ISOFLURANE VAPORIZER ANESTHESIA
Overview

- Liquid anesthetic is converted to a gas in the vaporizer and carried by oxygen to the animal.
- Easily and quickly allows for induction, maintenance, and recovery due to the inhalant route of delivery.
- Constant monitoring of the patient is required due to cardiac and respiratory depression by the anesthetic – never leave the room when your patient is anesthetized!
- Age, strain, health status, disease model, other medications may affect how the individual animal responds.
Definitions

- **Induction**: moving a patient from consciousness to unconsciousness; a higher dose of anesthetic is required to overcome the awake state.

- **Maintenance**: maintaining unconsciousness in a patient; typically, a lower anesthetic dose is required vs. Induction, unless procedure is identified to be painful/requiring more anesthetic to maintain a surgical plane of anesthesia.

- **Recovery**: allowing for a return to full consciousness; patient is no longer receiving an anesthetic.

- **Vaporizer**: converts liquid anesthetic to a gas for inhalational delivery to patient via lungs; all DCM vaporizers are marked for use with Isoflurane only.

- **Isoflurane**: one type of liquid anesthetic, converted to a gas in a vaporizer; induces & maintains a patient under anesthesia; allows for quick induction, recovery, and anesthetic depth changes in patient due to inhalational delivery route.
Definitions

- **Non-rebreather tubing**: provides uni-directional airflow to patient during anesthesia (i.e., gasses are not recirculated/reused)
- **Induction box**: clear, durable, enclosable space used to contain an animal while initiating anesthesia
- **Nosecone**: delivers gasses to an animal to maintain them under anesthesia during a procedure
- **F/air canister**: captures exhaled CO2, and excess O2 and isoflurane via activated charcoal granules so they do not dissipate into the room; requires regular weighing to determine replacement frequency – see manufacturer instructions
Oxygen

Oxygen carries isoflurane to the patient! It **must** be flowing or your animal will not become anesthetized!

Oxygen tank and line is always **green**

Large ‘H’ tank reads 2000-2200psi when full

- If gauge reads below 500psi, alert the husbandry staff or building manager that tank needs to be changed soon. It should be replaced prior to performing long procedures – DCM provides oxygen for use with vaporizers in their procedure rooms

Pressure-reducing gauge:

- Reduces pressure of oxygen coming from tank so you don’t cause lung trauma to patient
- Should read **no more** than 50psi – can adjust if needed
- Some regulator valves may be preset and only have 1 gauge for tank volume

Oxygen flowmeter (located on vaporizer):

- Adjusts flow rate of oxygen in Liters/minute
- For rodents set to 1 L/min - this is enough for their metabolic needs

*Oxygen flush button provides a burst of gas to quickly clear the line of anesthetic - you must turn the isoflurane off/to 0% before pushing!
Isoflurane

Anything used with isoflurane is colored purple.

Marginal changes in percentage setting required to increase/decrease anesthetic depth of patient.

Do not touch or allow animals to touch the liquid — it is caustic & may burn! Use gloves while handling & wash any skin that touches the liquid.

Has a distinctive ‘clean’ smell - check your connections if you suspect a leak or poor connection.

*Ask a husbandry technician or the building manager if you need more isoflurane.

Pregnancy warning: Isoflurane is teratogenic and may cause fetal complications — do not put your nose/mouth in the same area where the gas is flowing. If a hood/vent is in use or F/air canister is connected properly then gas leakage should not be a concern. Speak to your PI about lab duties if you are pregnant & prefer not to work with this agent.

*Contact Occupational Health for more information on the effects of isoflurane if you have concerns with exposure to this agent.
Fill-ports

*Note: fill-port and window design may vary by manufacturer and age of machine

Check liquid level via reservoir window prior to beginning procedure and refill if needed

- Liquid ‘bubble’ should be between lines/dots/triangles
- If there are two windows check both for liquid level, these usually have a single black dot in the center - the right-side window indicates the machine level is getting low

You can refill between surgeries but not while an animal is anesthetized!

You should be focusing on your patient during the procedure!

Turn off the vaporizer & oxygen flowmeter while refilling so the liquid doesn’t spray out/overflow the fill-well
Fill-ports

To fill:
- Remove large screw lid at front of vaporizer and pour liquid into the now-visible open well, replace screw when finished
- If fill-well is already visible, remove the small screw on top to un-block reservoir opening & add liquid, replace screw when finished. Do not remove the bottom screw - this is for draining the machine

Pour slowly! Isoflurane does not flow quickly into the liquid reservoir and may overflow the well

Do not overfill the machine - stop filling when the liquid level is at the maximum level indicated

Make sure to screw everything back tightly! Isoflurane vaporizes readily & you can dry out a machine if it is not completely closed
If there is a no-spill port, you will need to use a clear/purple plastic ‘key’ to refill:

- Remove cap & attach key to Isoflurane bottle - it will only fit one way due to notches in ring around bottle neck.
- If present, remove the metal placeholder key and set aside - this blocks the reservoir holes when the machine is in use.
- Loosen the upper top fill-port screw, if present may need to slightly loosen the lower top fill-port screw (1/4 to 1/2-turn) to unblock the reservoir access holes, depending on the machine.
- **DO NOT** loosen either screw on the bottom port, these are for draining the machine (Note: the example picture at left with has only 1 top fill-port screw).
- Line up the similar end of the plastic key into the fill-port - it will only fit one way, so the holes are lined up.
No-Spill Refill

- Slowly tilt the Isoflurane bottle up until you see liquid draining into the clear part of the key and into the fluid level window.
- When finished filling the reservoir, loosen the screw(s), remove plastic key, replace metal key, and tighten screw(s).
- Remove plastic key from Isoflurane bottle, replace bottle cap, and replace key & bottle where they came from.

Note: If you have 2 upper screws and no metal key (upper left picture), the lower top screw (A) blocks the reservoir holes while machine is in use – open this completely, tighten upper screw while filling key is in place, then tighten lower screw completely when finished filling liquid reservoir.

*If unclear, ask the building manager or RST to show you how to fill the machine before you need to use it for a procedure.

During Filling

Vaporizer with 2 fill-ports, upper and lower, with 2 holding screws each.
Isoflurane Spills

If using a ducted hood or vent, it should always be ON while using the vaporizer
- Hood sash should be at manufacturer level indicated
- Isoflurane fumes can cause unconsciousness & need time to dissipate

For a small spill inside the hood/under a vent:
- Liquid will vaporize within a few minutes

For a small spill outside the hood/vent:
- Soak with paper towels & place in hood, liquid will vaporize in a few minutes

For a large spill inside the hood/vent:
- Place paper towels and move to one side/away from your animal
- If doing a procedure, closely monitor your animal for any possible anesthetic changes until liquid has been completely vaporized - this may take several minutes
- Do not let an animal touch the liquid!

For a large spill outside the hood/vent:
- Soak with paper towels & place in hood
- Leave the room/area for at least 10 minutes to give fumes time to dissipate

If you are not using a hood/vent and have a large spill:
- Place paper towels
- Open all doors & windows in the immediate area
- Leave for at least 10 minutes to allow fumes to dissipate via the building ventilation system
- Alert others working in the area that there has been an anesthetic spill and they need to leave
- Alert EHS if needed/fumes do not seem to be dissipating

*For a small spill with no hood/vent, place paper towels and move them to an open area to let fumes dissipate

*For all spills, clean the area and use caution if picking up broken glass/bottle shards

*Small spill = few drops; large spill = requires more than 1-2 paper towels to soak up
Non-rebreather Tubing

Pop-off valve (top) with exhaust tubing attached (bottom)

Reservoir bag

Exhaust tubing

Exhaust end in hood or attached to FAIR canister (+/- adapter)

+- Connective tubing

Clear line attached here to nosecone (removes to attach to induction box)

Nosecone attaches here

End of clear line attaches to vaporizer here (+/- adapter)
Make sure all pieces fit snugly together and to the vaporizer before you need them for a procedure - an adapter or additional clear tubing may be needed:

- Attach the clear tube of the non-rebreather anesthesia tubing set to outlet port on machine.
- Attach other clear end to induction box via port or to non-rebreathing tubing set via L-adapter near nosecone.
- The clear tubing is moved between the induction box during Induction and nosecone during Maintenance.
- You can only work with 1 animal at a time while under anesthesia - either in the box or on the nosecone, but not both at the same time.

*Note: the clear tubing can be cut if it is too long or you need a small piece as an adapter.
Tubing

Rebreathing/reservoir bag attaches to far end of clear corrugated tubing, below pop-off valve

- The reservoir bag usually comes as part of a new tubing set, you can toss when worn and tape over end

Pop-Off switch/valve should **ALWAYS** be open!!!

- Gas exits through exhaust tubing
- Is only closed when breathing for/‘bagging’ the patient: putting manual pressure on reservoir bag to force lungs to expand

**Due to small lung capacity, you will never use with rodents!**

Reservoir bag should always lie flat, if it is expanding the pop-off valve is closed and your animal is not breathing! Open valve immediately and check your animal!

---

Minus reservoir bag (w/ end taped)

Pop-off valve open and internal view

Rebreathing bag attached to pop-off connector

Bag lying flat (not connected)
Exhaust tubing (usually blue, corrugated) moves waste gas (CO2 and excess O2/Isoflurane) away from the animal.

- Attaches to small side opening of pop-off valve
- End of exhaust tubing stays inside a ducted hood or under a vent
- Gasses are directed to the building ventilation system and to the outside via hood/vent

*Per EHS, if working without a hood or vent or if they are not ducted, you must use a scavenging system (i.e., F/air canister) to collect waste gas!
Isoflurane vaporizer is preferably used under a ducted hood or vent.

If working on an open benchtop, there must be either an active or passive scavenging system utilized to ensure waste gases are collected.

Prior to use, EHS will evaluate the tentative location of the vaporizer to ensure there is a minimum number of air exchanges in the area for any isoflurane gas that may potentially escape the system.

Once approved, the corresponding EHS form must be posted beside the Isoflurane vaporizer.

- Contact EHS @ 919-962-5507 to setup an appointment
- Email: chemsafety@ehs.unc.edu

Required References:
- EHS Isoflurane Fact Sheet
- Waste Anesthetic Gas
F/air canister

Passive scavenging utilized to capture anesthetic waste gas

- Gasses are trapped via activated charcoal

Attach the end of the non-rebreathing exhaust tubing into/on the canister port

- Adapters may be needed for smaller tubes - all connections should be snug to prevent gas leakage

Attach a 2nd canister to the induction box; turn off the Isoflurane & depress the Oxygen Flush button prior to opening the box/ removing the animal - this flushes the isoflurane gas into the F/air canister

Place at an equal level as the vaporizer

- Tape to the edge of work surface or set into holder on back of vaporizer (if present)

DO NOT lay canister on its side - gasses will not distribute evenly

DO NOT sit canister on a solid surface or block the bottom openings - air must flow freely (~1cm clearance needed)

Write the date & initial weight (in grams) before first use on the canister. Check and note the weight before every subsequent use

- When the canister gains 50g - bag and call EHS to pick-up & replace with a new one
Active Scavenging

Pressure or suction actively pulls WAGs (waste anesthetic gasses) into an appropriate container for later disposal, preventing exposure to personnel.

- If working in/under one, a ducted hood or chemical hood/vent performs the scavenging for you.
- Connects to a ventilation system or passive receptacle (F/air or other charcoal canister).

May require a means to protect patient from vacuum/positive pressure buildup.

- System must be attenuated prior to use for proper functionality.
- If suction is too high, may cause trauma to patient or not enough anesthetic exposure, resulting in patient awakening during procedure.

Machine must be turned on prior to use, and off after procedure is completed.

Typically required with USDA species as oxygen & isoflurane flowrates are higher, causing a greater exposure risk to personnel.

Required References: EHS SOP for Isoflurane
IACUC Standard for Inhaled Anesthetics
Split Outflow Tubing

If preferred, you can have one clear line to the induction box & one to the nosecone. Toggle between them using 3-way valves or tube clamps to stop gas flow to the other while performing the surgical procedure.

- This setup allows you to start the induction process with another animal while finishing up the previous one, when anesthetic gas is no longer needed.

Note: you cannot use two different vaporizer settings (%) if the machine setup does not allow for this! If it does, there will be multiple oxygen flowmeters and percentage settings for isoflurane.
*Induction box + Nosecone*

Ordered separately from tubing set

- Rubber replacement covers/diaphragms for nosecones also ordered separately

### Induction box

- Comes in various sizes
- Costly but repays itself quickly

*Store-bought plastic containers are not graded for anesthesia use and are not advised as an alternative

### Nosecone

- Comes in various sizes
- Rubber diaphragms are cut as needed to fit your animal's muzzle – typically in an 'X' or 'O'
- Should snugly fit the animal's nose/muzzle to prevent isoflurane from leaking around the opening
- Rubber is soft but can cause ocular trauma so do not let it touch the eyes

*Gloves are not graded for anesthesia use and should not be used as a diaphragm replacement for the nosecone*
Vaporizer

Settings are in percentages (%); percentage equals amount of fresh gas saturated with anesthetic.

Want the lowest setting needed for the procedure you are performing.

- To turn on, press button on the side or top of turn-wheel – it locks in ‘Off’ position but freely turns otherwise.
- Make changes in ½-percentage increments.

Vaporizers are heavy! If you need to move machine, lift by handles and do not tilt! If you tilt a machine >45° it will not function properly & will need to be recalibrated before using again.

Should be recalibrated every 2 years – DCM calibrates their machines yearly due to frequent use.

Note: At least 1 procedure room per building is equipped with a vaporizer machine, oxygen, and liquid isoflurane for PI use – all other equipment should be provided by the lab.
**Induction**

*Anesthetic overdose and cessation of breathing is highly possible during this stage, so always closely monitor your animal!*  

A higher isoflurane percentage/\% is preferred to induce quickly, a lower \% takes longer for animal to become unconscious & is stressful

- Place animal into induction box & close lid, turn on oxygen (1L/min), set isoflurane between 3.5-5\%
- Wait a few minutes until animal lays down (<5min, usually 2-3min) & does not respond to gentle tilting of the box (animal should slide across the bottom with no resistance)
- **Quickly** turn isoflurane to 0\% & flush induction box with oxygen before opening (using O2 flush button) if on a benchtop
- **Quickly** move clear tubing from induction box to nose cone. If using 3-way stopcock or tube clamps, open nosecone line & close induction box line
- **Quickly** move animal to nosecone & turn isoflurane to Maintenance percentage (1.5\%+, dependent on procedure)

The animal can wake up if they take 2-3 good breaths without anesthetic! This must be done **QUICKLY**!

Note: your IACUC protocol should list a range of induction percentages to use due to individual animal responses to isoflurane
Mouse unresponsive to gentle tilting of induction box

Moving mouse quickly onto nosecone and clamping tubes/switching gas lines

*Isoflurane percentage was turned down off-camera
Animal Placement

Nose & mouth only go into nosecone

Do not place the animal so rubber diaphragm is touching the animals’ eyes or over the entire face

• Diaphragm can cause eye trauma
• Gas/air flow into face can cause increased drying of eyes or worsened hypothermia

The blink reflex is the first to go - patients cannot blink while under anesthesia!

• Lubricate the eyes quickly after moving to nosecone/turning down isoflurane %
• Do not touch ointment tube directly to animal’s eyes – this may cause trauma or compromise sterility of lubricant
• Using a new cotton swab with each patient, touch only lubricant to eyes & apply liberally to ensure eyes are covered – the animal will clean any excess off when they awaken
• Can close lids together to spread lubricant evenly
• Recheck & reapply ointment if needed throughout procedure (~every 30min)
• Lubricant does not need to contain antibiotics or steroids
Animal Placement

You must always have a barrier (paper towel, etc.) between the animal and work surface, even if your procedure is ‘short’

- Animals under anesthesia cannot thermoregulate, so hypothermia is of high concern
- Vivarium work surfaces are typically metal or Formica and quickly wick warmth away
- Rodents lose a lot of heat from their tail, so it should be placed alongside the body and on the heat source
- Do not place animal directly on the heat source

Mouse on a wypall for a short procedure

Heat pack between wypalls
Heat

A heat source must be provided if procedure is more than a few minutes as patients cannot thermoregulate while under anesthesia.

Animal must be on heat from Maintenance through Recovery until fully awake and acting normally.

Preferred examples:
- Warmed surgical boards
- Chemical reusable heat packs
- Warm water/air circulating blankets

If using a heat lamp or electric heat blankets, make sure to maintain them well: check regularly for wear, heat gradients, or visible wires so your animal does not become overheated or burned.

Do not place animal directly on the heat source! Use a barrier option (towel, paper towel/wypall, etc.) between animal and heat source.

Can use a thermometer beside the animal to monitor temperature – ideally, between ~82-85°F.
Immediately after placing animal on the nosecone, turn isoflurane down, typically between 1-3% for most procedures.

- Your IACUC protocol should list a range of Maintenance percentages available for use due to individual animal responses.
- More painful procedures will require a higher setting (3-5%) (Examples: intra-cavity, ocular, bone reparation/healing, etc.)

*If you need to change the isoflurane percentage because the animal is too deeply under/not deep enough, the depth of anesthesia will not change instantly! It takes a few minutes for the new setting to reach the animal, then it has to infiltrate the bloodstream - give them a few minutes for the new setting to affect them.

*Once on the nosecone with the eyes lubricated, check your animal’s breathing pattern.
Breathing

Rodents’ breathing will always be fast due to high metabolism

- Even and steady with a regular/constant rhythm is the goal
- Thoracic = lighter anesthetic depth vs. abdominal = deeper
- May see breathing character differences if animal is lying on back or side – sternal is normal rest position for rodents and easiest for lungs to expand

Monitor breathing & extremity (tail, ears, paws) color regularly throughout procedure, and toe pinch occasionally to check depth of anesthesia
Agonal Breathing

YOU DO NOT WANT AGONAL BREATHING!

- Taking very slow/rare, very deep breaths
- Using lower abdominal muscles & appears to be struggling, may be open-mouth breathing
- Not getting enough oxygen/too deeply anesthetized

The animal is close to respiratory arrest!!!

- Immediately turn isoflurane down by a 0.5% and closely monitor patient for improvement of breathing
- Make sure oxygen flowmeter is set to 1L/min & nosecone is correctly placed over animal’s muzzle
- May need to decrease Isoflurane again if not improving/still struggling after a minute or two
- **Stop the procedure!** Can resume surgery once animal is stable
- If not in middle of surgery, can turn isoflurane off or remove animal from nosecone

DO NOTwake the animal if it has an open cavity!!!

- If still performing the surgery, keep isoflurane at 0.5-1% & quickly close cavity/end procedure as described in your protocol; when finished with the surgery, turn off isoflurane & let the animal breathe only oxygen until it awakens

If animal stops breathing:

- Take off the nosecone and let breathe normal air +/- awaken if possible (**do not** do this if patient has an open body cavity!)
- Postpone that animal’s procedure until a later time – you may need to replace if animal has repeatedly struggled while under anesthesia
- If you suspect patient is deceased, perform a secondary physical method to ensure euthanasia

If you notice blue-tinged (cyanotic: low oxygen) or pale (anemic: low iron/blood flow) extremities:

- Turn down Isoflurane % and/or finish procedure quickly
- May turn oxygen up to 1.5L/min but remember you are delivering more anesthetic to animal since you increased the flow rate - you may need to turn Isoflurane setting/% down to compensate
After checking the breathing pattern, perform a toe-pinch:

- Testing for a pain response before actually performing the surgery and initiating a painful technique
- Pinch the first joint of the toes &/or foot pads of all 4 feet using the fingernail of one finger to fingertip of the other – you may need forceps to reach front feet
- Pinch well/hard but not enough to break any toes!
- If animal moves the limb/body or the breathing changes, it is not ready for the procedure - wait several minutes and try again
- May need to increase isoflurane % if consistently getting a pinch response – increase in ½-increments until no response noted
- Still may need to increase % if you notice response to a potentially painful technique (i.e. cutting any tissue) – this indicates the animal can still feel a pain stimulus!
Recovery

Once surgery is completed, turn off Isoflurane and allow animal to breathe only oxygen until signs of awakening (movements) noted

- Anesthetic is breathed off more quickly than if just removed from nosecone
- Should take only a few minutes to return to normal awake status

Place animal into a clean cage with a solid medium (paper towel, etc.), no other animals, & no bedding (due to possible aspiration of dust or small particles)

Have heat source under half of recovery cage so animal can move toward or away as they prefer

Monitor for return to normal ambulatory status/sternal recumbency before moving to regular housing cage

- May see abnormal movements as brain reassesses functionality: rolling, head/limb stretching, wobbling gait - this is unconscious behavior and animal has no control/is not yet fully awake

Providing SQ fluids

Heat pack under cage
Injectable analgesic medications can be given just prior to awakening

- Do not give oral analgesics - animal must be fully awake before administering these medications so they do not aspirate!

Giving a small bolus of warmed fluids (saline or LRS, via SQ route) will help animal recover more quickly and prevent dehydration complications

If constrained due to time, can move to recovery cage without breathing only oxygen, but may take longer to awaken since breathing room air (<100% oxygen)

All animals should be fully awake and ambulatory (walking) before placing back with cage mates & returning to the animal housing room

To help them recover more easily, +/- place food pellets/moist food, hydrogel, caloric gels, a water bottle or other support options on the floor of the home cage for animals that had longer procedures performed (~30min+)

Remember to place appropriate cards and monitor post-op animals by the frequency indicated in your protocol
Cleaning

Non-rebreathing tubing + induction box + nosecone should be cleaned regularly so debris is removed & pathogens are not transferred to other animals in the colony.

Does not have to be after every procedure unless used heavily: ~weekly/biweekly if in regular use, less frequently if rarely used.

Chlorhexidine is a mild, cheap disinfectant:
- Dilute 2 oz (or 2 Tbsp) per gallon of water in a sink, etc.
- Soak for ~10min
- Rinse well with water & allow to air dry (hang tubes up to drip-dry)

Soap + water: rinse well to remove soap residue especially from tubing, allow to air dry.

Alcohol solutions should not be used for tubing and some induction boxes.

Check tubing, nosecone, & induction box regularly and replace pieces as needed as they become worn/develop holes/tear, etc.
Clinical Assessment of Hydration

1) Minimal Dehydration: 4%
   a. Skin resiliency: pliable
   b. Skin tenting: skin disappears slowly or persists up to 2 secs
   c. Eyes: bright, slightly sunken
   d. Mouth: moist, warm

2) Moderate Dehydration: 6-8%
   a. Skin resiliency: leathery
   b. Skin tenting: tented skin very slowly returns to normal or persists for 3+ sec
   c. Eyes: duller than normal, obviously sunken
   d. Mouth: sticky to dry, warm

3) Severe Dehydration: 10-12%
   a. Skin resiliency: no pliability
   b. Skin tenting: tent persists indefinitely does not return to normal, fur may come out with scruff
   c. Eyes: deeply sunken, corneas dry, ~2-4mm space between eye globes and bony orbits
   d. Mouth: dry, cyanotic, may be warm-to-cold

*In general, mild, moderate, and severe dehydration can be treated with 0.25-0.5ml, 0.5-1ml, & 1-2ml of fluids in mice, and 1-2ml, 2-5ml, and 5-7ml for rats, respectively

*For weanlings: offer ~1/2 of indicated volumes listed
*For pinkies: no more than 0.1-0.2ml for mice, 0.5-1ml for rats

Fluid Therapy

It is beneficial to offer rodents undergoing surgical procedures a means of maintaining hydration.

Can give pre-, peri-, or/ & post-operatively

Fluid dosing is independent of procedure length; amount to administer depends on degree of dehydration. Always warm the fluids to body temperature!

Split larger volumes between multiple locations to limit skin stretching.

Easiest method is via SQ injection with saline (0.9% sodium chloride) or LRS (lactated ringer’s solution)

- Maintenance Dose for mice = 100ml/kg or ~0.5-1ml q2hr
- Maintenance Dose for rats/hamsters/gerbils = 150ml/kg or ~2-3ml q2hr

Performing a Skin Tent to Test for Hydration:

Scruff and release any extra dermal tissue (typically @ the shoulders) to test for length of return to normal position

- Normal = <1 sec or too fast to note
- Mild = 1-2 sec
- Moderate = 2-3 sec
- Severe = 3+ sec or no return to normal
Remember to document all anesthesia procedures (IACUC requirement)

It is not preferred to hold the animal on nosecone to induce anesthesia – this is highly stressful to both the patient and handler.

You can use a large dead animal bag (rat-sized) in place of an induction box, but only if you are using a ducted hood. These are provided in all procedure rooms.

- Place animal into bag, hold top of bag closed around nosecone, turn on oxygen and isoflurane - wait until gentle shaking of bag does not receive a response (standing, trying to move) then remove animal from bag and quickly place onto nosecone.
- This is not an airtight seal & bag will not fill/balloon-out.
- Place rats on a surface (hood/bench-top) so they have weight support.

You can use a lower percentage to induce (as noted in your IACUC protocol):

- Less likelihood of animal overdosing/entering respiratory arrest.
- Takes several minutes longer to become unconscious as they are exposed to a lesser concentration of anesthetic.
- Check for blink reflex & apply lubricant if needed while still in induction box.

You can stimulate animals to waken after the procedure by lightly pinching the tail/rear feet or rubbing the back, but remember they have teeth and may use them.

Be mindful of where you place post-op injectable medications - if they are near your heat source or supportive fluids this may affect their release rate/length of effectiveness.
Anesthesia is very patient-dependent and you may see various responses even within the same cohort:

- Overweight, younger, or older animals will probably take longer to awaken
- Rats appear to be sensitive in general (easily go under, take longer to awaken)
- C57Bl6/J mice will hold out and stop breathing immediately after they stop moving (during induction) – watch them closely!
- CD-1 mice in general appear sensitive to anesthetics and struggle to breathe if placed on their backs

If the oxygen gauge or flowmeter does not completely go down when you turn off the tank, there may be gas left in the line: press the oxygen flush button to clear it.

- **DO NOT** do this with an animal on the nosecone! Remove the animal first!
Alternate Tubing

Some alternate nosecones and non-rebreather tubing

Note: if the exhaust tube is connected through the reservoir bag (as pictured in ‘B’), make sure the pop-off valve is still Open.
Anesthesia Planes

Patients display different body responses at different depths of anesthesia. There are 4 classic stages of anesthesia: disorientation, excitement, anesthesia, and moribundity.

**Stage 1**
- Animal is disoriented, respirations can range from normal to panting, heart rate is unchanged.
- Good muscle tone, all reflexes present.

**Stage 2**
- Animal becomes excited and may struggle, vocalize, chew or yawn.
- Breathing becomes irregular and patient may hold their breath or hyperventilate, heart rate increases.
- Good muscle tone, all reflexes present and may be exaggerated.

**Stage 3 includes 4 Planes of Anesthesia:**

**Plane 1** - light anesthesia
- Breathing becomes regular, heart rate is within normal ranges.
- Good muscle tone, all reflexes present.

**Plane 2** - light surgical anesthesia
- Breathing is regular, may be shallow, heart rate is within lower end of acceptable range, pulse is strong.
- Heart rate and respirations may increase with surgical manipulation.
- Muscle tone is relaxed, toe pinch may be present, patellar, palpebral, & corneal reflexes may be present.

**Plane 3** - deep surgical anesthesia
- Respirations are shallow and at low end of normal range, heart rate is slow, capillary refill time is increased.
- Muscle tone is greatly reduced, all reflexes diminished or absent.
- No response to surgical manipulation.

**Plane 4** - overdose of anesthesia
- Respirations are jerky, heart rate is below normal values, capillary refill time is prolonged and mucous membranes pale.
- Flaccid muscle tone and no reflex activity.

**Stage 4**
- Animal is moribund or dying.
- Respirations are apneic, there is cardiovascular collapse.
- Muscle tone is flaccid, no reflex activity.
## Isoflurane Vaporizer Log

<table>
<thead>
<tr>
<th>Date</th>
<th>Procedure/Protocol</th>
<th>Start Time Isoflurane</th>
<th>End Time Isoflurane</th>
<th>% Isoflurane (Range)</th>
<th>Animal Numbers</th>
<th>Toe Pinch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Post-operative monitoring/analgesia

- **Procedure**
  - Performed: __________________

- **Date**: ____________

- **PI name/Protocol #**: __________________

- **Cage Card #**: __________________

- **Analgesia name/dose**
  - (mg/kg) ____________

- **Give** (circle one)
  - 1x/day
  - 2x/day
  - Other _______ for _______ days

- **Date**
- **Initials**

- **Comments/postoperative observations**:
  - __________________
  - __________________
  - __________________
  - __________________
Additional Required Readings BEFORE Class

- **EHS Isoflurane Fact Sheet**
- **EHS Policy on Waste Anesthetic Gases**
- **Rodent Anesthesia Guidelines** (Open the PDF for this training listed on DCM page)
- **IACUC Standard for Anesthetic Vaporizers**
- **IACUC Anesthetized Procedure Standards for Rats and Mice (Non-Survival and Survival Procedures)**