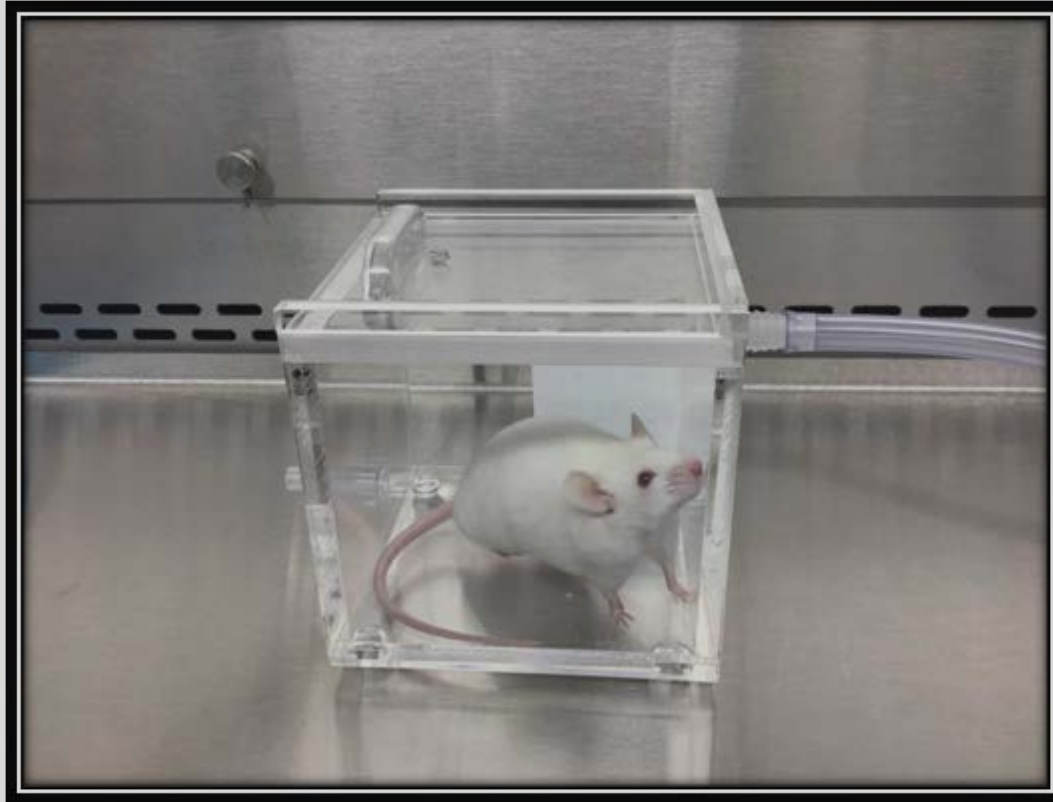




ISOFLURANE  
VAPORIZER  
ANESTHESIA

# Overview



- Liquid anesthetic is converted to a gas in the vaporizer and carried by oxygen to animal
- Easily and quickly allows for induction, maintenance, and recovery due to inhalant route of delivery
- Constant monitoring of patient is required due to cardiac and respiratory depression by 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 animal to maintain them under anesthesia during a procedure
- F/air canister: captures exhaled CO<sub>2</sub>, and excess O<sub>2</sub> and isoflurane via activated charcoal granules so they do not dissipate into the room; requires regular weighing to determine replacement frequency – see manufacturer instructions

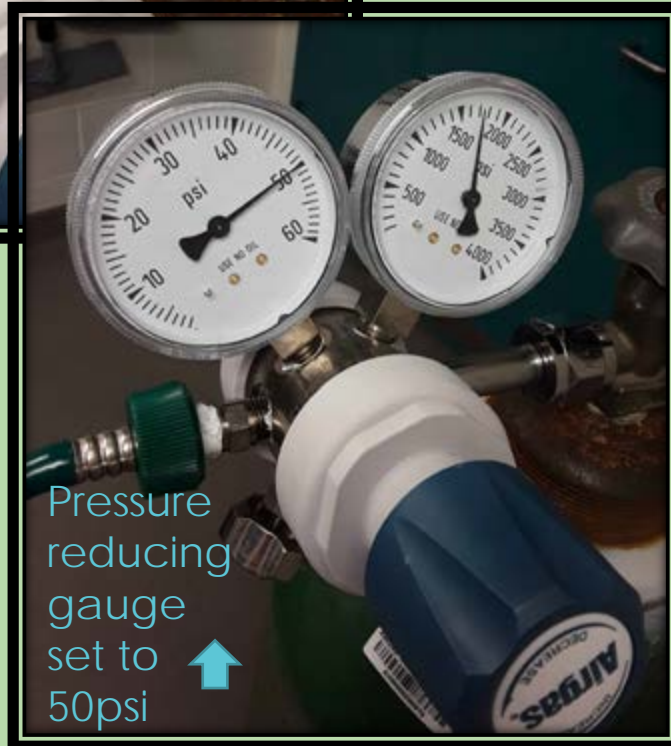


Tank Off

Oxygen Flush



Tank reading  
↓  
~1700 psi



Pressure reducing gauge set to 50psi ↑



Flowmeter Set to 1 L/min



Chained H tank

# 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

Upper window full



Reservoir Low



Lower window empty

## 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





Key attached to Isoflurane bottle



Filling key



Metal key in port during machine use



End of filling key and metal key

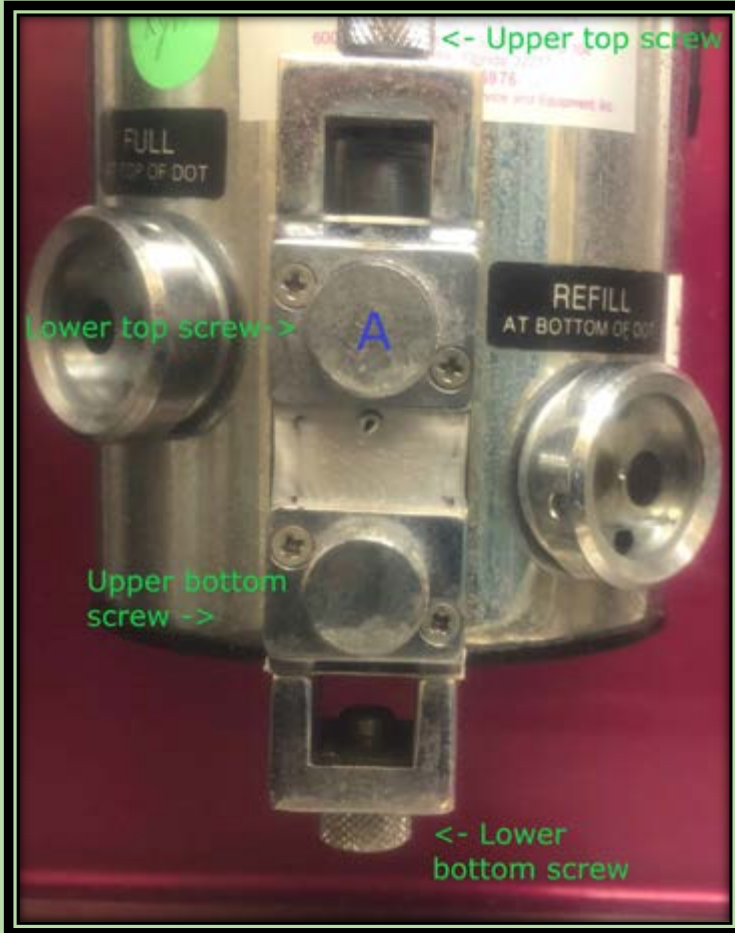


Holes in filling key will line up with holes in vaporizer port during proper placement

# No-Spill Refill

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



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

During Filling



## 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

# 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

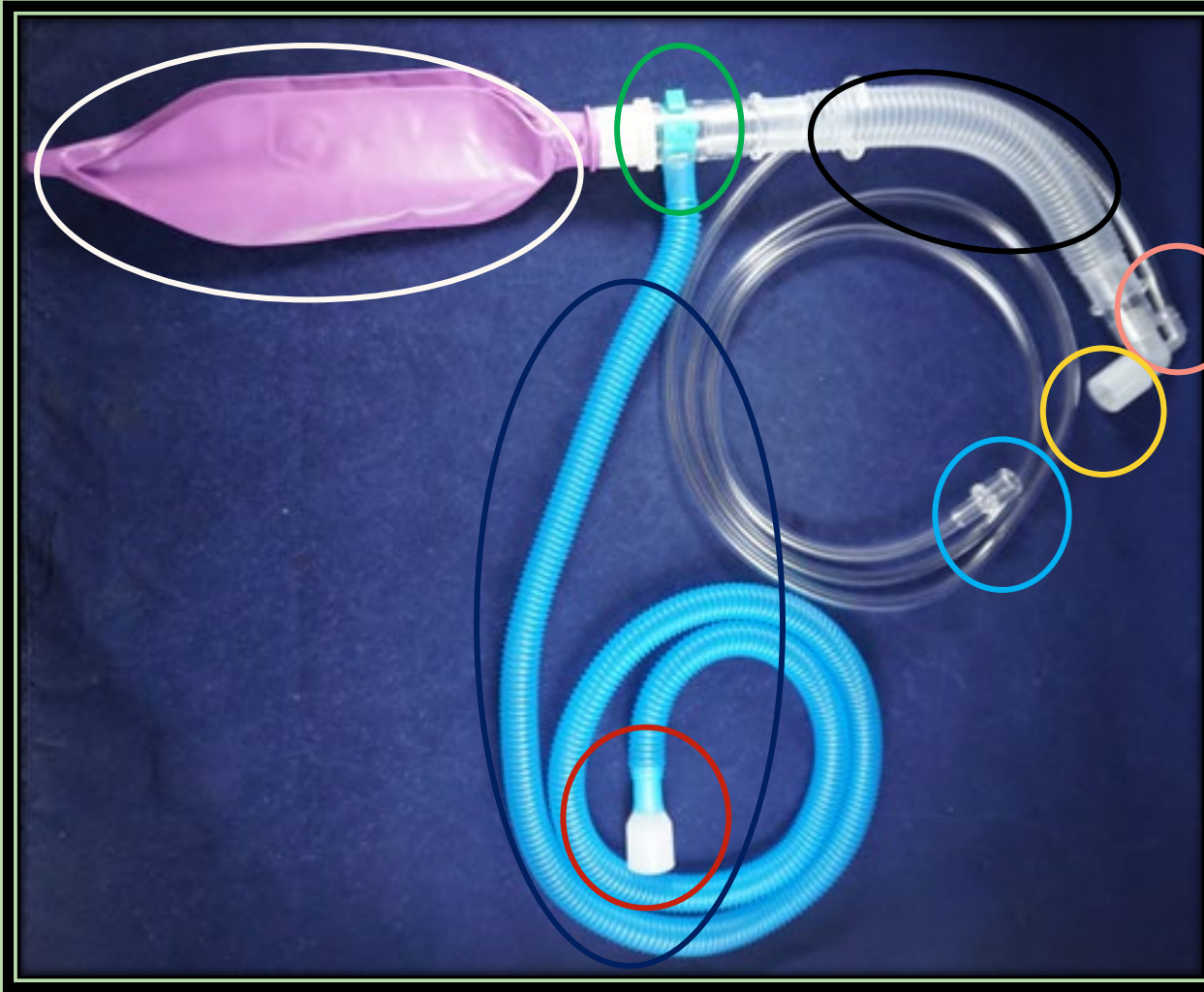
\*For all spills, clean the area and use caution if picking up broken glass/bottle shards

\*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 F air 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)



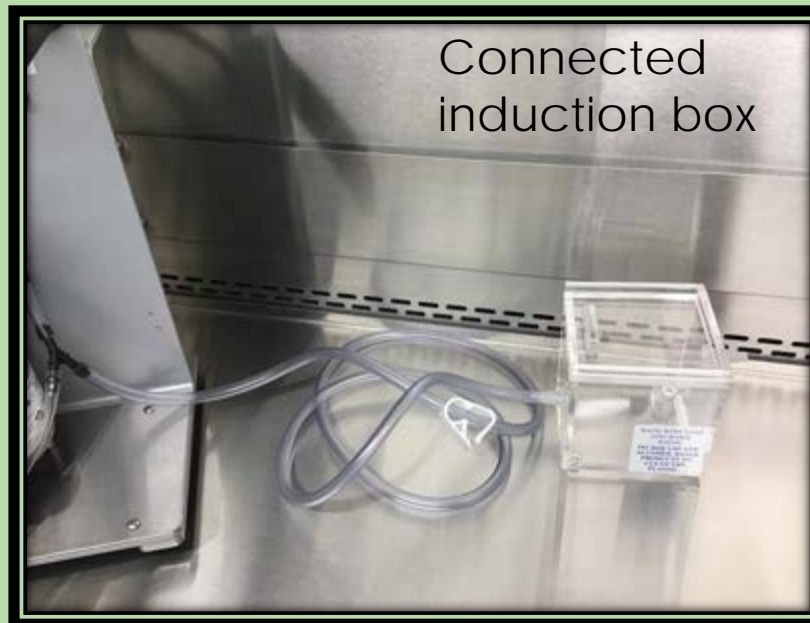
Outlet port



Clear line attached



Adapters



Connected induction box



Non-rebreathing tubing w/ nosecone

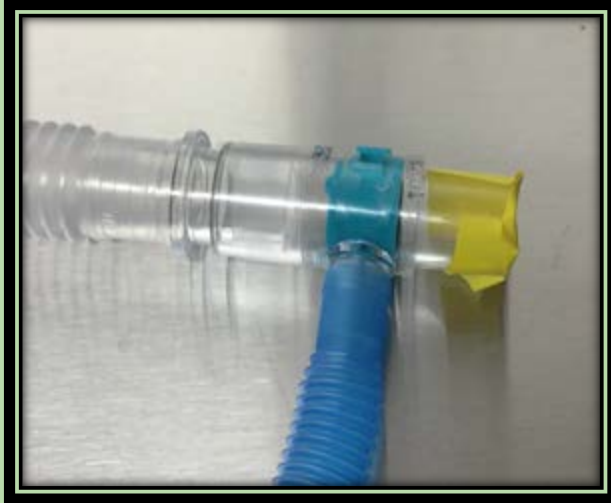
## Tubing

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

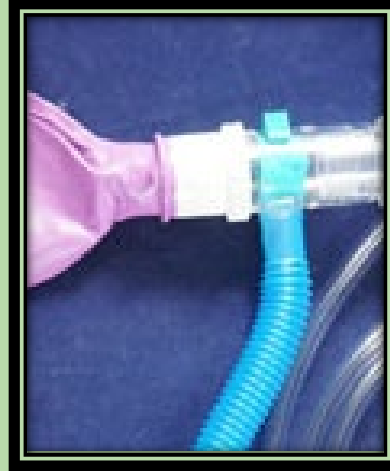
- Attach the clear tube of the non-rebreather anesthesia tubing set to outlet port on machine
- Attach other clear end to induction box via port or to non-rebreathing tubing set via L-adapter near nosecone
- The clear tubing is moved between the induction box during Induction and nosecone during Maintenance
- 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

Minus reservoir bag (w/ end taped)



Rebreathing bag attached to pop-off connector



Pop-off valve open and internal view



Bag lying flat (not connected)

## 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!



Exhaust tubing attaches to stem at pop-off valve



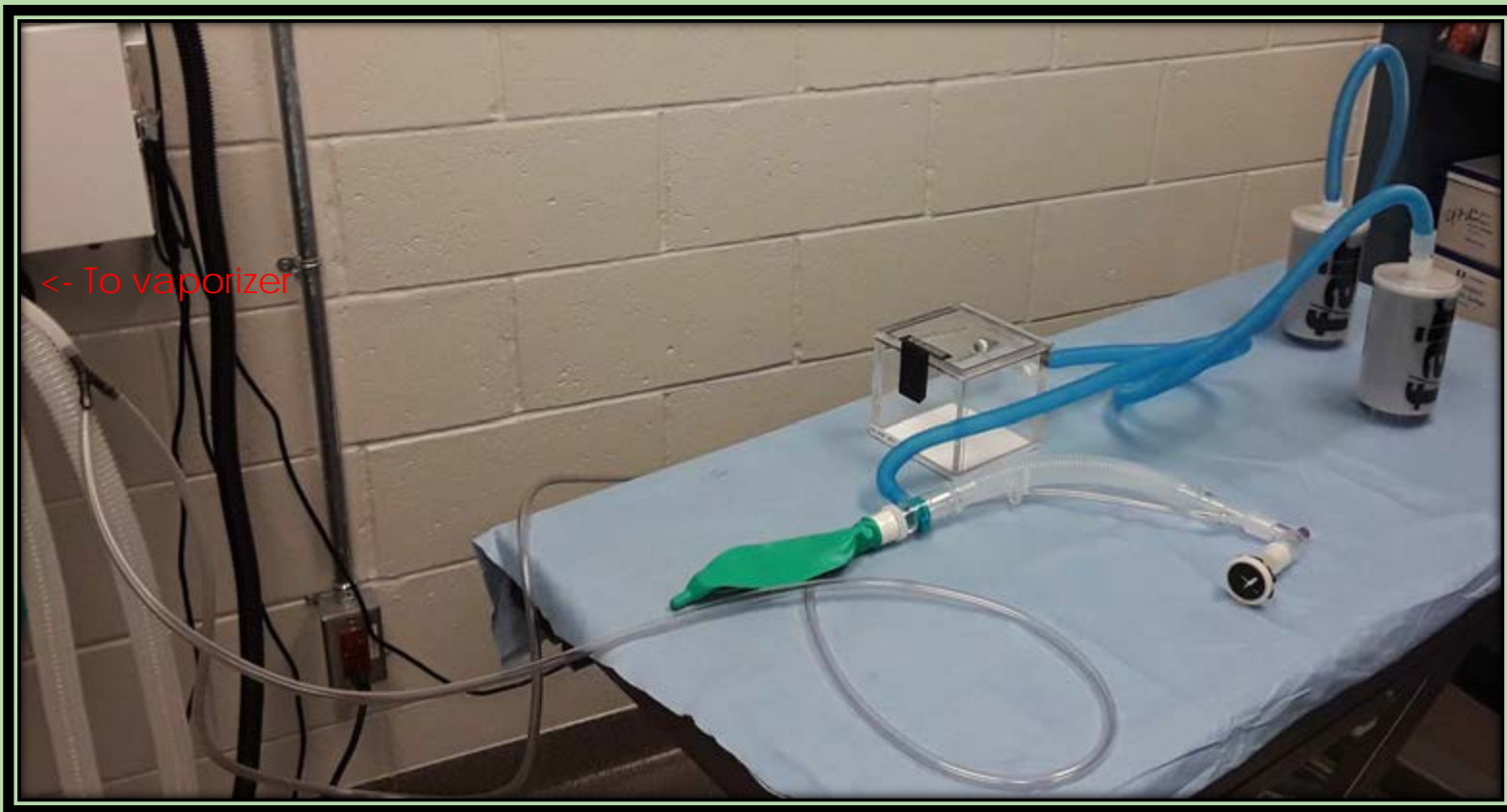
End of exhaust tubing inside ventilated hood

## Tubing

Exhaust tubing (usually blue, corrugated) moves waste gas (CO<sub>2</sub> and excess O<sub>2</sub>/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!



Full setup with F/air canisters (to show location in split setup)

## EHS Evaluation

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](mailto:chemsafety@ehs.unc.edu)

Required References:

[EHS Isoflurane Fact Sheet](#)

[Waste Anesthetic Gas](#)





Exhaust tubing attached



F/air slots on vaporizer



Weight before 1st use



Bottom openings for airflow

# 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 2<sup>nd</sup> 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 options

# 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

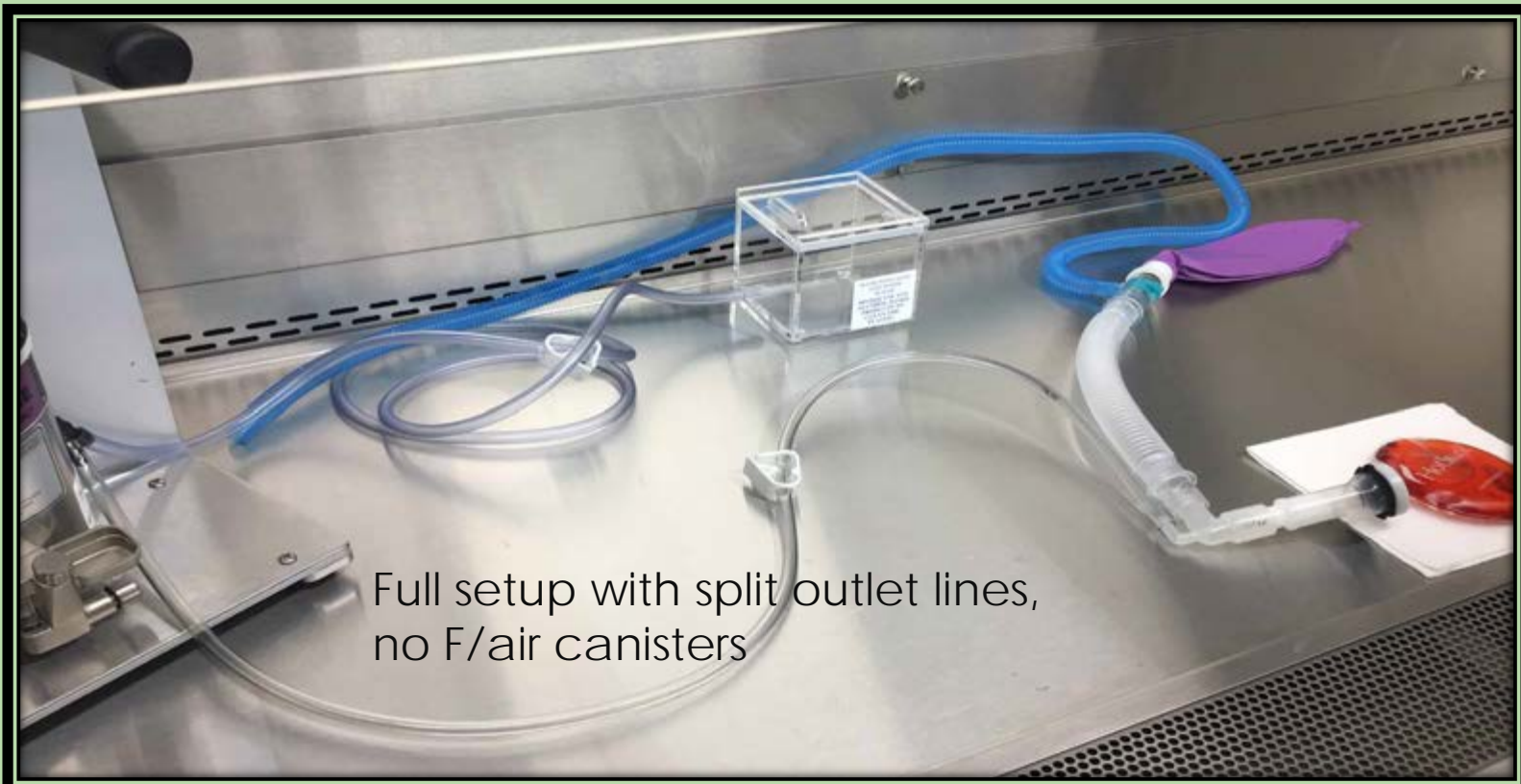
Required References: [EHS SOP for Isoflurane](#)  
[IACUC Standard for Inhaled Anesthetics](#)



Split  
outflow  
line



Tube clamp & 3-way valve



Full setup with split outlet lines,  
no F/air canisters

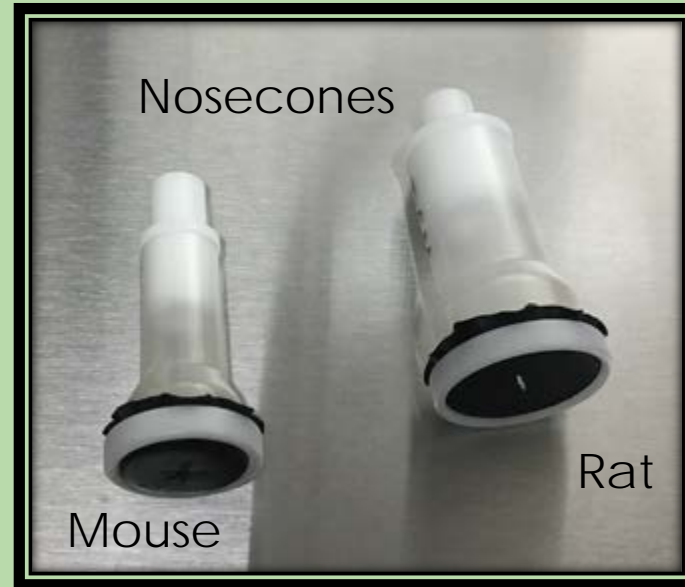
## Split Outflow Tubing

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

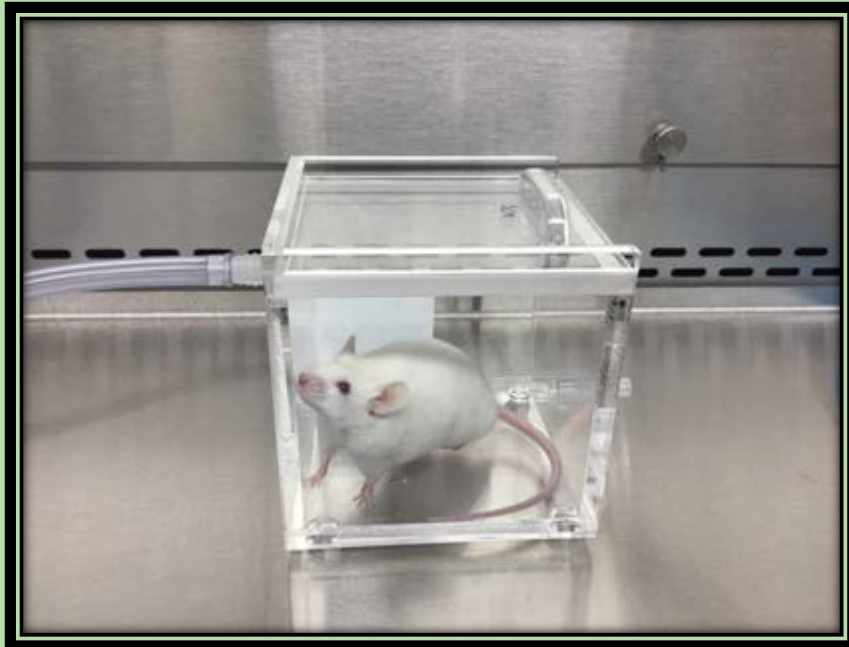
- To turn on, press button on the side or top of turn-wheel – it locks in 'Off' position but freely turns otherwise
- Make changes in ½-percentage increments

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

Vaporizer  
set to  
Induction %



Mouse just  
placed in  
induction box

# Induction

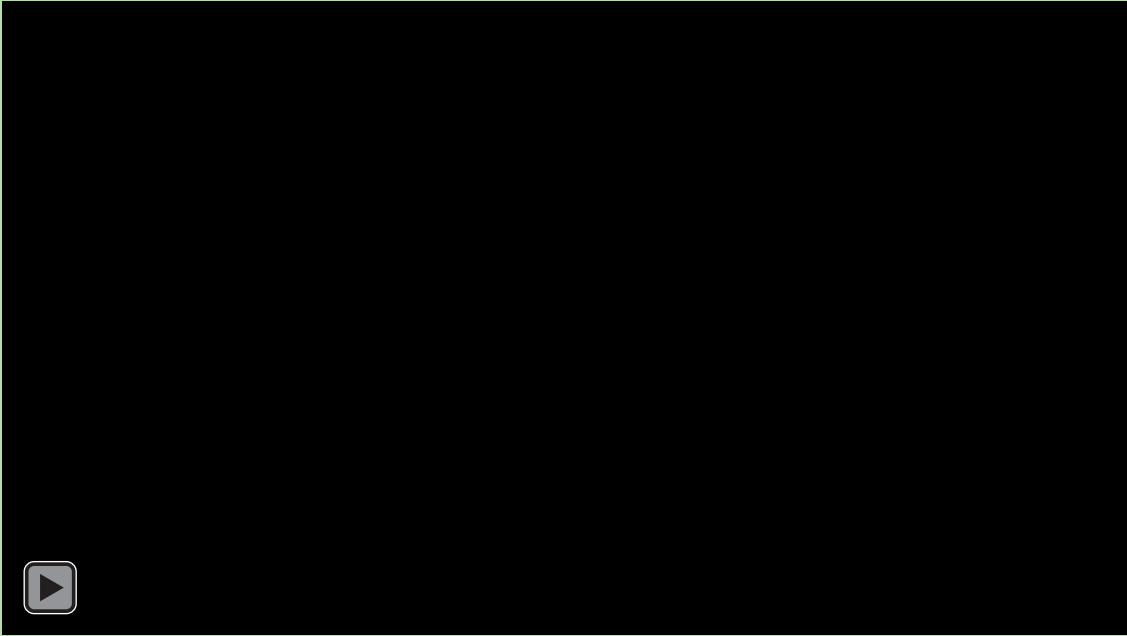
**\*Anesthetic overdose and cessation of breathing is highly possible during this stage, so always closely monitor your animal!**

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



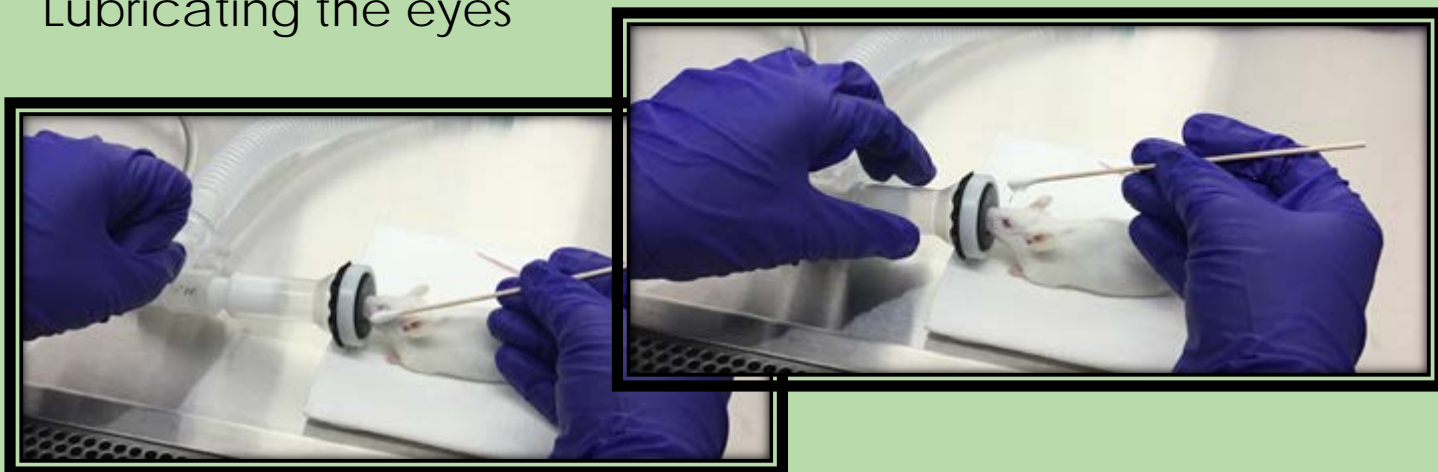
\*Isoflurane percentage was  
turned down off-camera



Eye lubricant on swab



Lubricating the eyes



## 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 waken
- 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



Mouse on a wypall  
for a short  
procedure



Heat pack  
between  
wypalls

## 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

Heat pack



Bair Hugger



Air circulating blanket



Water circulating blanket

# 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:

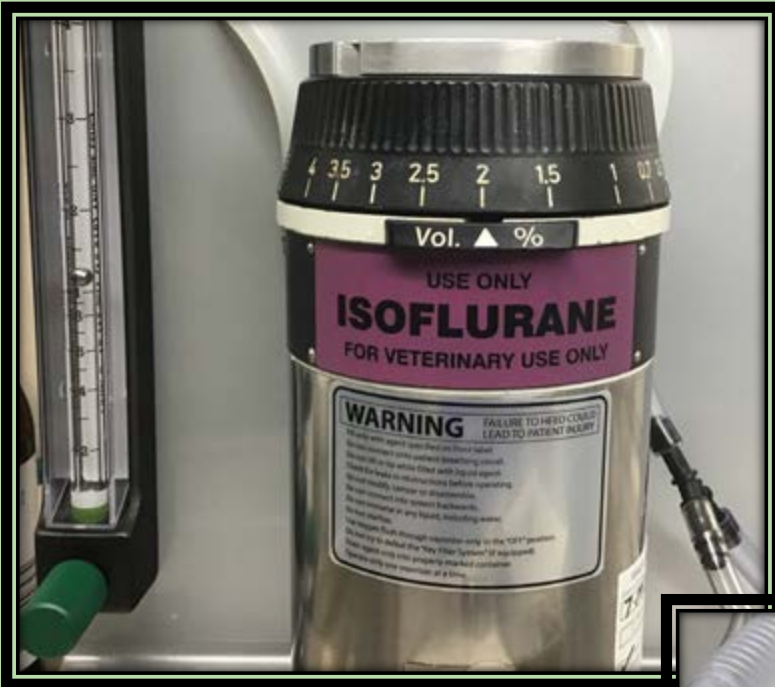
- Warmed surgical boards
- Chemical reusable heat packs
- Warm water/air circulating blankets

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

Vaporizer set to  
Maintenance %



# Maintenance

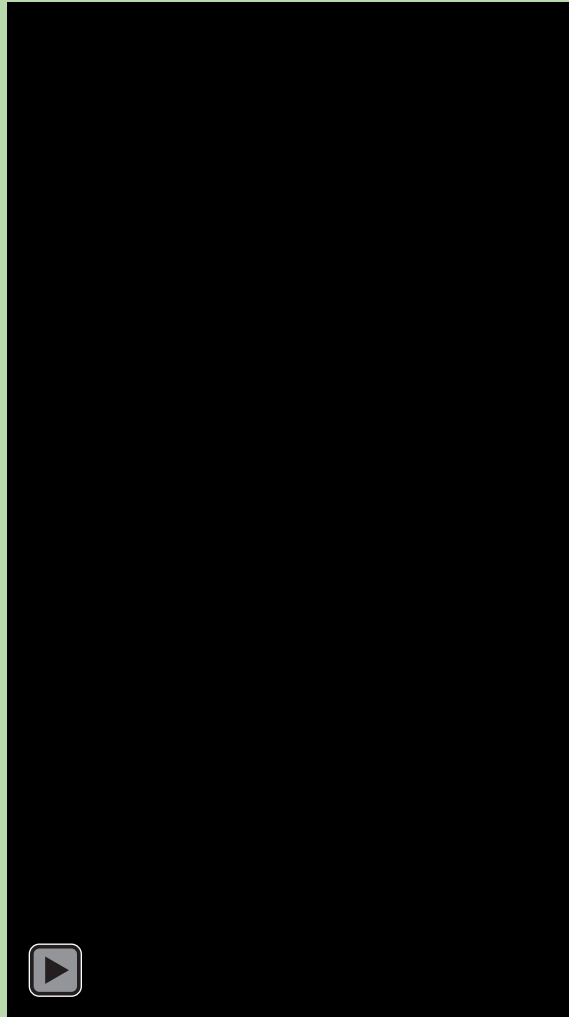
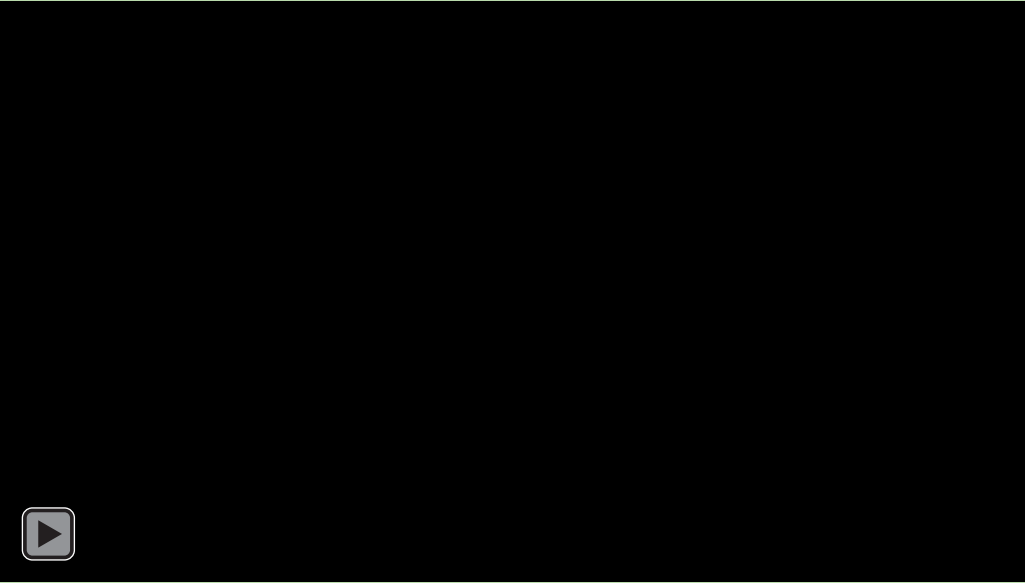
Immediately after placing animal on the nosecone, turn Isoflurane down, typically between 1-3% for most procedures

- 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

## Stable mouse breathing patterns



# 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 a 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 NOT wake 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

# Toe-Pinch Test

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!

Mouse under anesthesia with no response to a toe pinch test



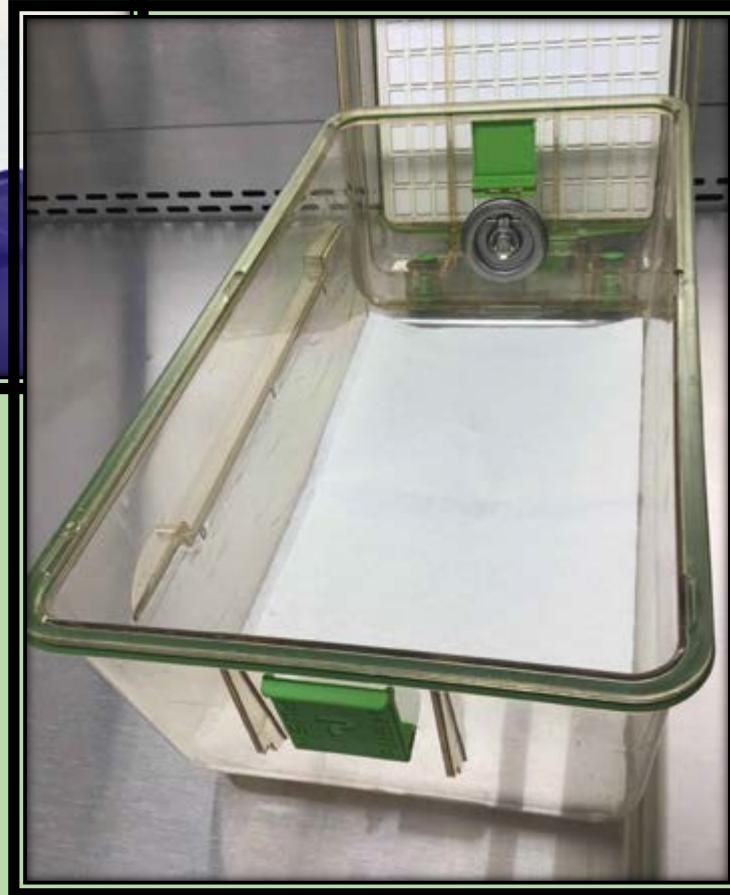
Toe pinching a rat



Providing SQ fluids



Recovery cage



Heat pack under cage

## 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

Mice  
waking up  
after  
anesthesia



# Recovery

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: tented skin disappears slowly or persists up to 2secs
- 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 = <1sec or too fast to note
- Mild = 1-2sec
- Moderate = 2-3sec
- Severe = 3+sec or no return to normal

# Miscellaneous Info

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 air-tight 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

# Miscellaneous Info

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

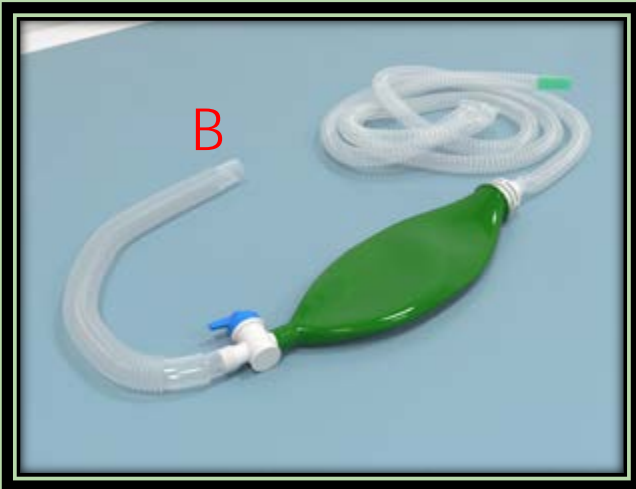
- DO NOT do this with an animal on the nosecone!  
Remove the animal first!

Oxygen Flush  
Button





← Low Profile  
↓ Tubing



# 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, reflexes are diminished
- Animal may respond to surgical manipulation

### 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 w/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



# 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 \(Non-Survival and Survival Procedures\)](#)