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Title: Avertin Preparation and Use

I. Purpose:

Tribromoethanol is a popular injectable anesthetic agent used in mice. It was once manufactured specifically for use as an anesthetic by Winthrop Laboratories under the trade name Avertin®, but this product is no longer available. Investigators who wish to use tribromoethanol as an anesthetic must make their own solutions. This document provides guidance on the accepted method of preparation and use of the substance.

II. Policy:

Avertin may be approved by the IACUC for use as an anesthetic on a protocol by protocol basis.

It must be prepared, stored , used and discarded in accordance with the below procedures.

In addition Investigators must be in compliance with the IACUC policy regarding the use of non pharmaceutical grade drugs, justification for use is documented in the animal care and use protocol. <http://safetyservices.ucdavis.edu/article/use-non-pharmaceutical-grade-compounds-animals>

III. Procedure:

Uses:

Tribromoethanol is appropriate for short term procedures in mice, it is used most commonly by experienced personnel performing embryo transplants in transgenic colonies. Only one injection of Tribromoethanol may be given to an animal for one survival procedure. If a mouse is not adequately anesthetized by the initial injection, the animal must not be used and allowed to recover from anesthesia. If a second injection of Tribromoethanol is given to the animal, the procedure must be terminal.

Advantages:

Tribromoethanol induces anesthesia rapidly and provides adequate surgical analgesia for up to 30 minutes. Since it is given by injection, one is spared the occupational health risks and technical difficulties associated with volatile anesthetics. If used appropriately, tribromoethanol has a good margin of safety.

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Disadvantages:

Only one injection may be administered to a mouse. If for any reason a mouse receives a second injection of Tribromoethanol, the mouse must be euthanized prior to the animal recovering from anesthesia. Use of this anesthetic requires the surgeon to be experienced. Inexperienced surgeons should be trained to master the surgical technique prior to performing the technique on mice anesthetized with Tribromoethanol since inexperienced surgeons may take longer to perform the surgery. Tribromoethanol is an irritant, especially at high doses, high concentrations, or with repeated use. Adhesions are sometimes seen in the abdominal cavity after IP injections.

Tribromoethanol degrades in the presence of heat or light to produce toxic byproducts. Degraded solutions can be both nephrotoxic and hepatotoxic. Administration of degraded tribromoethanol solutions has been associated with death. Another sequella, thought to be associated with degraded solutions, higher than recommended doses, or higher than recommended concentrations, is the development of intestinal ileus.

The effects of tribromoethanol are also somewhat unpredictable in mice younger than 16 days, or in animals with altered carbohydrate metabolism, such as various mouse strains used for diabetes or obesity models (db/db mice or ob/ob mice).

IV. Procedure:

Chemicals:

Two chemicals are necessary to formulate Avertin. The first is 2,2,2 Tribromoethanol; the second is amylene hydrate (tertiary amyl alcohol), both obtainable from Aldrich Chemical. There may be other sources as well.

100% Stock preps

The following documentation must be provided for stock preps:

2,2,2, Tribromoethanol Date received _____ Exp Date: _____

Tertiary amyl Alcohol Date received _____ Exp Date: _____

Using 10ml glass serological pipette, add 10ml tertiary amyl alcohol (Batch # _____) to newly opened 10 g bottle of 2,2,2, Tribromoethanol (Batch # _____)

Add stir bar, place on the magnetic stirrer and let dissolve for at least 2 hours at room temperature. (Elapsed time of stirring (HH:MM): _____)

Date prepared: _____ Lot #: _____ Expiration Date: _____ (6 months maximum)

(Fischer Scientifics' Tribromoethanol's powder has a shelf life up to 5 years)

Determine pH of the stock solution. When using pH paper to test the pH it is not recommend to insert the paper directly into vial of the solution as this may contaminate the solution. **Acceptable pH is >5.0; pH of < 5 is considered toxic and solution should be discarded. Do not attempt to adjust pH if not initially correct.**

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Keep container wrapped in foil. Label container with the following information: "100% Avertin", prep date, Lot #, expiration date (6 months maximum), pH, and initials of preparer. Store at 4°C.

1.2% working solution

Use 100% Avertin stock (see above) and sterile water

Determine appropriate volume of components using this table:

Final Volume	20ml	40ml	50ml	60ml	80ml	100 ml
100% Stock (ml)	0.240	0.480	0.600	0.720	0.960	1.20
Sterile water (ml)	19.76	39.52	49.40	59.28	79.04	98.8

1. Check the pH of the 100% stock Avertin prior to making a working solution. Stock prep pH must be >5.0. Stock prep pH <5.0 must be discarded.
2. Measure appropriate volume of sterile water into sterile, glass bottle using pipette and protect from light.
3. Add stir bar and begin stirring volume using pipette, add appropriate volume of 100% Avertin to volume of sterile water
4. Stir until completely dissolved. Determine pH of the stock solution. Working solution pH must be 7.0-7.4.
5. Filter sterilize using a 0.2 micron filter into fresh, sterile glass bottle wrapped in foil at 4°C.
6. Label with "1.2% Avertin", prep date, Lot #, pH, and expiration date (**2 weeks maximum** from preparation date)
7. Refrigerate the aliquots and protect them from light. The material degrades rapidly in the presence of heat or light. Even refrigerated and wrapped in foil, the material is stable for only about two weeks. If the material degrades, it becomes toxic.
- 8. pH test working solution prior to each use**

Dosage - Use:

Induction requires only 1-2 minutes and the righting reflex returns in approximately 40-90 minutes.

Use the following chart for dosing:

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Weight (g)	1.2% Avertin (ml)	Weight (g)	1.2% Avertin (ml)
20	0.62 – 0.70	30	0.94 – 1.06
20.5	0.65 – 0.73	30.5	0.96 – 1.08
21	0.67 – 0.75	31	0.98 – 1.10
21.5	0.67 – 0.77	31.5	0.98 – 1.12
22	0.69 – 0.77	32	1.00 – 1.12
22.5	0.71 – 0.79	32.5	1.02 – 1.14
23	0.73 – 0.81	33	1.04 – 1.17
23.5	0.73 – 0.83	33.5	1.04 – 1.19
24	0.75 – 0.85	34	1.06 – 1.21
24.5	0.77 – 0.87	34.5	1.08 – 1.23
25	0.79 – 0.89	35	1.10 – 1.25
25.5	0.79 – 0.89	35.5	1.10 – 1.25
26	0.81 – 0.92	36	1.12 – 1.27
26.5	0.83 – 0.94	36.5	1.14 – 1.29
27	0.83 – 0.96	37	1.17 – 1.31
27.5	0.85 – 0.98	37.5	1.17 – 1.33
28	0.87 – 1.00	38	1.19 – 1.35
28.5	0.89 – 1.02	38.5	1.21 – 1.37
29	0.92 – 1.02	39	1.23 – 1.37
29.5	0.92 – 1.04	39.5	1.25 – 1.39
		40	1.25 – 1.41

Cautions:

Do not administer non-sterile solutions, outdated solutions, more concentrated solutions, or higher doses than recommended above. Store the solution under refrigeration and in the dark. Containers should be wrapped in foil.

Summary of expiration dates:

Tribromoethanol powder = 5 years from received date

100% Stock solution = 6 months from the date of preparation

1.2% working solution = 2 weeks from the date of preparation