Utarbeidet av AÅ Godkjent av AÅ	Standard operating procedure **Working with inhalational anesthesia on rodents**	Versjon: 3,0 Utarbeidet: 19.12.2012 Revidert og oversatt: 08.03.24 AÅ Revidert 15.08.24 AÅ

1. Purpose

The purpose of this SOP is to make sure the use of inhalational anesthesia on rodents is performed in a safe manner for both staff and animals, providing good animal welfare and a safe working environment.

2. Scope

The SOP covers all procedures involving inhalational anesthesia on rodents at CoMed. You also need to read the SOP "General rules for the use of inhalational anesthesia at CoMed".

3. Responsibility

The facility manager is responsible for implementing the SOP among staff and researchers. All personnel are responsible for following all the rules in this SOP.

4. Definitions

Inhalational anesthesia: general anesthesia by breathing a gas, for example Isoflurane or Sevoflurane. The anesthetic gas is mixed with air in a vaporizer. Because all types of anesthesia cause some respiratory depression, inhalational anesthesia is used with extra O2 to avoid hypoxia.
N2O, nitrous oxide: laughing gas. Weak anesthetic effect but provides analgesia and improves the effect of other gases. Might have a teratogenic and reproductive toxic effect after long time exposure.
Isoflurane: halogenated ether. Some animal studies have shown reproductive toxic effect after long time exposure.
Sevoflurane: halogenated ether with faster effect and less irritation of mucus membranes than Isoflurane. Possibly less/no reproductive toxicity.
Doxapram**:** respiratory stimulant.

5. Execution

Information and training

Everyone working with inhalational anesthesia at CoMed must read the safety sheets of all relevant gases. Local training in the use of vaporizers and the rest of the setup is mandatory for everyone before they can start working with inhalational anesthesia. This training must be given by facility staff and the training should be documented in the facility.

Control of equipment

All vaporizers should be labeled with an ID. The setup, including vaporizer, tubes, mask and chamber, should be inspected by all users before starting the procedure. Look for any tear or leaks from the system. If you find anything abnormal, contact facility staff immediately.

A leak test using a leak detector should be performed every time the setup is changed and minimum once a month by the facility staff.

General rules for working with inhalational anesthesia on rodents

- Inhalational anesthesia is only allowed in labs with proper ventilation.
- Inhalational anesthesia should only be used on a downdraft table, unless a specific risk assessment is performed, and the work is considered safe.
- When using a heating pad, cover as little as possible of the downdraft area. The ventilated area must always be uncovered close to the operator, to make sure waste gases are removed.
- Place all other equipment outside of the downdraft area, preferably on another table.
- The mask should stay as close as possible to the head of the animal, covering the nose and mouth.
- The chamber should if possible be placed on the downdraft area when opened. If this is not possible, make sure the suction is at max speed when opening the chamber.
- Do not use a higher flow or concentration of gas than necessary.
- If you smell Isoflurane, stop your work immediately and ask for help.
- People that are pregnant or planning to get pregnant should not be working with inhalational anesthesia. See separate SOP for pregnant personnel.
- If your procedure is of a long duration and you have lost animals in anesthesia earlier, you might ask the facility for Doxapram to be prepared for you in advance.

How to use the system

- Check the vaporizer setup, make sure it is prepared for your species (mice or rats).
- Check the level of Isoflurane, refill if necessary. You can buy Isoflurane from the facility staff.
- Prepare a heating pad and any additional equipment you will need during your procedure.
- Turn on the suction and make sure the vents for anesthesia and suction are switched to the chamber side of the system.
- Place the animal in the anesthesia chamber. Add a mixture of carrying cases to the vaporizer according to your plan and add 3-4 % Isoflurane to the carrying gases. The standard settings are written on a poster on the wall by the vaporizer.
- Monitor your animal during the induction phase and do not transfer it to the mask before it is properly asleep and breathing in a regular manner. This might take some minutes. Make as little noise as possible during this phase.
- Switch the vent for the anesthesia to the mask side of the system before opening the chamber to transfer the animal.
- Make sure the suction is set to maximum speed before opening the chamber to transfer the animal. If the chamber is difficult to open because of the suction, you can open the chamber slightly on minimum suction and then increase the suction again before opening it completely.
- Transfer the animal from the chamber to the mask.

- Close the chamber and switch the suction to the mask side of the system immediately after transferring the animal. Make sure the animal is positioned properly into the mask, with as little room as possible between the face of the animal and the mask. Remember to lubricate the eyes, also for procedures of short duration.
- Adjust the % of Isoflurane to 1,5-2 %, and check reflexes on a regular basis to evaluate the depth of anesthesia. Adjust the level of Isoflurane if necessary.
- After finishing your procedure, switch off the Isoflurane and the N2O. If the procedure was of a long duration, it might be a good idea to leave the animal on the mask, breathing O2 alone for a minute or two, before you transfer it back to the cage for recovery. Keep a close eye on the animal if you do this.
- If the respiratory rate falls progressively or seems labored and the surgery is in progress, assist ventilation by gentle compression of the chest. Reduce Isoflurane levels if the concentration is high and/or the animal has no reflexes. Finish your procedure as soon as possible. If the surgery is completed, switch off Isoflurane and N2O, continue oxygen and administer Doxapram if available, 5-10 mg/kg ip or sublingual.

6. HSE aberrations

- If staff is exposed to anesthetic gases, the exposed person must be transferred to fresh air as soon as possible. Call 113 if the person is unconscious, has respiratory problems or irritation of the airways.
- Spill on skin: wash with water and soap for 15 minutes. Contact your doctor if the skin is irritated.
- Spill in eyes: flush with large amounts of water for 15 minutes. Equipment for flushing eyes is available in all surgery rooms. Contact your doctor.
- Spill on equipment: Use tissue paper to absorb the liquid, put the paper in a yellow container and close it immediately. If you spill large amounts/break a bottle – leave the area immediately and contact facility staff. Do not enter the area without proper protection.
- All unwanted incidents must be reported in NTNUs system for aberrations.