Isoflurane Safety Guidelines

1. Background

Isoflurane is an anesthetic gas that is widely used at MIT for rodent anesthesia. Isoflurane is used because of the rapid recovery of the animal after surgery and the high survival rate. There are different set-ups in laboratories that potentially will expose researchers and employees to isoflurane. This Standard Operating Guideline (SOG) will provide all necessary information in order to protect lab members who administer isoflurane in laboratories and imaging facilities at MIT.

2. Scope

- Researchers or employees who work with isoflurane for animal anesthesia
- Researchers who do the surgery using isoflurane
- Department of Comparative Medicine (DCM) laboratories
- Operators of imaging equipment

3. Health Effects

Isoflurane is a general inhalation anesthetic gas that is widely used in animal research laboratories, imaging facilities, and rodent surgeries. Isoflurane is a halogenated anesthetic gas and one of the most commonly used inhalation anesthetics in experimental and veterinary animal procedures.

Isoflurane is a clear, colorless, stable liquid with no additives or chemical stabilizers. Isoflurane has a mildly pungent, musty, ethereal odor. Surgical levels of anesthesia, 1.5-3.5%, may be required when isoflurane is given mixed with oxygen. Short term symptoms (acute exposure) for isoflurane include headaches, dizziness, fatigue, temporary blurring of vision, and nausea. Isoflurane is an irritant agent for skin and eyes, and it's also toxic to the central nervous system.

Chronic health effects (long term health effects) include headache, nausea, fatigue, reduced mental performance, malignant hyperthermia in some individuals, hypotension, hepatotoxicity, and a slight increase in risk for miscarriages, liver, and kidney disease, and possible reproductive effects. To date, studies do not show that chronic exposure to isoflurane is carcinogenic (National Institute of Health (NIH)).

4. Exposure Limits

In 2022, the threshold limit value (TLV or exposure limit) of 50 parts per million (ppm) as an 8-hour time weighted average (TWA) for isoflurane was published by ACGIH (American Conference of Governmental Industrial Hygienists).

5. Exposure Assessment

For an isoflurane exposure assessment, the TVOC activated charcoal badges are usually used (Image 1). They are used as a passive sampling method and adsorbed isoflurane will be reported as TWA for 8 hours. The MIRAN (infrared spectrophotometer) device could be used as a real-time monitoring device (Image 2).

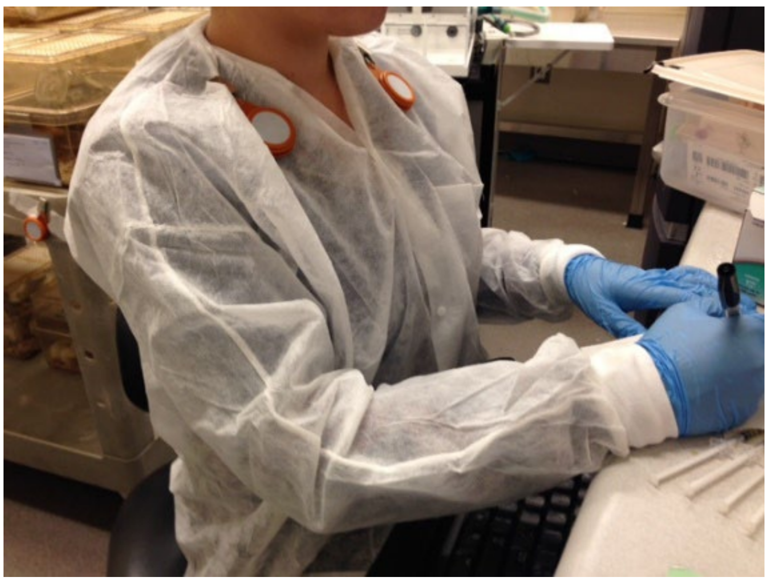

Image 1: TVOC badge for personal sampling

Image 2: MIRAN (infrared spectrophotometer)

6. Waste Gas Scavenging

The waste gas scavenging is usually used when anesthetic gas use cannot be completed using local ventilation. When connecting into local ventilation is not possible, the following solutions may be utilized:

6.1 Active Scavenging Systems:

Active scavenging chambers consist of an isoflurane vaporizer connected to 1-4 chambers (see Images 3-4 below). The system has a carbon filter unit and an air pump that pulls the contaminated air above the chamber's sliding lids. It actively extracts waste gas to a charcoal filter purification unit and discharges clean air into the lab. Be aware that carbon filter chambers are not running in 100% efficiency and may release isoflurane in the room, so dilution ventilation of the room will be necessary at all times. New imaging equipment is often equipped with active scavenging pumps or exhaust vents (see Image 5 below).

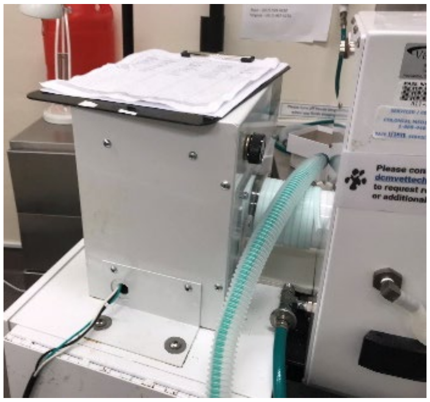

Image 3: Induction chamber with active scavenging system on back

Image 4: Induction chamber chambers connected to active scavenging system

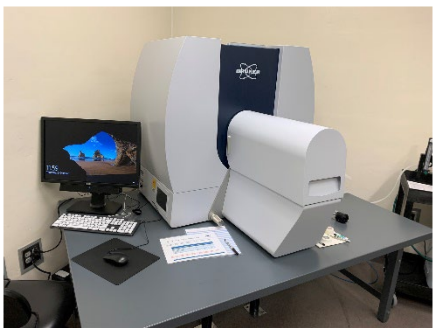

Image 5: MicroCT equipment with built-in active scavenging system

Image 6: Induction chamber connected to a pump

There is also equipment developed by VetEquip which can actively scavenge the waste gas from the induction chamber (see Image 6 above from http://www.vetequip.com/item.asp?cat=14&catalogID=901820).

6.2 Intermediate system:
Using the house vacuum system connected to the nose cone. This isn't recommended because there is not enough airflow to capture waste isoflurane sufficiently. It can be used as an alternative when there are no other active controls in place.

6.3 Passive Scavenging System:
Passive scavenging chambers have the same vaporizer and chambers with no air pump and purification system (Image 7 below). The chambers may have hinged or sliding lids and a carbon filter canister is connected to the discharge line. There is no forced air inside the tubes, and waste anesthesia gas (WAG) will be captured in carbon cartridge

passively (Image 8 below). This control system is not effective enough if none of the active controls are in place.

Image 7: Induction chamber with passive scavenging Canisters

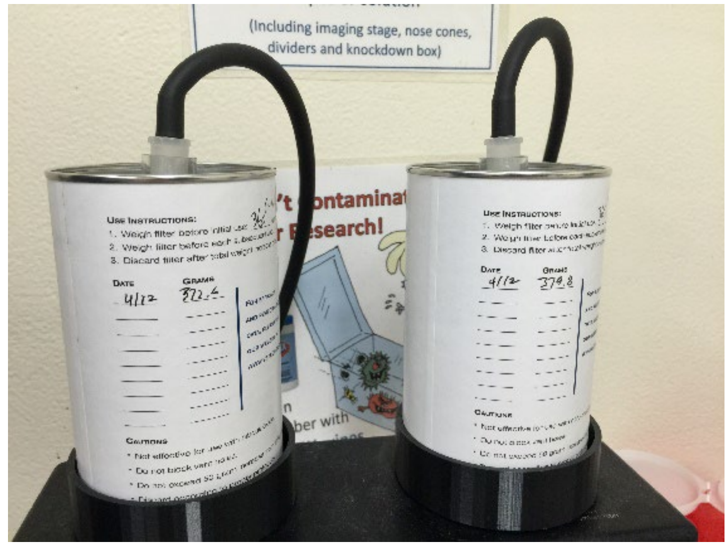

Image 8: Activated charcoal canisters

7. Engineering Controls (Ventilation)

7.1 The dilution ventilation should be 6 air exchange per hour (ACH) or higher to dissipate any isoflurane contamination from the general lab area. The local exhaust ventilation is able to capture the isoflurane vapor right after release from the person's breathing zone. All exhaust ventilation is tested by EHS Office every three years. The following considerations will effectively minimize the potential exposure in isoflurane setups:

7.1.1 It is strongly recommended to use downdraft tables, slot hoods and snorkels as the first choice to capture isoflurane gas and vapors effectively (Images 9-11 below).

Image 9: Downdraft table connected to the house exhaust ventilation

Image 10: Slot hood connected to the house exhaust ventilation

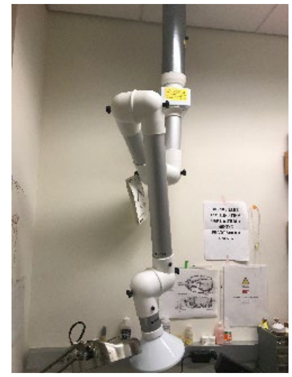

Image 11: Snorkel connected to the house exhaust ventilation

7.1.2 Often, the snorkels aren't located correctly near the source of the gas release point and capture velocity is not enough to remove the entire waste gas. They may be connected to the exhaust drop in the lab. However, it is often difficult to get snorkels close enough to adequately remove all waste anesthetic gas (WAG), resulting in some exposure, depending on how close the snorkel is to the gas release zone. It is recommended to locate the snorkels 4-6 inches from the gas release zone. If a snorkel with a rectangular hood is chosen, the capture velocity will be improved (see Figure 1 below from https://lcicorp.com/benchtop.html):

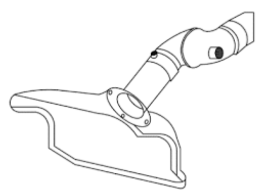

Figure 1: Rectangular hood

7.1.3 Using slot hoods creates a similar issue if the releasing zone is away from the slot location (poor capture velocity). The nominal slot hood airflow is usually between 250-300 cfm. The airflow measurement inside the slot hoods shows that the capture velocity of 75 foot/minute (fpm) is available in 12 inches (horizontal distance) from the slots. Increasing the distance from slots will significantly decrease the air velocity and performance of the slot hood. Therefore, put the isoflurane surgery setup or anesthesia chambers within 12 inches radius from slots.

7.1.4 The special bench-top gas/vapor extractors or downdraft enclosures are also recommended if the lab does not have an adequate or proper local exhaust ventilation (ECD) (Images 12-14 below). This equipment has appropriate capture velocity and less restriction for the surgery zone. For more information, please contact the EHS Office (environment@mit.edu or 617-452-3477).

Image 12: Downdraft enclosure with carbon filter chamber

Image 13: Benchtop gas/vapor extractor with carbon filter

Image 14: Benchtop gas/vapor snorkel connected to the carbon filter/HEPA

7.2 Isoflurane can be used inside the biosafety cabinet only if:

7.2.1 The biosafety cabinet is hard ducted (Type II B2) (100% exhausted).

7.2.2 The biosafety cabinet has a thimble/canopy connection (Type II A2) (30% exhausted).

8. General Recommendations

8.1 The highest exposure during work with Isoflurane has been found when opening the induction chamber. Reducing the exposure during this step will greatly reduce the overall exposure to Isoflurane. Some new models of induction chambers have a connection for the exterior exhaust drop in the lab.

8.2 Researchers should follow and encourage safe work practices at their workplace, including those recommended by the manufacturers, when handling Isoflurane.

8.3 Always try to use active scavenging chambers.

8.4 General ventilation in the laboratory is essential to dissipate the released WAG (at least 6 air change per hour).

8.5 Wherever possible, use Exposure Control Devices (ECUs) (i.e. local exhaust ventilation including snorkels, slot hoods, downdraft tables).

8.6 If there is no house exhaust ventilation available, use a benchtop ventilation unit with a carbon filter.

8.7 Ensure that the flow of isoflurane to the chambers and/or nose cone is switched off in between animals.

8.8 Ensure that the induction chamber lids are closed and locked when Isoflurane gas is being delivered.

8.9 Keep the induction chambers lids closed if they are not in use.

8.10 Use the lowest anesthetic gas flow rates possible.

8.11 Make sure the chamber gaskets are intact with no leak.

8.12 Flush Isoflurane from the anesthesia chamber with oxygen before opening for the recommended time per manufacturer.

8.13 Inspect all tubing, valves, and fittings for leaks. Make sure that you seal all leaks before starting work.

8.14 Regularly leak test the anesthesia chambers, system, interfaces, and charcoal canister using a refrigerant leak detector.

8.15 If possible, place the induction chambers or vaporizers inside a fume hood or slot hood (Image 15-16 below).

Image 15: Isoflurane induction chamber inside a fume hood

Image 16: Isoflurane vaporizer inside a slot hood

8.16 Keep laboratory doors closed when anesthetic gas is in use. This will keep the differential pressure for removing the vapors.

8.17 All Isoflurane setups should be equipped with passive waste scavenging canisters (charcoal canisters).

8.18 If it is possible, immediately after removing the saturated charcoal canister, it should be placed in a fume hood as waste gas may desorb from the filters.

8.19 Ensure that anesthesia chamber lids are closed and locked when Isoflurane gas is being delivered.

8.20 You can use a gasket anesthesia chamber with an exhaust port to scavenge waste gas and flush Isoflurane from the anesthesia chamber with oxygen before opening.

8.21 Nose cones should be chosen based on the size of the animal to be anesthetized. Do not use a nose cone that is too large or too small. A good nose cone option is one with a diaphragm that can provide a seal around the nose of the animal. Nose cones that allow for waste gas to be exhausted are the best method of reducing exposure to waste gas.

8.22 Whenever possible, use fume hoods when opening the bottle of Isoflurane or filling the vaporizer. You can use the anti-spill adapters to reduce the chance of isoflurane being spilled while filling the machine (Image 17 below).

Image 17: Anti spill adaptor for isoflurane bottle

8.23 In lack of any ventilation system, a respirator with an organic vapor cartridge must be used. First contact the EHS Office for assistance in determining whether a respirator is required, what size and type would best protect you, and what type of filters/cartridges to use. For additional information, see the EHS web page on PPE here: https://ehs.mit.edu/workplace-safety-program/personal-protective-equipment/

8.24 EHS recommends using a new innovative low-flow anesthesia delivery system designed specifically for mice and rats (Image 18 below). This device has a precision syringe pump and an integrated digital vaporizer that uses either room air (no need for an oxygen tank) or compressed gas to deliver anesthesia at low flow rates (flow rates from 25 mL to 1 L) proportionate to the animal's size. Using less anesthesia not only benefits the animal during procedures but also significantly reduces the risk of exposure to lab personnel from waste anesthesia gas.

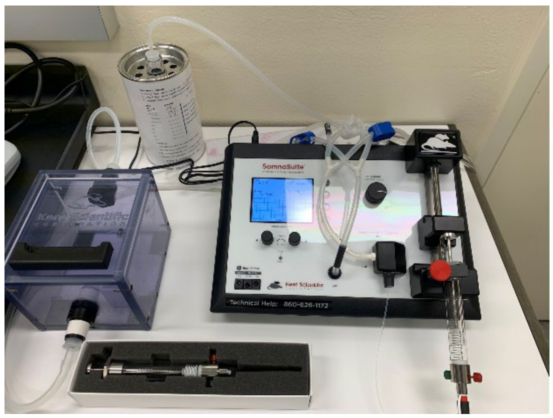

Image 18: SomnoSuite® Low-Flow Anesthesia System

8.25 EHS recommends using an induction chamber with a slide-top lid or vapor-vac hooded induction chamber (See Patterson Scientific, VetEquip, or Harvard apparatus) instead of an induction chamber with a hinged lid to minimize the Isoflurane waste gas exposure (Image 19 below).

Image 19: Pumped induction chamber with sliding door

9. Specific recommendations

9.1 Induction Chambers

9.1.1 If you use the **<u>active scavenging induction chamber,</u>** in addition to the general recommendations, you will need to check and service the machine regularly to ensure that the carbon filter is not saturated and efficiently remove the Isoflurane gas.

9.1.2 If you use **<u>passive scavenging induction chambers,</u>** you will need to follow the general recommendations and following recommendations.

9.1.2.1 Do your work in a well-ventilated area in the lab (not in air-trapped corners).

9.1.2.2 Use exhaust ventilation like a snorkel if it exists; locate 3-5 inches from point of gas discharge (see Image 20 below).

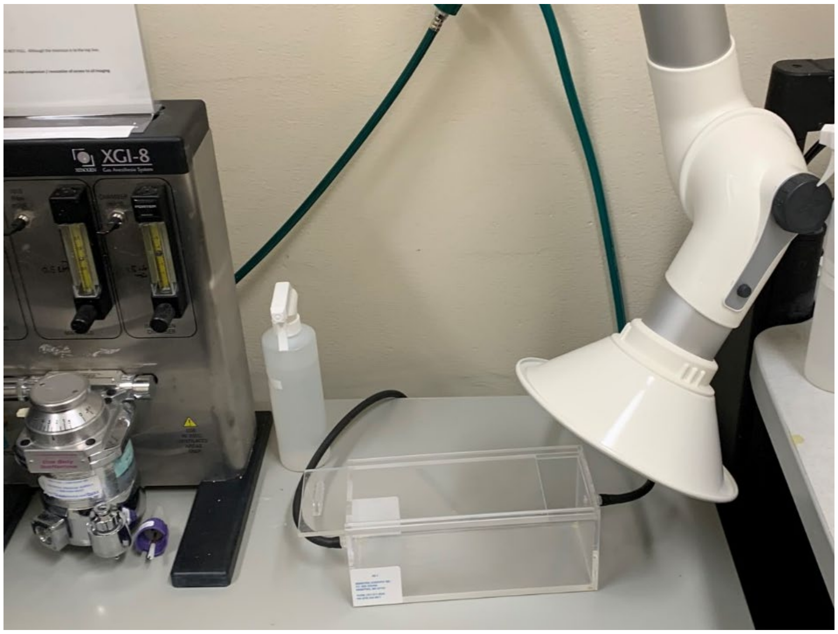

Image 20: Exhaust snorkel close to the induction chamber for better isoflurane capturing

9.1.2.3 Induction chambers with sliding doors are recommended because local exhaust ventilation can be located on the back of the chamber more efficiently.

9.1.2.4 After removing the mice, close the chamber immediately.

9.2 Carbon cartridges

9.2.1 The carbon filter canister should be located upright (vertical) preferably lower than the height of the chamber (isoflurane is 6.3 times heavier than air).

9.2.2 Make sure that the carbon canister is properly installed and tested before starting using Isoflurane.

9.2.3 Do not block cartridge holes.

9.2.4 Weigh carbon filter cartridge before first and every use.

9.2.5 Weigh canisters periodically and dispose of as hazardous waste when sorbent gains 50 grams.

9.2.6 If a carbon filter cartridge becomes saturated, put it inside a sealed bag and red tag it as hazardous waste. The charcoal filter cartridges should be collected separately and prepared for waste pickup by the hazardous waste vendor.

Note: The Department of Comparative Medicine (DCM) has a different approach for contaminated carbon filter cartridges. If you are working in

DCM, you should dispose of them as regular trash after they are saturated.

9.2.7 If carbon canisters are saturated, place charcoal filter cartridges in a fume hood, as waste gas may desorb from the filters at room temperature.

9.3 Imaging equipment

9.3.1 Check for adequate general ventilation of room (negative pressure and minimum 6 Air change per hour (ACH)).
9.3.2 Choose equipment with an integrated active scavenging system.
9.3.3 You can leave the discharge tube of the active scavenging system inside the fume hoods, qualified biosafety cabinets (Type II A2 or B2), slot hoods, or snorkels.

10. Spills and Waste Disposal

Small volumes of liquid isoflurane evaporate readily at normal room temperatures and may dissipate before any attempts to clean up or collect the liquid are initiated. However, when large spills occur (one or more bottles), specific cleaning and containment procedures are necessary, and appropriate disposal is required.

Because of the volatility of isoflurane, rapid removal and cleanup is suggested. The large spills in poorly ventilated areas or in storage areas should be absorbed using absorbent material. Commercially available absorbents, Vermiculite, and carbon-based sorbents are some of the materials that regularly used for this purpose. In the case of broken bottles, caution should be taken for handling the sharp objects.

Empty isoflurane bottles, if properly dried under a fume hood, could be disposed of as trash. At MIT, all contaminated wastes with isoflurane are considered hazardous material and should be collected as chemical wastes. You should follow the hazardous waste collection procedure in your lab.

Note*: *DCM does not have satellite accumulation areas for hazardous chemicals in labs, so if any contaminate waste with isoflurane is generated, collect and transfer the waste to your lab and dispose of it based on waste disposal procedures.

The isoflurane vapors may be present in the area even after finishing the cleaning; therefore, make sure that all vapors have dissipated before entering the area. Empty isoflurane bottles are not considered regulated waste and may be discarded with ordinary trash or recycled.

For transferring the isoflurane bottles between labs, use a secondary container, sealed Tupperware container, or bottle totes to prevent accidental breaks.

To minimize exposure to waste liquid isoflurane during clean-up and disposal, the following general guidelines are recommended:

- If Isoflurane spills in the lab, leave the laboratory and call EHS.
- If the spill is inside the fume hood, close the sash and let it be evaporated.
- If you are comfortable, use required PPE (safety glasses and protective nitrile gloves) and wipe down the residues.
- Where possible, ventilate area of spill or leak. If ventilation is not available, appropriate respirators with organic vapor cartridge should be worn.
- Restrict persons entering the spill area until clean-up is complete.
- Collect the spilled liquid and absorbent materials in an appropriate glass or plastic container. Tightly cap and seal the container, and red tag the container clearly to indicate its contents (see SOP EHS-0032: Hazardous Waste Management for details).

11. References

- **Anesthetic Gases: Guidelines for Workplace Exposures:** https://www.osha.gov/dts/osta/anestheticgases/index.html
- **University of IOWA; Isoflurane Anesthetic Gas Guidelines:** https://ehs.research.uiowa.edu/isoflurane-anesthetic-gas-guidelines
- **University of Georgia; Isoflurane Safety Guidelines:** https://research.uga.edu/docs/policies/compliance/oacu/UGA-IACUC-Isoflurane-Guidelines.pdf
- **2019 Waste Anesthetic Gas, DOHS WAG Program Manager, NIH:** https://www.ors.od.nih.gov/sr/dohs/Documents/Waste%20Anesthetic%20Gas%20(WAG)%20Surveillance%20Program.pdf
- **Sentry Air System:** https://www.sentryair.com/specs/Laminar-Flow-Hood-Spec-200-WS.htm
- **Patterson Scientific:** https://pattersonscientific.com/products/vapor-vac-hooded-induction-chambers/
- **VetEquip V-1 Tabletop with Active Scavenging:** http://www.vetequip.com/item.asp?cat=14&catalogID=901820#
- **Extract-All:** https://www.airsystems-inc.com/product/the-extract-all-model-s-981-2b-bentch-top-fume-extractor/
- **SomnoSuite®, Low-Flow Anesthesia System:** https://www.kentscientific.com/products/somnosuite/
- **Harvard Bioiscience Minivac, Gas Evacuation Unit:** https://www.harvardapparatus.com/minivac-gas-evacuation-unit-1.html
- **Nederman FX-75 snorkel:** https://www.nederman.com/en-us/products/capture-and-extraction-units/bench-top-extraction-arms-fx/benchtop-extraction-arm-fx2-75-chem/c-24/c-1733/p-415123
- **American Association of Nurse Anesthetists (AANA):** Management of waste anesthetic gases. Park Ridge, IL: 1992. Pp. 16-17.

Appendix A: Isoflurane Set-up Failure Tips