

Policies and Procedures

SECTION: IACUC	NUMBER: 7.06			
CHAPTER: Miscellaneous Experimental Animal Use Policies	ISSUED: 11/2007	REV. A: 4/7/2009	REV. B: 11/2012	REV. C: 08/2016
POLICY: Volatile Anesthetics	REV. D: 04/2019	REV. E: 08/2019	PAGE 1 OF 3	

Application

The use of variable by-pass precision vaporizers for accurate delivery of volatile anesthetics is a recognized method of delivering anesthetic gases for certain applications:

- Anesthetic gases may be delivered via a properly placed endotracheal tube or laryngeal cuff;
- Anesthetic gases may be delivered via an appropriately sized nose-cone device; and
- Precision vaporizers must be calibrated annually by a certified technician. A sticker with the last certification date must be placed on the vaporizer.

Rodent Anesthesia Using Open- Drop Exposure or Nose Cone exposure to Isoflurane

Purpose: To anesthetize mice or rats for brief non-surgical or minor surgical procedures where using a precision vaporizer is impractical. The Longer Procedure with nose cone may be used when performing transcardial perfusion.

Materials:

- Cotton pads (cotton ball, gauze pad), this should be replaced between each animal, as reusing the cotton pad will cause the isoflurane to be further diluted by propylene glycol and result in inadequate and unpredictable anesthesia
- Clear sanitizable, non-porous container of known volume with secure lid. Should be large enough to comfortably accommodate animal. Must allow constant visualization of the animal.
- Wire mesh that fits bottom of container, or alternate method to prevent animal from contacting isoflurane-soaked cotton pad.
- Mixture of 20% v/v isoflurane in propylene glycol (Mice)*
- Mixture of 30% v/v isoflurane in propylene glycol (Rats)*

*Note: Use of undiluted isoflurane is not permitted, as the vapor pressure of isoflurane can lead to lethal accumulations of anesthetic in the vapor phase. **Undiluted isoflurane may be used for terminal procedures or euthanasia. Use of undiluted isoflurane for survival procedures must be justified and approved by the IACUC.**

Important Safety Information: Because of risks to human health the use of the open drop method is not allowed on open bench tops. These procedures may only be performed in a certified ducted chemical fume hood, a type B2 biosafety cabinet or a Class II Type C1 biosafety cabinet. These hoods must be approved by OEHS. **This method cannot be used in animals housing rooms.** It can be used in the Class II Type C1 biosafety cabinet located in the Procedure Room 613 in the ARF. For questions or training please contact the Attending Veterinarian.

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Brief Procedures:

- Under an approved hood, wearing appropriate PPE, wet the cotton pad with the diluted isoflurane at a rate of 1 ml/500cc volume of container.
- Place gauze in container under the wire mesh or perforated floor or using an alternate method to prevent animal from contacting the isoflurane soaked cotton pad.
- Transfer the animal to the container and tightly close the lid. Monitor the animal closely. A deep plane of anesthesia is indicated by a lack of the righting reflex when the jar is tipped slightly and the respiratory rate should be about 80-100 breaths/minute. This should take approximately 1 minute for mice and 2 minutes for rats.
- Quickly remove animal from container and place on clean work surface. Replace lid on jar immediately.
- Apply a toe pinch to the animal to ensure adequate plane of anesthesia. If no response is noted, proceed with procedure. If animal responds, return it to the jar and monitor respiratory rate and righting reflex as previously described.

Longer Procedures:

- To maintain anesthesia for a longer period of time (up to ~8 minutes) a nose cone can be constructed using a 15 ml conical tube for a mouse and a 50 ml conical tube for rats. Place a small piece of gauze moistened with the isoflurane mixture inside the tube so it will not come into direct contact with the animal. (30 ul for 15 ml cone, 100ul for 50 ml cone)
- Anesthetize the animal as described in the Brief Procedure section, when the animal has reached the appropriate anesthetic plane, remove it from the container and place the animal's nose at the edge of the nose cone- DO NOT create a complete seal. Adjust the depth of anesthesia by moving the nostrils/nose closer or farther away from the end of the cone. Moisten the animal's eyes with artificial tears.
- Closely monitor the anesthetic depth of the animal by applying toe pinch and monitoring respiratory rate.

Clean up/Waste Disposal

Wash induction container and nose cone with warm soapy water after use. Dispose of remaining isoflurane/propylene glycol mixture and used cotton pads into appropriate tightly closed

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container dedicated to waste isoflurane and labelled as hazardous waste. This will be disposed of by EH&S.

*** Isoflurane is considered non pharmaceutical grade once diluted.** For IACUC protocols, please check the non pharmaceutical grade box in the appropriate sections (E2 (if using for euthanasia and terminal procedures) or E3.) Please include the volume of isoflurane and volume of container and/or nose cone in the dosage/route column, be sure this is at a 1 ml/500cc ratio. In section E6 please include the following information.

Rationale: Isoflurane- the use of undiluted isoflurane can lead to lethal accumulations of anesthetic in the vapor phase.

Source/purity: Please complete with your source of pharmaceutical grade isoflurane and USP grade propylene glycol

Formulation: Under an approved hood, wearing appropriate PPE, Isoflurane will be mixed with propylene glycol at a ratio of 1:4 for mice and 3:7 for rats in a glass container with a secure lid. This container must be labelled with the correct name, date of preparation and original expiration date of isoflurane. This mixture must be made immediately prior to use, excess discarded in an appropriate tightly closed container dedicated to waste isoflurane and labelled as hazardous waste to be disposed of by EH&S.

References:

1. Itah et al. 2004. A replacement for methoxyflurane (Metofane) in open-circuit anaesthesia. Lab. Anim. 38:280-5.
2. Risling et al. 2012. Open-dr