Tribromoethanol Guidelines (Avertin)

IACUC Guideline
Effective Date: January 2023

UCSF Office of Research
Institutional Animal Care and Use Program

Purpose: The purpose of this document is to describe the necessary information to obtain IACUC approval for the use of non-pharmaceutical grade tribromoethanol (Avertin).

Background: Avertin® was the trade name for the injectable anesthetic tribromoethanol (TBE). Avertin® was once manufactured as a pharmaceutical-grade drug, but it is no longer available as such. The use of non-pharmaceutical grade compounds can present a risk to animal welfare due to concerns over consistency, contamination, or preparation. There are multiple reports in the literature of physiologic harm to animals including ileus, adhesions, and mortality from the use of tribromoethanol. The NIH Office of Laboratory Animal Welfare (OLAW) has advised IACUCs to critically evaluate the proposed use of tribromoethanol and the consideration of alternative methods that avoid or minimize discomfort, distress and pain.

UCSF GUIDELINES
IACUC approval is required to use TBE. Justification for the use of non-pharmaceutical grade TBE must be approved in the IACUC protocol. Scientific justification must be provided for the inability to use alternative pharmaceutical-grade anesthetics such as isoflurane or ketamine-combinations.
The UCSF IACUC recognizes regulatory efforts to strongly justify non-pharmaceutical-grade substances used in animal care and use protocols and does not recommend the use of TBE in rodent studies.
In light of the body of literature detailing serious post-anesthetic effects, inconsistent and variable anesthesia time, effect variability based on rodent strain, and the availability of pharmaceutical grade alternatives (xylazine, ketamine, isoflurane, etc.), the use of TBE in IACUC protocols is limited to that which is scientifically necessary. The goal of this guideline is to reduce risks to animal health and reduce interference that may alter experimental outcomes.

The following are guidelines for possible justifications.

Inadequate justifications:
- Cost savings
- Administrative burden of acquiring and maintaining a Controlled Substances Authorization (CSA)

Generally acceptable justifications:
- A long-term ongoing study where a significant amount of data has been collected with the use of TBE, or a study where known data must be compared with historic data collected using TBE; TBE use may then be continued until the end of these studies
- Known impact on measured outcomes, which is substantiated by data or published reports (see References below for some examples in which TBE's effect on models is compared with that of other anesthetics).

- Unpublished, anecdotal experience on benefits of TBE for the model or detrimental effects of alternatives on the strain or model

Justification that is always acceptable:

- An investigator is specifically studying the effects of TBE.

Additional Considerations: While any TBE use requires justification and standardized procedures of preparation, storage, and use, the committee will be more inclined to approve TBE in terminal procedures, as most documented health and welfare concerns with TBE take hours to weeks to manifest. For single-use, survival procedures, justifications concerning immunology, genetics, cardiovascular studies or others may be considered. Please refer to the UCSF Guidelines on Non-Pharmaceutical Grade Compounds posted on UCSF IACUC website for more information.

Stock Concentration, Working Solution, Dosing:
General recommendations:

- Filter sterilize using a 0.5 micron (or smaller) filter
- Preparation under sterile conditions including the usage of sterile compounds
- Use fresh solutions (< 1week) and lowest dilution
- Container should protect contents from light as solution is light sensitive
- Store wrapped in tin foil the solution under refrigeration at 4oC and in the dark

Stock concentration(1g/ml):

- Add 5 ml Amyl-Alcohol to 5g Tribomethanol powder, invert several times, incubate in 37oC to dissolve thoroughly, wrap in tin foil at 4oC. Can be used up to 1 year.

2.5% working solution:

- Add 0.25 ml stock solution to 9.75ml 0.9% saline, mix well, filter with 0.22um filter cap to remove undissolved particles to a new tube, wrap in tin foil at 4oC. Can be used up to 4 weeks.

IP inject mouse:

- 0.50ml to 25g body weight

UCSF IACUC and NIH policies and guidance:

Non-pharmaceutical grade compounds policy

Anesthesia and Analgesia Mouse Formulary

NIH Office of Laboratory Animal Welfare. (Jerry Collins, PhD). (2012). "Use of Non-pharmaceutical-Grade Chemicals and Other Substances in Research with Animals Webinar".

NIH Office of Laboratory Animal Welfare

Model-specific references comparing tribromoethanol to other anesthetics:

1. Pekny T, Andersson D, Wilhelmsson U, Pekna M, Pekny M. "Short general anaesthesia induces prolonged changes in gene expression in the mouse hippocampus". *Acta Anaesthesiologica Scandinavica*. 2014. 58: 1127-1133. [link] This study shows that some of the effects of short general anesthesia on gene expression in the mouse

hippocampus persist for at least 4 days, and that there are differences between isoflurane's and TBE's effects.

2. Norton W, Scavizzi F, Smith C, Dong W, Raspa M, Parker-Thornburg J. "Refinement for embryo implantation surgery in the mouse; comparison of injectable and inhalant anesthesias – tribromoethanol, ketamine and isoflurane – on pregnancy and pup survival. [link] *Lab Animal*. 2016. 50 (5): 335-43. Based on a direct comparison of pregnancy status, number of pups born, and number of pups weaned for each agent, we found no statistical difference among the three anesthetics (TBE, ketamine, & isoflurane).

3. Pachon R, Scharf B, Vatner D, Vatner S. "Best anesthetics for assessing left ventricular systolic function by echocardiography in mice." [link] *Am J Physiol Heart Circ Physiol*. 2015. 308: H1525-H1529. Ketamine alone exerts the least depressant effects on LV function and heart rate, with Avertin second. Isoflurane and ketamine-xylazine were also evaluated.

4. Sena E, Bart van der Worp H, Howells D, Macleod M. "How can we improve the pre-clinical development of drugs for stroke?" [link] *Trends in Neurosciences*. 2007. 30 (9): 433 -439. Ketamine anesthesia

5. Brown ET, Umino LT, Solessio E, Barlow R. 2005. Anesthesia can cause sustained hyperglycemia in C57/BL6J[sic] mice. Visual Neurosci. 22: 615-8.

6. Kiatchoosakun S, Kirkpatrick D, Hoit Bd. 2001. Effects of tribromoethanol anesthesia on echocardiographic assessment of left ventricular function in mice. Comp Med. 51(1):26-9.

7. Meyer RE, Fish RE. 2005. A review of triboromoethanol anesethesia for production of genetically engineered mice and rats.

8. Gardner, D.J., J.A. Davis, P.J. Weina, et al. 1995. Comparison of tribromoethanol, ketamine/acetylpromazine, Telazol/xylazine, pentobarbital, and methoxyflurane anesthesia in HSD:ICR mice. Lab Anim. Sci. 45:199-204.