ml).
6. Label the stock solution with name, date of preparation, and a **"use-by" date 6 months** from preparation.

Ingredient	To make 50% stock solution
2,2,2-tribromoethanol crystal	2.5 g
2-methyl-2-butanol	5 ml

7. The stock solution can be frozen at -20°C (protected from light) or diluted immediately to the **working solution** as follows:

Ingredient	To make working solution	
	1.25% working solution (12.5 mg/ml)	2.5% working solution (25 mg/ml)
TBE 50% stock solution from above	5 ml	10 ml
phosphate buffered saline	195 ml	190 ml

8. Mix working solution thoroughly. Filter the working solution through a 0.22 micron filter into a sterile 250 ml bottle.

Storage of Compound

1. Aliquot the **working solution** into sterile 10-15 ml containers. Label and mark working solution containers with expiration date (**4 weeks** from the date of preparation). Store at 4°C.
2. Toxic decomposition products can occur when TBE is improperly manufactured, prepared, and/or stored. In critical applications, it is advisable to anesthetize and recover 1-2 test mice before starting work on study mice. Immediately report any adverse effects to the DCM veterinary staff.

Use of Compound

1. Warm an aliquot of TBE **working solution** to 37˚C and shake well before use.
2. Administer recommended dosage IP to mouse.

TBE working solution concentration	Mouse dose of TBE	
	ml/gm body weight	ml/ 10 gm body weight
1.25%	0.020 ml IP	0.20 ml IP
2.5%	0.010 ml IP	0.10 ml IP

   a. The anesthetic dose for mice (see above) is approximately 250 mg/kg IP.
   b. Males and fat mice need relatively greater doses; young mice need relatively less anesthetic.
   c. TBE should not be used in ICR mice until further characterization is available[6].
   d. Onset of TBE anesthesia -- approximately 5 minutes after IP injection
   e. Duration of TBE anesthesia -- approximately 15-20 minutes of surgical anesthesia.
   f. TBE can be re-dosed, but additional doses should be administered before mouse becomes too "light."
   g. Full recovery from TBE anesthesia will take up to 90 minutes. Do not place an anesthetized mouse in a cage with awake mice. The conscious mice may attack and kill the incapacitated mouse.

3. The most common complication of rodent anesthesia is **hypothermia**. During induction, maintenance, and recovery from anesthesia, mice should be provided with a means to maintain body temperature. This can be accomplished by placing anesthetized mice on a circulating hot-water blanket, slide warmer and/or disposable hand warmers.

Disposal of Compound

Excess or expired TBE should be given to EH&S as unwanted material (details available at : http://ehs.wustl.edu/hmm/Pages/default.aspx).

References

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3. Zeller, W. B. Meier, K. Burki, and B. Panoussis. 1998. Adverse effects of tribromoethanol as used in the production of transgenic mice. Lab Anim. 32:407-413.

4. Buetow, B. S., L. I. Chen, L. Maggio-Price, and K. Swisshelm. 1999. Peritonitis in nude mice in a xenograft study. 38(6):47-49. Source: US National Institutes of Health
5. Lieggi CC, Fortman JD, Kleps RA, Sethi V, Anderson JA, Brown CE, Artwohl JE. 2005. An evaluation of preparation methods and storage conditions of tribromoethanol. Contemp. Top. Lab. Anim. Sci. 44(1):11-16.
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