

Standard operating procedure Rodent Euthanasia

ISOFLURANE- CO₂ BOX

1. Purpose

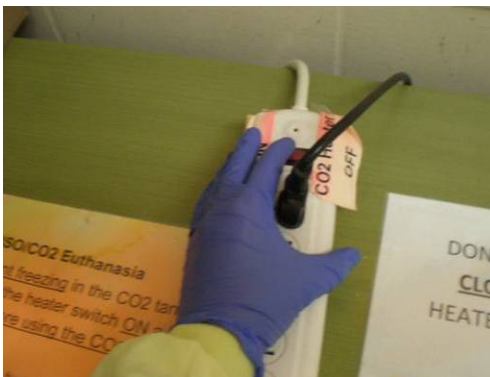
This Standard operating procedure (SOP) describes acceptable procedures for rodent euthanasia. It ensures that animals are euthanized in the most humane way possible.

2. Responsibility

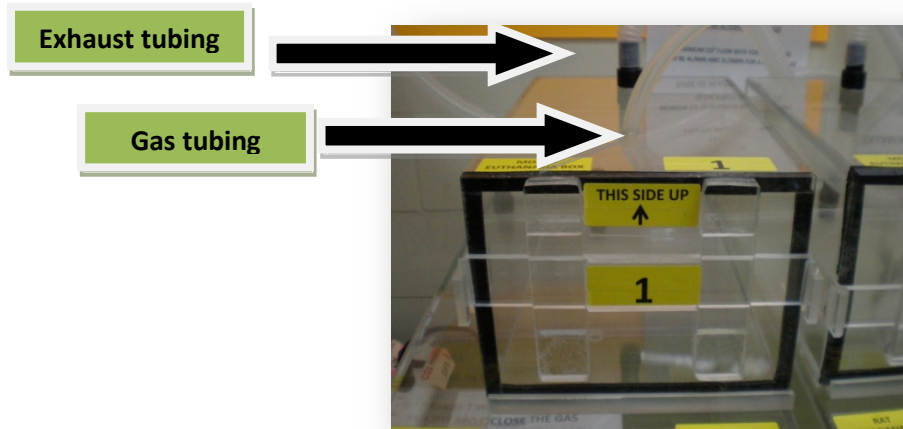
Animal health Technician, animal care staff, principal investigator and their research staff.

3. Euthanasia of rodents

- In order to minimize stress, animals should be euthanized in their home cages with a maximum of: 5 adult mice, 2-3 rats or one litter per cage (rat or mouse)
- Neonatal animals (up to 10 days of age) are resistant to the effects of ISO- CO₂, therefore, alternative methods are recommended. For neonatal, decapitation using sharp blades is recommended.
- To prevent freezing of CO₂ tubing, please turn the CO₂ heater switch **ON** a few minutes before using.



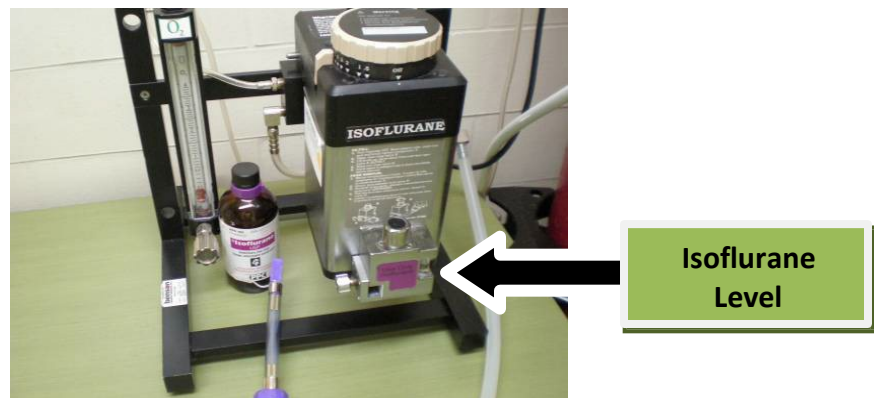
- Connect the hose (or silicone tubing) from the box to the Isoflurane machine. Be sure the hose is tightly connected to the Isoflurane machine.
- Connect the exhaust tubing to the Plexiglas box. One or two boxes can be connected in the same time.



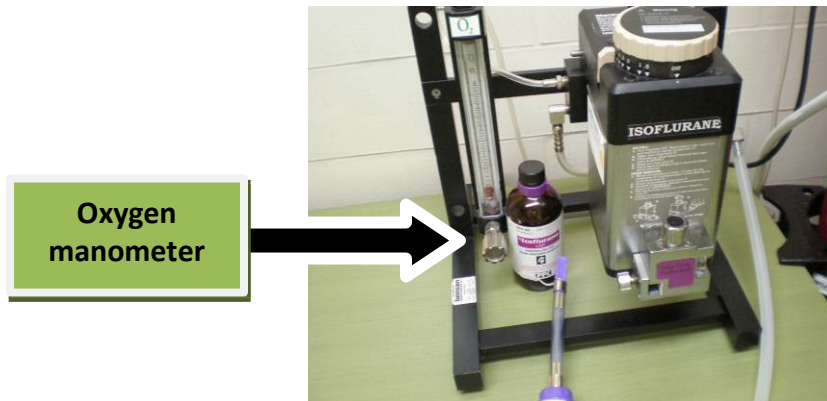
- Turn on the valves of Oxygen (white tank) and CO₂ (red tank) tanks completely. If you are not sure about the gas content of these tanks, ask animal facility technicians for assistance.



- Check Isoflurane level to make sure that it is full.



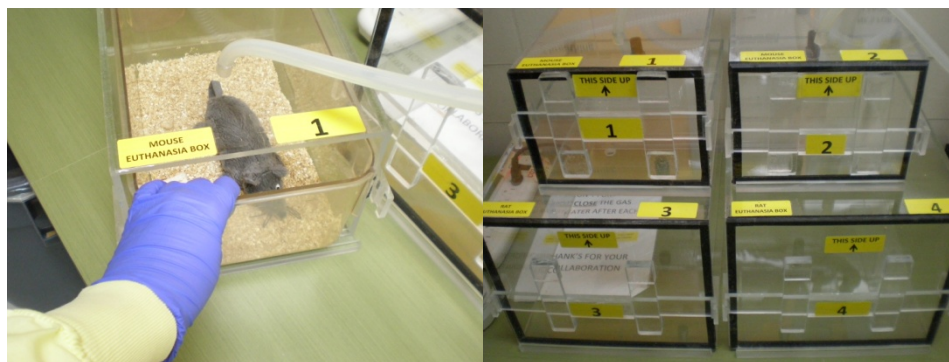
- **To fill vaporizer:** Remove filler cap and carefully pour Isoflurane into opening, Observe proper level of Isoflurane through sight glass. Replace cap.
- Open the oxygen manometer. Put the flow rate at 400ml/min. Be sure the CO₂ manometer is close.



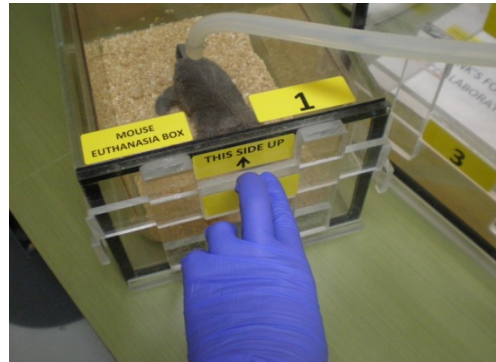
- Take your animal cage and remove the water bottle and the grid.



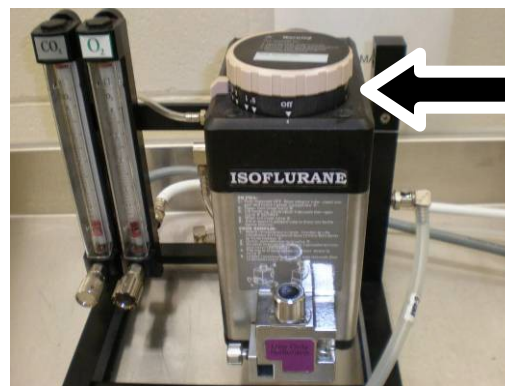
- Insert the cage inside the Plexiglas box (rat or mouse box). To avoid gas lost, choose the right euthanasia box size. One or two Plexiglas boxes can be used in the same time.



- Choose the euthanasia lid that is associated with the good euthanasia box. (Ex: box # 1 with lid # 1)
Pay attention to the side of the lid (Side up). Close the lid properly.

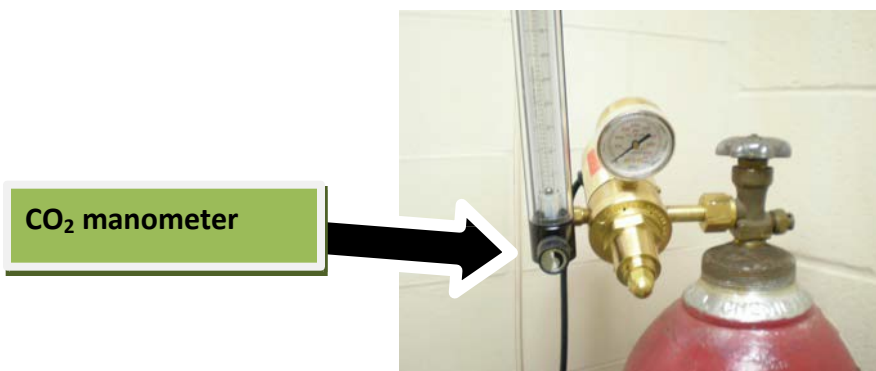


- Open the Isoflurane vaporizer at 5%.



Isoflurane Vaporizer

- **Once the animal has been rendered unconscious, turn the Isoflurane vaporizer setting to OFF.**
- Close the O₂ manometer and open the CO₂ manometer. **The maximum CO₂ flow rate for a rat cage should be 4L/min and 2L/min for a mouse cage.**



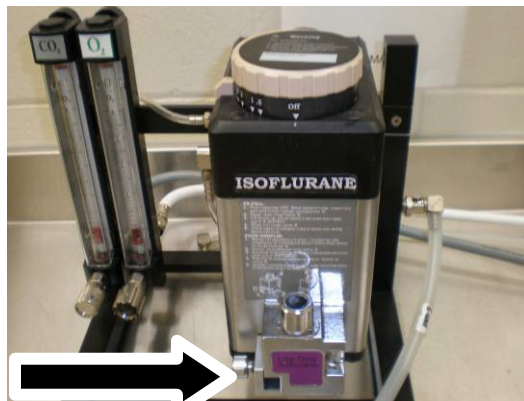
CO₂ manometer

- Maintain the CO₂ flow until the animal has stopped breathing.
Please note that the time required for euthanasia can be several minutes.
- When the animal has stopped breathing, close CO₂ manometer.
- Leave the animal in their cage for an additional 1-2 minutes.

- To confirm death, monitor animal following signs:
 - ✓ No rising and falling of the chest
 - ✓ No palpable heart beat
 - ✓ Poor mucous membrane color
 - ✓ No response to toe pinch
 - ✓ Color change or opacity in eyes
- Physical methods of euthanasia, **such as cervical dislocation and or pneumothorax should be done on your animals before disposal to ensure that they have been correctly euthanized.**
- Turn off Oxygen and CO₂ tanks.
- Please turn the CO₂ heater switch OFF.



- Before leaving it is mandatory to refill the Isoflurane machine.



- Clean and disinfect all area with Peroxygard.
- Put carcasses inside a bag and dispose them inside the indicated freezer.

