

Standard Operating Procedure

ISOFLURANE

Overview

Vapors of inhaled anesthetic agents, such as isoflurane, are commonly used in laboratory animal research protocols. Isoflurane is a clear volatile liquid at room temperature and standard pressure, with a boiling point of around 120F/49C. It also has poor warning properties. The 8-hour Cal/OSHA Permissible Exposure Limit is 2 ppm and the odor threshold is commonly listed as 50 ppm. The odor is often **described as ether-like or sweet and if you can smell it, you are likely being overexposed**. Occupational exposure occurs primarily through inhalation of excess or waste anesthetic gas (WAG) unintentionally released into the laboratory environment. All use of Isoflurane outside of a fume hood should be reviewed by EH&S prior to taking place.

Hazards:

Potential exposure routes include inhalation and skin contact. Possible health effects of overexposure to WAG may include, but are not limited to the following hazards:

1. Acute effects: drowsiness, eye and skin irritant, depression, headache, dizziness, nausea, fatigue, and problems with coordination, audiovisual ability, or judgment.
2. Chronic effects: central nervous system toxicant, liver and kidney disease, adverse reproductive effects or cancer.

Exposure Limits:

Cal/OSHA has established an 8-hour time weighted average permissible exposure limit (8-hour PEL) of 2 ppm.

Special Handling and Storage Concerns

Personal Protective Equipment

Minimum PPE: Nitrile gloves, lab coat, and eye protection (safety glasses, goggles, or face-shield)

Additional PPE: Respiratory protection (minimum half-face air-purifying respirator with organic vapor cartridges) may be required if the exposure limit is exceeded. Particulate respirators such as filtering facepiece respirators (N95s) commonly used for exposure protection against lab animal allergens (LAA) do not provide wearers protection from isoflurane. All use of respiratory protection equipment including N95s must be reviewed and approved by the EH&S Industrial Hygiene Division. Please contact the EH&S Industrial Hygiene Division (ehs-ih@ucsb.edu) with any questions about the use of respiratory protection or to schedule an exposure assessment.

Engineering Controls

Isoflurane must be used in a well-ventilated room from which there is no recirculation of exhaust air.

Waste Management

Unused solutions of isoflurane will be disposed of as a hazardous material through EHS.

First Aid and Emergencies

Spill

Do not attempt to clean-up if you feel unsure of your ability to do so or if you perceive the risk to be greater than normal laboratory operations.

- Small volumes of isoflurane evaporate readily at normal room temperatures and may dissipate before any attempts to clean up or collect the liquid are initiated.
- If a small spill occurs rapidly, absorb any liquid with absorbent pads or paper towels and place in a chemical fume hood for safe evaporation.
- If a large spill occurs, notify others in the area and evacuate the room immediately.
- Contact EHS emergency number (805-893-3446) for assistance.

Fire

Standard firefighting measures apply.

Personnel Exposure

Signal Word: Warning

Hazard Statements: May cause drowsiness or dizziness.

Precautionary Statements: Avoid breathing mist or vapors. Use only outdoors or in a well-ventilated area.

IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell.

Storage: Store in a well-ventilated place. Keep the container tightly closed. Store locked up.

In case of skin or eye contact: Rinse immediately off the skin for 15 minutes. In the case of eye contact, go to the nearest eyewash and rinse for 15 minutes. Follow up with Urgent Care or Student Health.

Laboratory Specific Information

Prior Approval Required

NO

YES (describe):

Designated Area

Entire Laboratory Area

□ **Other (describe):**

Experimental Conditions of Use

Temperature Range:

Pressure Range:

Scale Range:

Other Relevant Details:

The following summarizes some basic methods for minimizing exposures when using isoflurane:

Drop Method/Bell Jar

- Since waste gas scavengers cannot be used with this method, it is required to be used in a fume hood, under an exhaust arm, or in a ducted biosafety cabinet.
- Use a fume hood when soaking the cotton ball with isoflurane or the isoflurane/propylene glycol solution

Induction Chamber Use

- If possible, use in a fume hood, under an exhaust arm, or in a ducted biosafety cabinet.
- Use a gasketed chamber with an exhaust port to scavenge waste gas.
- A slide-top lid is preferred over a hinged lid to prevent isoflurane gas from being pushed into the user's breathing zone.
 - If this is not possible, then facing the hinge opening of the lid away from the user is recommended.
- Once the animal is anesthetized, purge the induction chamber with oxygen for 5-10 seconds prior to opening.

Anesthesia Machine Use

Procedures:

1. Before using the anesthesia machine, the following procedures shall be performed:
 - . Review the Safety Data Sheet (SDS) for isoflurane. A copy of the SDS is found in the Environmental Health and Safety binder.
 - a. Verify that the anesthesia machine is not out-of-service by checking the certification sticker on the anesthesia machine. The anesthesia machine shall **NOT** be used if the date of use is more than one year from the certification date. Contact the ARC Manager if the anesthesia machine lacks a certification sticker.
 - b. Check for adequate isoflurane anesthetic liquid in the vaporizer (see photo below).
 - Ensure that the oxygen flow through the vaporizer is turned off.
 - Refill the anesthetic liquid directly from the bottle by removing the reservoir screw cap and decanting into the reservoir using a pouring adapter. Watch the fluid level rise in the reservoir window and stop adding fluid when it reaches the “full” line or spot.
 - d. Check for sufficient flow/supply of compressed oxygen and check for any leaks in the anesthetic circuit:
 - Ensure that the machine is connected to the oxygen source and open the oxygen line and that the vaporizer is turned off.
 - Turn on the oxygen flow by turning the flow meter knob counter-clockwise and observing the flow probe rising to between 1-2 L/min.
 - Cover the corrugated anesthetic hose at the point where it connects to the face mask and observe an increase in the pressure gauge. When it reaches 20 cm of water, turn off the oxygen flow. If there are no leaks the increased pressure should be maintained until you uncover the anesthetic hose. If there is no pressure gauge on the anesthesia machine, then cover the tube from the output side of the vaporizer at the point of connection to the non-rebreathing anesthetic circuit. Listen for any audible leaks and for a change (bobbing) in the oxygen flow probe immediately after uncovering the tube.
 - e. Connect an induction chamber and/or face mask to the non-rebreathing anesthetic circuit appropriate for the patient and procedure to deliver the oxygen anesthetic gas mixture to the patient, and to reduce anesthetic gas leakage. If you are unfamiliar with the induction chamber and the non-rebreathing anesthetic circuits, or how to connect them to the anesthetic machine, please ask your PI for assistance.
 - When purging the induction chamber via the oxygen flush button, use an appropriate scavenging system to ensure no WAG escapes into the workspace.
 - f. Connect an activated carbon filter (i.e. [F/-Air canister](#)) to the exhaust side of the induction chamber and breathing circuit. Activated carbon filters are not necessary if working on a downdraft table, however, ensure that the exhaust port of the induction chamber and breathing circuit is located over the table.
 - Activated carbon canisters have a limited capacity for isoflurane adsorption (i.e., F/Air canisters have a useful life per canister of 12 – 15 hours), and this adsorption process is not irreversible. You must perform the following tasks to ensure that the activated carbon canister has enough adsorption capacity:
 - . Weigh the activated carbon canister **on the first use and for each use afterward**.
 - i. Record the weights and the dates on the canister itself. Refer to the manufacturer’s maximum weight gain (typically 50g greater than the unpacked/initial weight) and replace the canister when the adsorptive

capacity has been reached. Again, remember that the adsorption process is not irreversible, and therefore canisters that are not in routine use may lose weight

- ii. Always maintain the carbon filter in an upright/vertical position and do not block the holes on the bottom of the canister that allow for filtered air to vent out.
- g. Active scavenging systems use a fan to capture and exhaust the WAG and are available in some of our rodent surgery stations. Examples of active scavenging systems include down-draft tables, self-contained evacuation systems (Extract-All), and snorkel ducts that connect directly to the exhaust ventilation system.

1 “Activated charcoal canisters will effectively adsorb the vapors of halogenated anesthetics but not N₂O. The effectiveness of individual canisters and various brands of charcoal vary widely. Different potent inhaled volatile agents are adsorbed with varying efficiencies. The efficiency of adsorption also depends on the rate of gas flow through the canister. The canister is used where portability is necessary. The disadvantages are that they are expensive and must be changed frequently. Canisters must be used and discarded in the appropriate manner, as recommended by the manufacturer.” <https://www.osha.gov/waste-anesthetic-gases/workplace-exposures-guidelines>

The use of the self-contained Extract-All systems is as follows:

- Turn on the fan in the Extract-All scavenging system.
 - Position ducts as close as possible to potential points of WAG release (i.e., animal face mask, induction box).
 - Keep the user's breathing zone at a maximal distance away from the WAG source. Gas concentrations decrease rapidly with distance away from the source.
 - Record the following information on the log sheet provided by the ARC after each use of the Extract-All scavenging system:
 - Oxygen flow rate ((L/min),
 - % isoflurane concentration, and
 - duration of anesthesia)
 - Periodically review the log and replace the activated carbon based on usage of the system, the amount of activated carbon in the machine (i.e., useful lifetime), and per the manufacturer's recommendation.
1. During use, the following procedures will be performed with the anesthesia machine.
 - a. Adjust the gas flow rate using the oxygen flow meter to provide adequate patient ventilation and anesthetic vapor delivery.
 - Flow rates between 1-2 L/min are generally sufficient for ventilation of rodent species using a non-rebreathing circuit.
 - b. Adjust the anesthetic concentration by turning the dial on the vaporizer until the desired level of anesthesia is achieved.
 - Induction of anesthesia for rodents generally requires 3-4% anesthetic concentration.

- Maintenance of anesthesia for rodents generally requires 1-2.5% anesthetic concentration
2. After use, the following procedures shall be performed to clean and maintain the anesthesia machine and non-rebreathing circuit in good working condition.
 - a Clean the anesthesia machine, induction box, and working surfaces (e.g. surgery table or down-draft table) to remove any surface contamination using an approved disinfectant.
 - b Dismantle the non-rebreathing anesthetic circuit and clean the components, especially the nose cone, with an approved disinfectant.
 - c Return the cleaned breathing circuit, and induction box to the storage cabinet.\
 3. Once per year, have a qualified service representative certify the anesthesia machine, calibrate the vaporizer (if needed) and perform a vaporizer efficacy test for the anesthesia machines in the shared procedure rooms of the vivarium. The service representative shall document that this service was performed by placing a certification and calibration sticker on the anesthesia machine, which lists the service date.

