

Policies and Procedures

SECTION: IACUC	NUMBER: 7.08			
CHAPTER: Miscellaneous Experimental Animal Use Policies	ISSUED: 12/2007	REV. A: 4/7/2009	REV. B: 11/2012	REV. C: 7/29/2013
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Purpose

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

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). Although this peritonitis is only rarely associated with morbidity or mortality (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.

Regulatory Issues:

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:

100 mg/kg Ketamine and 10 mg/kg Xylazine IP or SQ is recommended as an alternative. This dose produces approximately 30 minutes of surgical anesthesia in mice; contact the attending veterinarian for doses for other species, or for alternatives

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using inhalant or other anesthetics. {In direct comparisons of Ketamine-Xylazine versus tribromoethanol, Ketamine-Xylazine was reported to cause no peritonitis, and to result in a comparable success rate in embryo transfer. (Zeller 1998)}

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 can be stored at room temperature for up to one year.

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 through a 0.2 micron filter into a dark or foil covered container and store at 4° 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 4°C or prepare fresh before use. Never use a solution that is yellow or contains a precipitate because this indicates that oxidation has occurred.

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.

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