Isoflurane Use and Safety

The use of isoflurane (also known as "aerrane") in small animal research as an anesthetic is routine, and the small animal imaging facility utilizes isoflurane vaporizers at each instrument and procedure bench to facilitate research. However, exposure to the investigator to isoflurane vapors may occur, and if prolonged exposure occurs users may feel unwell. The long-term exposure risks are poorly understood, but it is the purpose of this document to train users in optimal use of the different vaporizers to minimize their exposure. We will continue to review and improve waste anesthetic gas (WAG) scavenging, and thus information in this document may become outdated as upgrades are incorporated into the imaging facility.

1. ISOFLURANE BACKGROUND: SAFETY AND HANDLING

Please note that the MSDS can be found at
http://web.ncifcrf.gov/rtp/LASP/intra/forms/msds/msds_isoflurane.pdf
A descriptive leaflet can also be found in the cupboard over the sink where isoflurane stocks can be found. More leaflets are available in each box of isoflurane. The Stanford EH&S Anesthetic Gas Fact Sheet can be found at
http://www.stanford.edu/dept/EHS/prod/researchlab/lab/safety_sheets/11-108k.pdf

1.1 Occupational Exposure Regulations and Recommendations: The National Institute for Occupational Safety and Health Administration (NIOSH) recommends that no worker should be exposed at ceiling concentrations greater than 2 ppm of any halogenated anesthetic agent over a sampling period not to exceed one hour, and the Cal/OSHA Permissible Exposure Limit (PEL) is 2ppm as an 8 hour time weighted average, according to Cal/OSHA PEL Table AC-1 (http://www.dir.ca.gov/title8/5155table_ac1.html).

1.2 Adverse Reactions: Adverse reactions encountered in the administration of isoflurane are in general dose dependent extensions of pharmaco-physiologic effects and include respiratory depression, hypotension and arrhythmias. Shivering, nausea, vomiting and ileus have been observed in the postoperative period. As with all other general anesthetics, transient elevations in white blood count have been observed even in the absence of surgical stress. There have been rare reports of mild, moderate and severe (some fatal) postoperative hepatic dysfunction and hepatitis. Isoflurane has also been associated with perioperative hyperkalemia. There have been rare post-marketing reports of hepatic failure and hepatic necrosis associated with the use of potent volatile anesthetic agents, including isoflurane. Due to the spontaneous nature of these reports, the actual incidence and relationship of isoflurane to these events cannot be established with certainty.

The predicted effects of ***acute overexposure*** by inhalation of isoflurane include headache, dizziness or (in extreme cases) unconsciousness.

There are no documented adverse effects of ***chronic exposure*** to halogenated anesthetic vapors (Waste Anesthetic Gases or WAGs) in the workplace. Although results of some epidemiological studies suggest a link between exposure to halogenated anesthetics and increased health problems (particularly spontaneous abortion), the relationship is not conclusive. Since exposure to WAGs is one possible factor in the findings for these studies, operating room personnel, and pregnant women in particular, should minimize exposure. Precautions include adequate general ventilation in the operating room, the use of a well designed and well-maintained scavenging system, work practices to minimize leaks and spills while the anesthetic agent is in use, and routine equipment maintenance to minimize leaks.

2. USE OF RESPIRATORS

It is currently **required** that any user of the imaging facility that spends greater than 90 minutes per day working with animals in the laboratory should wear a respirator. You may also wish to wear a respirator if you spend less time than this in the facility. Note that you may wish to wear a respirator in the facility even if you are not using isoflurane for your experiments, since other users may be using it, and potentially exposing you.

For employees who are required to wear respirator, the following requirements apply:

1) Obtain a medical clearance to wear an air-tight-fitting respirator (non-N95). Medical clearance can be obtained through the Occupational Health Center (725-5308). This will typically involve filling out a questionnaire and meeting with a medical personnel.
2) After obtaining a medical clearance, the individual needs to contact EH&S –occupational health & safety program (650-723-0448) to schedule a time for fit testing and training.
3) Fit-testing and training will take ~1 hour and is conducted at Environmental Safety Facility (480 Oak Road, Stanford).
4) After the fit testing, EH&S will provide the user with the model/ type of respirator and cartridge they need to purchase. Respirator and cartridges are purchased after the fit testing by the department.
5) The selection and replacement schedule of the cartridges vary depending on manufacturer; EH&S will provide cartridge replacement information to the users during fit testing and training.

Note: Such procedure is repeated at least every year.

3. ISOFLURANE BOTTLES AND ADAPTERS

Stocks of isoflurane can be found in the cupboard over the sink in S040. Older vaporizers use the "Funnel Filler Adapter" (middle bottle). Please do not remove the cap to these fillers. Use of these adapters allows a more controlled flow than pouring directly from the bottle. If you empty a bottle of isoflurane, please transfer the adapter to a new bottle, which should be done in the fume hood. The "Keyed Filler Adapter" (right bottle) is used on the newer vaporizers, and if used correctly should result in little spillage. A piece of tape over the end of these may reduce evaporation. Please note that although highly volatile, isoflurane liquid is a solvent, and will melt the polycarbonate induction chambers. Therefore do not allow isoflurane to pour onto these chambers (it can glue the slide doors closed), or use paper towels soaked in isoflurane placed into the box to euthanize/anesthetize animals. This can result in damage to the boxes, and is not an approved APLAC procedure.

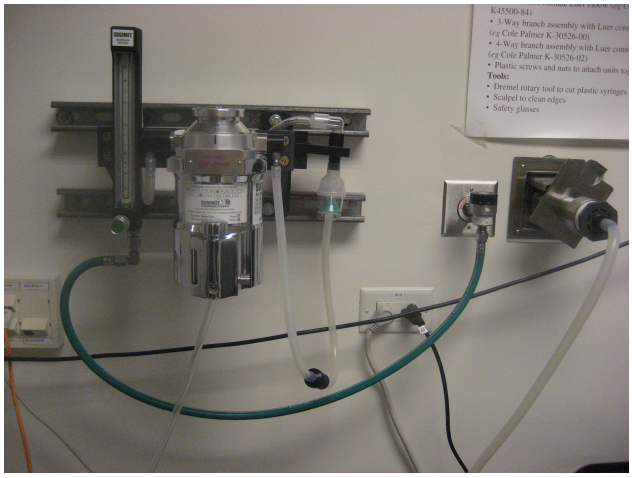
4. ISOFLURANE INDUCTION CHAMBERS

All induction chambers in SAIF laboratories now use sliding panels to open from the top. The newer style has vents at the top with an exhaust port above vents to attached to waste gas exhaust lines. Clean paper towels should be placed in the bottom of these chambers before placing animals in them. The newer vented chambers each have an exhaust pump attached at the top of the chamber, and this pump should be switched on throughout the use of the chambers, even if the isoflurane flow is turned off during placement in/removal of animals from the chamber. Older

style chambers have a passive venting of the waste gas from the top of the chamber – isoflurane flow should be turned off when the chamber lid is opened.

Do NOT use ethanol or isopropanol on these chambers. Do NOT use paper towels soaked in isoflurane in these chambers to anesthetize/euthanize animals. Do NOT use heat lamps to keep animals warm in these chambers – the heat lamps will melt and warp the lids making the lid harder to open and creating leak points for isoflurane to escape. Also, anesthetized animals can be over heated by improper use of heat lamps – they cannot retreat from the heat, and may be killed by a heat lamp left over the induction chamber. Maximum isoflurane flow rate to induction chambers is 2 litres per minute (*lpm*).

5. WALL MOUNTED ISOFLURANE VAPORIZERS

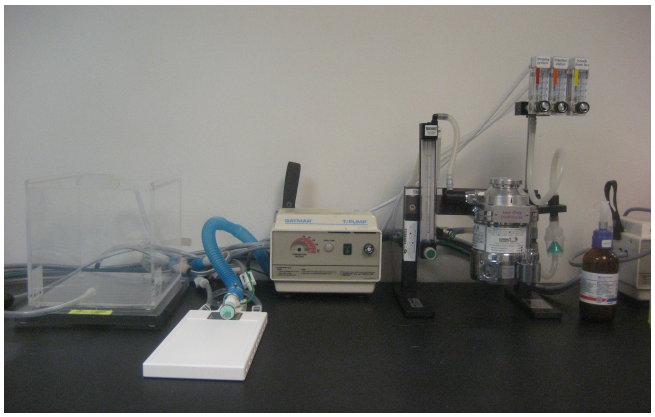


Oxygen to all wall mounted units is turned on at the wall (black tap). Some units have an additional flow controller upstream of the vaporizer unit (as shown left), and some systems have additional flow controllers downstream of the vaporizer to allow control of flow to different outlets (such as induction chambers and imaging instruments, and shown on the bench-top unit image below).

Flow rate for animals in imaging instruments should be set to a maximum of 0.5 *lpm*, and if available, flow rates to induction chambers should be set to a maximum of 2.0 *lpm*. Note that the tidal volume of rodents is very small, and using higher flow rates may result in the extra gas being exhausted into the room, and potentially exposing you to the isoflurane.

Vaporizers may have nebulizers attached (shown here attached to the right of the vaporizer). These allow the isoflurane-charged oxygen to be humidified, so that during prolonged anesthesia, the lining of the trachea and lungs is not otherwise dried out by the oxygen. Small nebulizer chambers should be filled with deionized water. Waste gas exhaust is provided by the metal outlet on the wall (shown here on extreme right) – this works well for the non-rebreathing nose cones if correctly set up; the metal slider on the wall outlet can be adjusted by means of a thumb screw to increase or reduce waste gas scavenging. Instructions for the correct operation of the standard isoflurane vaporizer can be found in the Isoflurane Calibrated Vaporizer.pdf document.

6. BENCH-TOP ISOFLURANE VAPORIZER STATIONS



These stations may be quite elaborate, as shown left. In this case, the induction chamber (older style) is shown on a heated water pad (left), which will keep the animals warm (mice and rats cannot thermo-regulate when anesthetized, so should be kept warm if they are kept anesthetized for long periods). A white surgery pad with warm water heating and a NON-REBREATHING nose cone assembly (see below) is next to the induction chamber, and the water pump that flows water at ~38°C is in the middle

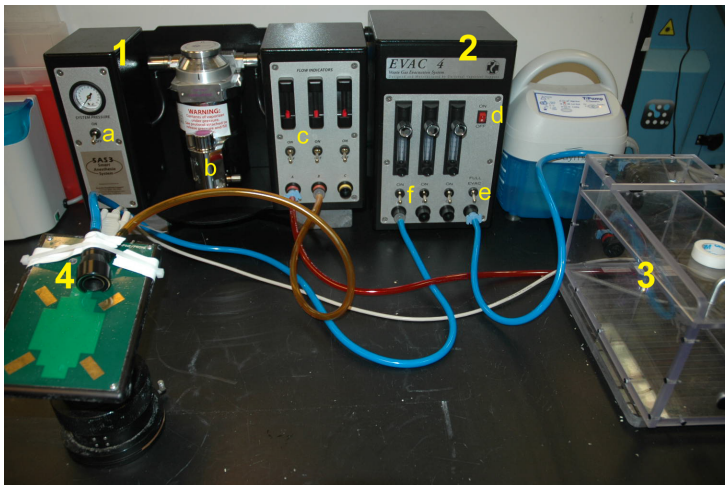
of the picture. Ensure that the water level is above the minimum line before turning on. Should the pump overheat, an orange light will illuminate, and the system will need to cool down before it will work again. The vaporizer shown is similar to the wall-mounted unit, and has secondary flow controllers to adjust flow to (in this case) the imaging system (red), the white surgery/injection station (orange), and the induction chamber (yellow). Oxygen flow to the imaging chamber and injection station should be set to a maximum of 0.5 *lpm*, and the induction chamber to a maximum of 2 *lpm*. Flow to any outlet should be turned off if not in use, or in the case of the induction chamber, it should be turned off temporally while opening the lid to place/remove animals. Waste isoflurane gas on this system is collected from the top of the induction chamber and the large blue tubing, and is exhausted though the wall mounted exhaust outlets. Careful balancing of exhaust and oxygen flow should prevent leakage and potential exposure to users.

Instructions for the correct operation of the standard isoflurane vaporizer can be found in the Isoflurane Calibrated Vaporizer.pdf document.

7. XGI-8 (XENOGEN) ISOFLURANE VAPORIZER WITH FLOW CONTROL AND EVACUATION PUMP

The XGI-8 vaporizer units currently installed on the IVIS imaging systems and the CRI Maestro scanner allow controlled flow of isoflurane to both the imaging instrument and the induction chamber (newer style). A green knob on the lower left of the instrument allows oxygen to be turned on to the unit, and a switch at the upper left of the unit controls an evacuation pump. When this pump is engaged, the flow meter should register at least 7 *lpm*, and will draw waste gas from both the top of the induction chamber, and the isoflurane manifold on the heated pad in the IVIS imaging chamber. Exhaust flow should be sufficient to remove waste gas without creating a vacuum at either system. The new style induction chambers allow fresh gas to enter via a port at the bottom rear of the chamber, and expelled gas is vented through holes on the lid (when closed) to be collected by the exhaust pump output above these holes. Isoflurane flow can be controlled to both the Imaging System (set to a maximum of 0.25 *lpm*) and to the induction chamber (set to a maximum of 2 *lpm*) by the flow controllers on the upper right of the unit. Gas flow can also be turned off temporarily while animals are being placed in or removed from the induction chamber, thus minimizing exposure to users. If no animals are present in either of the induction chamber or the Imaging System, the oxygen flow should be turned off by the flip knobs adjacent to the flow controllers. The evacuation pump should be left running for the duration of experiments.

8. SAS3-EVAC4 ISOFLURANE VAPORIZER WITH WASTE GAS PUMP



The new SAS3-EVAC4 systems allow controlled isoflurane delivery and scavenging for up to three outlets. The SAS3 unit (1, right) has a switch on the left side of the unit (a) that will pressurize the entire unit to ~6psi, and three flow controllers (c) to the right of the vaporizer (b) to deliver anesthetic gas to the induction chamber (red outlet) and two other outlets (orange and yellow). Note that the flow to each of these outlets is preset. The EVAC4 pump (2) is turned on by the red switch on the upper right face of the instrument (d), and inputs from the induction chamber (right most switch, e) and 3 other inputs are controlled by flip switches (f). Exhaust to the induction chamber (3) is fixed, and is higher than to the other three inputs, and these three inputs have flow adjusters to allow different scavenging rates. Typically, you will want to set these to their maximum setting (at least 5 *lpm*). The nose cone (4) is also different from the non-rebreathing style described above. Isoflurane is delivered at a low flow to the inner cone, in which the rodent nose is placed. The outer skirt scavenges gas at a higher flow, effectively removing the waste gas from the vicinity of the nose cone. Note that this unit should NOT be sealed with a latex seal, as this will result in potential scavenging of gas before the animal can breath it. Note also that the operation of this unit is more complicated, and instructions should be read and understood before use.

8.1 SAS3-EVAC4 Operation

8.1.1 Pressure relief for the SAS3 Unit

When the oxygen is turned on to the unit, the entire system is pressurized to about 6psi, and the pressure needs to be relieved when you plan to re-fill the vaporizer unit, and when you have finished at the end of your experiments. Since the vaporizer itself has some control on the pressure both upstream and downstream, it is important that the vaporizer be set to "0" rather than "OFF" during the pressure relief protocol. To release pressure, first turn off the oxygen at the left of the unit (a, left). Set isoflurane concentration to "0" (b) and open the flow on channel A (c). The red flow meter will pop up if any pressure is in the system. Allow oxygen to vent until this red flow indicator drops. Turn the flow (c) and reset isoflurane to "OFF" (b). The system is now ready to fill with isoflurane, or leave for the next user.

8.1.2 Filling the SAS3 Vaporizer

After venting any pressure (above), make sure the fill knob (1a, right) is in the "closed" position. Insert the bottle with filler key into fill hole (1b) with bottle below unit. Tighten thumbscrew (1b). Open fill knob (2a) and raise bottle (3). The vaporizer chamber will now fill, and you should see bubble enter bottle. When full, turn fill knob (4a) to "closed" position, and lower bottle to table and wait until isoflurane has drained from hose connection back into bottle. Release thumbscrew (1c) and remove bottle. Do **NOT** open the fill knob if the adapter is not inserted – this will result in the contents of the vaporizer emptying onto the bench.

8.1.3 Using the SAS3-EVAC4 unit

Turn oxygen flow on (1a) and ensure system pressurizes (1b). Turn on evacuation pump (1c) and open "Full Evac" (1d, to induction chamber) and exhaust flow to devices being used (1e). Adjust exhaust flow (1f) to maximum possible flow. Turn on oxygen flow to induction chamber (1g) and devices (1h) – flow meters (1i) will indicate correct operation. Finally, adjust isoflurane (1j) to required concentration. Note that isoflurane is delivered to the nose cone (2a), while waste gas is scavenged from the surrounding skirt (2b). Do **NOT** use a latex seal for this type of nosecone.

Wall-mounted vaporizer operation

1. If necessary, fill vaporizer chamber with isoflurane.
2. Turn on oxygen flow at wall.
3. If present, adjust upstream flow meter (this will allow lower flow rates than wall setting).
4. If present, adjust downstream flow meters to individual outlets
    a. Use <2.0 *lpm* for induction chambers
    b. Use <0.5 *lpm* for instruments/nose cones.
5. If present, line induction chamber with clean paper towels.
6. Adjust isoflurane concentration to required level.
7. Remember to stop flow to induction chamber when the door is open, and flow to nose cones/instruments if no animals are present.
8. If vaporizer needs to be re-filled during a session, turn off oxygen flow to chamber prior to filling.
9. When finished, turn isoflurane concentration to "Off", and turn off oxygen at wall. Clean induction chamber and nose cones with disinfectant.

Bench mounted (portable) vaporizer operation

1. If required, fill vaporizer with isoflurane.
2. Turn on oxygen at wall outlet, if present.
3. Adjust flow to vaporizer with upstream flow meter.
4. Adjust down-stream flow meters to each outlet
    a. Use <2.0 *lpm* for induction chambers
    b. Use <0.5 *lpm* for instrument or nose cones on bench-top.
5. Line induction chamber with clean paper towels.
6. Adjust isoflurane concentration to required level.
7. Remember to stop flow to induction chamber when the door is open, and flow to nose cones/instruments if no animals are present.
8. If vaporizer needs to be re-filled during a session, turn off oxygen flow to chamber prior to filling.
9. When finished, turn isoflurane concentration to "Off", and turn off oxygen at wall. Clean induction chamber and nose cones with disinfectant.

Xenogen XGI-8 vaporizer operation

1. If necessary, fill vaporizer with isoflurane.
2. Turn on exhaust pump on the main XGI-8 unit.
3. Open green tap to allow oxygen to flow.
4. Open flow taps to Induction Chamber and IVIS and adjust flow rates
    a. Use <2 *lpm* for the induction chamber
    b. Use <0.25 *lpm* for the IVIS chamber.
5. Turn off flow to IVIS and/or chamber if no animals are present.
6. Insert nose cones into manifold on IVIS chamber stage – use rubber stoppers in outlets not to be used.
7. Place a sheet of clean black paper on the IVIS stage where animals will be placed, and clean paper towels into induction chamber.
8. Adjust isoflurane concentration to required level.
9. Turn off gas flow to induction chamber while opening door.
10. If vaporizer needs to be filled during session, first turn off oxygen at green tap before opening vaporizer (animals will remain anesthetized for the short time required to refill the vaporizer).
11. When finished, turn vaporizer to "OFF", turn off oxygen flow to each outlet and then turn off the green oxygen switch. Finally turn off exhaust pump (this should be left running during entire experiment time).
12. Clean nose cones and induction chamber with disinfectant and discard black paper and paper towels lining IVIS stage and induction chamber.

SAS3-EVAC4 vaporizer operation

1. If necessary, fill vaporizer with isoflurane, but first confirm that there is no pressure in system:
    a. Make sure main switch on SAS3 is off
    b. Turn vaporizer to "0"
    c. Open flow "a", and ensure there is gas flow (red flow meter should stay at bottom).
2. To fill vaporizer:
    a. Ensuring isoflurane bottle is kept low, insert filler key into vaporizer and tighten thumbscrew
    b. Raise bottle vertically, and turn fill knob to open – the isoflurane will now fill the reservoir
    c. When filled to desired level, turn fill knob to close, lower bottle back to bench level, and allow any residual isoflurane in filler tube to pour back into bottle – failure to do this can result in isoflurane spillage
    d. Loosen thumbscrew and remove filler key.
    e. Do NOT open fill knob on vaporizer if filler key is not inserted and secured with thumbscrew – the isoflurane in the reservoir will empty onto bench!
3. Turn on power to EVAC4 pump, and open taps to induction chamber and any other outlets to be used. Ensure flow rates to each outlet is ~5 *lpm* by adjusting flow meters. Leave EVAC4 pump running for entire experiment.
4. Line induction chamber with clean paper towels.
5. Turn on oxygen on SAS3, and adjust isoflurane concentration to desired level.
6. Turn on flow to induction chamber (red outlet and tubing on SAS3) to charge induction chamber with isoflurane, but remember to turn off flow while the chamber is open for insertion/removal of animals.
7. Turn on/off flow of isoflurane to each outlet on SAS3 as required. Note that flow rates on these systems are fixed, and cannot be adjusted.
8. If vaporizer reservoir requires refilling during experiment, ensure that the pressure is released prior to refilling (see step 1 above).
9. When finished, turn isoflurane concentration to "0", and turn off main oxygen input. Allow flow on each outlet of SAS3 to drop to zero, and then turn isoflurane concentration to "Off".
10. Turn off evacuation pump, and turn off all flow controllers on SAS3 and EVAC 4 units.
11. Clean induction chamber and nose cones *etc.* with disinfectant, discarding any paper towels used to line chambers.