**Purpose**

The purpose of this policy is to establish clear guidelines for the use of Tribromoethanol (TBE) (Previously available as Avertin). Under normal circumstances, TBE is not to be used for survival and/or non-survival surgery. **If investigators have a need to use TBE, written scientific justification must be provided for IACUC review and approval.** If justification is approved then the following methods must be followed for TBE use.

Abbreviations

TBE- Tribromoethanol

K-X- Ketamine- Xylazine

**Application:**

Tribromoethanol, also known as "Avertin" is a commonly used anesthetic for surgery. Literature shows that Tribromoethanol has toxic breakdown products that if not stored properly can kill mice. Even when stored properly (or freshly made) it has been reported to cause peritonitis in mice (Zeller 1998) and rats (Reid 1999) and morbidity/mortality in mice (Lieggi et al. 2005a, 2005b). Although this peritonitis is only rarely associated with morbidity or mortality (Lieggi et al., 2005b ; Pappianou 1993), we have to presume that peritonitis is painful and should be avoided when possible. A prior report (Pappiannou 1993) found that Avertin was "safe and effective", but the authors were primarily measuring clinical illness, not histological changes. There can be variability in purity of commercially available TBE powder and there is some evidence that toxicity of administered TBE solution can be associated with storage of TBE powder (Lieggi, et al. 2005a).

**Regulatory Issues**:  
http://iacuc.yale.edu/resources/pixel.gif  
The drug Avertin was long ago discontinued as an anesthetic in the medical and veterinary fields and is no longer commercially available. When using "Avertin" what is actually used is a mixture of non-medical grade ingredients that duplicate Avertin.

USDA policy on Pharmaceutical-Grade Compounds in Research is as follows:

Investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute procedures. Non-pharmaceutical-grade chemical compounds should only be used in animals after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings alone are not an adequate justification for using non-pharmaceutical-grade compounds in regulated animals.

**Guidelines**:  
http://iacuc.yale.edu/resources/pixel.gif  
K-X anesthetic combination given IP or SQ is recommended as an alternative. This dose produces approximately 30 minutes of surgical anesthesia in mice; contact the attending veterinarian and reference the Creighton University Anesthesia and Analgesia Formulary for doses or for alternatives using inhalant or other anesthetics. {In direct comparisons of K-X versus TBE, K-X was reported to cause no peritonitis, and to result in a comparable success rate in embryo transfer. (Zeller 1998) Another study determined that “both TBE and K-X produced adequate anesthesia, although K-X was associated with significantly less inflammation when compared with TBE” (Lieggi et al., 2005b, p. 20).}

**How to Make/Dilute Avertin**

Avertin (2, 2, 2-Tribromoethanol)

Stock Solution (1.6 g/ml):  
25 g Avertin (2, 2, 2-Tribromoethanol) [Sigma-Aldrich, #T4, 840-2]  
15.5 ml tert-amyl alcohol (2-methyl-2-butanol) [Fisher, #A730-1]  
Mix at room temperature for ~12 h in a dark bottle (the bottle that the Avertin comes in works well). Stock solution should be stored protected from light at 5◦C for up to 8 weeks (Lieggi et al., 2005a). If the entire bottle of TBE powder is not used, ensure the lid is tightly closed.

Working Solution (20 mg/ml):  
0.5 ml Avertin stock  
39.5 ml 0.9% saline  
Mix in an airtight dark or foil covered container. Filter the solution though a 0.2 micron filter into a dark or foil covered container and store at 5° C. The working solution should be replaced each month.

**Caution**—Avertin is hygroscopic and subject to photo degradation. The degradation products are lethal. Always store in the dark at 5ºC or prepare fresh before use. Never use a solution that is yellow or contains a precipitate because this indicates that oxidation has occurred. From an evaluation of stored and newly prepared TBE, “the most favorable storage condition was considered to be 5 ◦C in the dark” and “monitoring of pH does not necessarily correlate to the presence of toxic components in or the potential lethality of a TBE working solution” (Lieggi et al. , 2005a, p. 21)

**Usage:**  
Dosage is 250-500 mg/kg (0.25-0.5 mg/g) given intraperitoneally (IP) in mice. Avertin is lipid soluble so fat mice may require a larger dose.

Similar to the use of any anesthetic, an animal should not respond when the footpad is pinched between the thumb and forefinger. A reflex response would indicate that the animal has not been given sufficient drug. If three animals have a reflex response to Avertin when administered an appropriate dose based on weight, it would suggest that the solution had been made inappropriately or is no longer effective and should be discarded.

Refer to Creighton University IACUC policy on the Use of Secondary Containers for labeling purposes.

**References**:

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