Guidelines for the Use of Isoflurane

Introduction
Isoflurane is a volatile halogenated anesthetic agent frequently used in animal research. It is a clear, colorless, volatile liquid at room temperature and pressure. It has an odor that has been described as ether-like or sweet. Exposure to isoflurane waste anesthetic gases has been associated with several health concerns and can occur when vapors escape into the work environment during the administration of anesthesia.

Health Effects
Acute effects – Headaches, dizziness, lightheadedness, nausea, fatigue irritability, depression.
Chronic effects – Liver and kidney disease.
Reproductive effects – Some halogenated anesthetics have been associated with birth defects or miscarriages.

Minimize Exposure
The NIOSH Recommended Exposure Limit for isoflurane is 2 parts per million (ppm). If you can smell isoflurane you are being over exposed. Email labsafety@uthsc.edu for assistance with exposure assessment.

Active Scavenging (Preferred): Whenever possible work in a chemical fume hood or hard-ducted Class II B2 Biological Safety Cabinet. Other ducted local exhaust ventilation systems such as a downdraft table or point ventilation (e.g. snorkel) may also be useful. Ductless fume hoods that rely on activated charcoal filters also help to minimize isoflurane exposure.

Passive Scavenging (Less Effective): When a fume hood or other local exhaust ventilation is not available charcoal canisters must be used to scavenge waste isoflurane vapors. These devices must be properly maintained in order to be effective. Follow manufacturer instructions as printed on the label. Weigh the canister before and after each use and replace when the weight reaches maximum capacity.

Anesthetic Equipment Maintenance:
Anesthesia machines must be maintained annually or as directed by the manufacturer. Fittings, tubing and connection must be checked routinely with seals and damaged components replaced as necessary. A refrigerant leak detector or soap bubble test should be periodically used to check for gas leaks.

Checklist for Working with Isoflurane
There are many types of anesthesia machines on campus. Ensure personnel have been trained to use the specific piece of equipment they will be using. Review and understand manufacturer’s instructions prior to use.

- Verify equipment is currently certified and in working condition.
- Fill vaporized with isoflurane either in a fume hood or using an anti-spill bottle adaptor. Wear chemical resistant (e.g. nitrile) gloves, lab coat and eye protection while filling.
- Use a certified local exhaust ventilation system, preferably a fume hood or ducted B2 biological safety cabinet to remove waste anesthetic gas.
- Avoid high concentrations (>4%) of isoflurane for induction or for prolonged periods. Turn off the vaporizer when not administering anesthetic to animals.
- Close the induction chamber lid during anesthetic gas delivery. If possible, use a sliding top chamber, purge the chamber with oxygen for 3-5 seconds before opening and stand as far back as possible to keep waste anesthetic gas from entering your breathing zone.
- Use a coaxial nose cone. Select the best-fitting nose cone with a tight-fitting diaphragm to minimize leakage.
- Exhaust waste anesthetic gas to either a fume hood, ducted Class 2 B2 biological Safety cabinet or properly weighed and maintained charcoal canister.
- During stereotaxic procedures waste anesthetic gas can escape below the animal’s head. Use a downdraft table, perform in a Class II B2 biological safety cabinet or obtain a device specially made for this procedure.

**One-Drop or Bell Jar Method**

This procedure must not be performed on an open bench top. It must take place in a fume hood or suitable ventilated enclosure. It should be reserved for instances when only a short duration of anesthesia is required – typically 30 seconds for mice or 60 seconds for a rat. Eye bleeds or tail biopsies are examples of such procedures. Follow the standard operating procedure established for your protocol. Wet a cotton pad or comparable material with 1.0 cc of isoflurane/propylene glycol mixture per 500 cc volume of the anesthesia jar. Place the cotton pad inside the container under a wire mesh or perforated floor. 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. 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. Indications of a deep plane of anesthesia in rodents include the lack of a righting reflex upon gently tipping the jar and a 50% reduction in respiratory rate compared to pre-anesthesia levels. 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. Apply a stimulus such as a toe pinch to ensure adequate plane of anesthesia. If no response is noted, the procedure can be initiated. If the animal responds to the stimulus, return it to the jar and monitor respiratory rate for signs of deep anesthesia.

**Spills and Waste**

**Spills** – Do not attempt to clean isoflurane spills. Evacuate personnel and allow anesthetic to evaporate. Call Research Safety Affairs (8-6114) for assistance with large spills of two or more bottles.
**Waste** – Unused, expired or unwanted isoflurane or stock solutions should be collected as hazardous waste and Campus Safety contacted for waste pickup. Email labsafety@uthsc.edu for pickup. Spent charcoal canisters can be discarded in the conventional trash.