

Rodent Anesthesia using Open-Drop Exposure to Isoflurane

Recommended Best Practices

Purpose: To anesthetize mice and rats for brief non-surgical or surgical procedures using open-drop exposure to isoflurane inhalant anesthesia.

Materials:

Cotton pad (i.e. nestlet or gauze pad)
Bell jar or other container (with known volume)*
Wire mesh to fit in bottom of glass container
Mixture of 20%v/v isoflurane** in propylene glycol (for mice)
Mixture of 30% v/v isoflurane** in propylene glycol (for rats)
OEHS-approved hood

* Any type of container with a secure lid may be used, provided it is constructed of non-porous material that is sanitizable and allows for constant visualization of the animal. The jar should be of sufficient size to comfortably accommodate the animal, but not so large as to require excessive anesthetic.

** Isoflurane is available from Veterinary Clinical Service or any major veterinary supply vendor.

Important Safety Consideration: *Because of risks to human health, the use of inhalant anesthetics is not allowed on open bench tops. The following procedures must only be performed in a certified ducted hood, biosafety cabinet or portable ductless hood equipped with a charcoal filter. OEHS approval is required for all other hood options.*

Optional Materials:

To maintain anesthesia for up to 8 minutes duration, a simple nose cone can be constructed from a 3cc syringe (for mice; larger syringe for rats), with the plunger removed. A pre-cut section of gauze is also required for the nose cone.

Note: There are strain and species differences in the response to isoflurane: hypertensive rats (SHR, WKY) are more sensitive than normotensive (SD) rats. Also, transient post-operative immune suppression has been noted in mice following use of isoflurane (Markovic and Murasko, 1993).

Procedure A (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 Procedure B.*

1. Don gloves. Open bottle containing appropriate isoflurane/propylene glycol mixture in an approved hood. Wet a cotton pad (e.g., nestlet, gauze) with an exact amount of isoflurane mixture. A 1.0 cc volume of appropriate mixture should be used for every 500 cc volume of the anesthesia jar.

Note: Use of *undiluted* isoflurane is not acceptable, as the vapor pressure will lead to *lethal accumulations* of anesthetic in the vapor phase.

2. Place the cotton pad inside a small container under a wire mesh. The use of the mesh ensures that the animal does not contact the isoflurane-soaked pad, which can cause skin irritation and potential overdosing since isoflurane is also absorbed through skin.

3. Transfer animal to anesthesia jar and close lid tightly. Monitor animal closely. Within approximately one minute for mice and 2 minutes for rats, the animal will become anesthetized. Initially, respiratory rate will increase and then decrease. Clinical indications of a deep plane of anesthesia in rodents include the lack of a righting reflex (upon tipping jar gently) and a 50% reduction in respiratory rate compared to pre-anesthesia levels (ie. to ~80-100 breaths per minute).
4. Allow the animal to remain at a deep anesthetic plane for ~10 seconds before proceeding. Quickly, yet carefully, remove the animal from the jar and place it on a clean work surface. Replace the lid on the jar immediately.
5. Apply a noxious stimulus (ie. toe pinch) to ensure adequate plane of anesthesia. If no response is noted, the procedure can be initiated. If the animal responds to noxious stimulus, return it to the jar and monitor respiratory rate as in step #4.
6. For retro-orbital blood sampling: if the animal reaches a lighter plane of anesthesia, evidenced by increased respiratory rate, whisker twitch, or purposeful movement, stop the procedure and apply pressure to the eye to control any bleeding. Transfer the animal back to the bell jar, until the animal again reaches a deep plane of anesthesia. Proceed with step #4.

Procedure B (for more prolonged anesthesia): This method uses an isoflurane mixture/nose cone system and can provide at least 8 minutes of deep anesthesia, appropriate for minor surgical procedures, such as an Alzet pump placement or subcutaneous tumor implantation. Procedures lasting longer than 8 minutes should be performed using a precision vaporizer system.

Slightly moisten the end of a small piece of gauze with the isoflurane mixture. Insert the gauze into syringe nose cone of appropriate size, with moistened end away from open end of the nose cone. Place prepared nose cone into anesthesia jar, until ready to use. Anesthetize the animal as described in steps #1-4. Also retrieve the nose cone from the jar, and still working in the hood, place the animal's muzzle at the edge of the nose cone. Moisten the animal's eyes with Paralybe. Check the depth of anesthesia as described as step #5, and if appropriate, begin to perform the procedure. The depth of anesthesia can be adjusted by moving the nostrils closer to or further from the end of the cone. Care must be taken not to create a complete seal around the muzzle.

7. Allow the animal to recover on a piece of clean paper towel in a bedding-free recovery cage to prevent aspiration injury or death. Monitor the animal closely until it can maintain sternal recumbancy, then transfer to the home cage.
8. Proceed to anesthetize the next animal, as described above. If the animal does not reach a surgical depth of anesthesia, remove used isoflurane/cotton pad and replace with a fresh pad. Proceed from step 3.
9. Air dry used cotton pad(s) inside the anesthesia jar in hood for 15 minutes, and then discard them by wrapping in a glove and transferring to a biomedical waste disposal bag or bucket.

For questions or training, please contact Regulatory & Safety Services at:
regulatory.services@yale.edu

Approved by VCS; Regulatory and Safety Services, 11/16/04

References:

Itah et al. 2004. A replacement for methoxyflurane (Metofane) in open-circuit anesthesia. *Lab.Anim.* 38:280-5.

Markovic SN, Murasko DM. 1993. Anesthesia inhibits interferon-induced natural killer cell cytotoxicity via induction of CD8+ suppressor cells. *Cell Immunol.* 151:474-80.