Open-drop and/or Nose Cone Method of Isoflurane Anesthesia in Mice and Rats.

University of Mississippi Institutional Animal Care and Use Committee

Purpose: This procedure details how to use isoflurane to anesthetize rodents. Isoflurane is an inhalant anesthetic designed for use with a precision vaporizer. The Open-Drop method should be reserved for instances where short duration anesthesia is desired, or it is impractical to use a precision vaporizer.

The open-drop method of isoflurane exposure can be used to anesthetize mice and rats for brief non-surgical procedures. To maintain anesthesia for 5-10 minutes duration, a simple nose cone can be constructed from a conical tube.

Note that even when diluted, this method does not allow for more than crude control of the concentration of the anesthetic and therefore the potential for inadvertent overdose with this method is significantly greater than when a precision vaporizer is used. Mice and rats need to be watched very closely and observed during induction to avoid anesthetic overdose and death. Precision vaporizers provide a much safer and more reliable method of inducing isoflurane anesthesia. The open-drop and nose cone procedures are not acceptable for major surgical procedures or procedures requiring greater than 10 minutes of surgical anesthesia or where the use of a precision vaporizer is required to maintain an adequate and safe level of anesthesia.

Important Safety Information: The Open-drop and/or Nose Cone method must only be performed in a fume hood, portable ductless hoods like a Snorkel with a charcoal filter, or on a surgical downdraft table. This procedure CANNOT be performed in Animal Housing Rooms.

Materials:

- Fume hood, surgical downdraft table, or portable ductless hood with charcoal filter.
- Cotton ball or gauze.
- Container with known volume and tightly fitting lid (Open-Drop procedure)
- Wire mesh to fit in bottom of container.
- Isoflurane
- Recovery cage with supplemental heat.
- Conical tube for anesthesia maintenance (15ml for mice/50ml for rats)

Open-Drop Procedure: (For brief procedures): Mice will remain deeply anesthetized for approximately 30 seconds and rats for one minute. This method can be used for retro-orbital blood sampling, tail biopsies and similar rapid procedures. To maintain longer anesthetic times, see Nose Cone Procedure.

Concentration of Isoflurane (%)

% isoflurane	Internal Volume of Anesthetic Chamber				
	1L	2L	3L	4L	5L
1	0.05	0.10	0.15	0.20	0.25
2	0.10	0.20	0.30	0.40	0.50
3	0.15	0.30	.045	0.60	0.75
4	0.20	0.40	0.60	0.80	1.00
5	0.26	0.51	0.77	1.02	1.28

Volumes in the shaded area are in mL and indicate the volume of isoflurane to be applied to a cotton swab or gauze in the bell jar.

From: "*Anesthesia and Analgesia in Laboratory Animals*" 2nd edition pg. 86

1. Put on gloves. Open Isoflurane bottle in an approved hood. Using the chart above, wet a cotton ball or gauze with the appropriate amount of Isoflurane.
2. Place cotton ball inside a small container under a wire mesh. The mesh ensures that the animal does not contact the isoflurane-soaked cotton. Isoflurane can cause skin irritation and potential overdosing since isoflurane is also absorbed through skin.
3. Place the animal in anesthesia container and close lid tightly. Monitor the animal closely. A deep plane of anesthesia is indicated by lack of a righting reflex when the jar is tipped slightly, and respiratory rate is reduced by 50% from pre-anesthetic rate (i.e., 80-100 breaths/min). This should take roughly 1 minute for mice and roughly 2 minutes for rats.
4. Allow the animal to remain in deep anesthesia for roughly 10 seconds before proceeding. Remove animal from the anesthesia container and replace lid immediately.
5. To ensure an adequate plane of anesthesia, use the toe pinch method to test withdrawal reflexes before proceeding. If the animal responds to the toe pinch, return it to the anesthesia container for roughly an additional 10 seconds. Remove the animal and repeat toe pinch test.

Nose Cone Procedure: This method can be used with the Open Drop Procedure above to maintain anesthesia for up to 10 minutes duration and is appropriate for minor surgical procedures, such as subcutaneous tumor implantation. Procedures lasting longer than 10 minutes should be performed using a precision vaporizer system.

1. In a fume hood, downdraft table, or portable ductless hood with charcoal filter, slightly moisten the end of a small piece of cotton/gauze with isoflurane. Insert the cotton/gauze into a conical tube (15ml for mice/50ml for rats) with moistened end facing away from the open end of the tube. Cap the conical tube until ready to use.
2. Anesthetize the animal as described in the Open-Drop Procedure above.
3. Still working in a hood etc., place the animal's muzzle at the edge of the conical tube. Check depth of anesthesia with toe pinch method described in sept 5 of the Open-Drop procedure. Adjust the depth of anesthesia by moving the tube closer or further from the muzzle of the animal (figure 1). Do not create a complete seal around the muzzle, allow for some space on either side (figure 2).

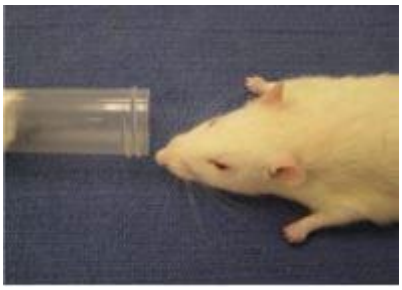

Figure 1.

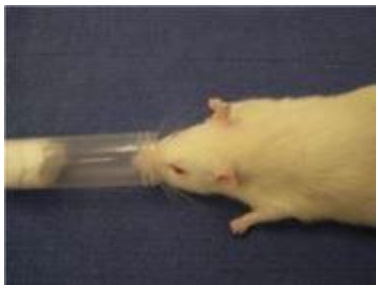

Figure 2.

4. If the animal does not respond to toe pinch, begin the procedure.
5. Allow the animal to recover on a piece of clean paper towel in a bedding-free cage to prevent aspiration injury or death. Monitor the animal closely until it can maintain sternal recumbency. Return the animal to the home cage.
6. Air dry isoflurane exposed cotton/gauze and conical tube in the hood for 15 minutes and discard by wrapping in a glove and transferring to a biomedical waste disposal container.