Standard Operating Procedures (SOP) for Euthanasia of Research Rodents – Supplement to University Standard for Rodent Euthanasia

Request for Division of Comparative Medicine (DCM) Euthanasia of Research Rodents

For a fee, the DCM staff can perform euthanasia of research animals. When requesting this DCM service, research personnel must do the following:

- Complete and submit a "Request for Euthanasia of Animals" form [available on the DCM website]. Ensure clear euthanasia instructions (e.g., 'euthanize dam and neonates' or 'euthanize only the pre-weanling animals, not the dam').
- Provide proper documentation on the euthanasia request form. DCM is not responsible for errors on the form or miscommunications that may occur during the euthanasia process. Do not make verbal arrangements with DCM staff.
- Leave the animal(s) requiring euthanasia in their cage. All unweaned animals should stay with the lactating female until the time of euthanasia.
- Place a euthanasia card on the cage so that DCM can readily identify the animal(s) slated for euthanasia.

Guidelines for Decapitation:

- Remove mouse or rat from its home cage or experimental environment, anesthetized (if applicable) and carry animal to the guillotine.
- A minimal number of animals should be brought into the decapitation room at a time while decapitations are being conducted. Ideally, each animal should be brought into the room individually. The amount of time the animals are in the decapitation room with the guillotine should also be kept to a minimum to prevent stress.
- Make every effort ensure the animal is not agitated prior to placing the animal in the guillotine. The use of plastic cones (Decapicone® or other similar device) when using a guillotine, is optional but may reduce stress from handling, minimize the chance of injury to personnel, and improve the positioning of the animal in the guillotine.
- Holding the rodent securely, place the rodent on the stage at the entrance to the guillotine and place the rodent's head through the guillotine opening. Once the head is in the correct position, rapidly depress the guillotine lever. One good technique is for the researcher to grasp the rodent gently but firmly around the back and push his/her hand slightly forward. This will push the front legs up, which prevents the rat from biting the investigator and facilitates placing the animal in the guillotine.
- After use on an individual animal, the guillotine **must** be rinsed and cleaned to remove blood, tissue and gross contamination. This is a critical step to prevent contamination and stress due to the smell of blood on the guillotine.

Maintenance of Guillotines/Decapitators:

- A clean guillotine helps the rodents to remain calm, thereby making the procedure much easier and safer for the animal and the investigator. Following decapitation sessions, and once gross contaminants have been removed, the entire unit should be thoroughly cleaned. Rinse a final time with 70% alcohol to ensure evaporation and reduce the need to hand-dry the equipment. Turn the guillotine upside down with blades open to facilitate drying. Ensure the guillotine is lubricated properly by applying silicon or '3-in-1 oil' as necessary.
- Guillotine blades must be kept sharp. Frequency of sharpening may vary depending on the frequency of use and the species and number of animals decapitated. The IACUC recommends that blades be sharpened as frequently as needed to maintain sharpness. Any reputable company may provide blade sharpening at a minimal cost. It is recommended to have a spare guillotine available due to potential turnaround times for blade sharpening.

> **Note**: The Physics Department's Instrument Shop, located in Phillips Hall 115A, will sharpen blades for a small fee (919) 962-1183.

Guillotine - Safety of Personnel:

- Always make sure hands and fingers are clear of the blade path.
- Only trained personnel should sharpen blades, lubricate the guillotine, or take it apart.
- Do NOT use decapitation equipment unless properly trained.
- Old guillotine blades should be discarded in a 'sharps' container.
- Maintain documentation of the date of blade sharpening or replacement available for audit.

Hypothermia of Neonatal Rodents

Hypothermia is not the preferred method of euthanasia; however, if hypothermia for euthanasia is approved in protocol, the following guidelines should be followed:

- Pups may be placed in a latex sleeve or glove and immersed up to the neck in crushed ice and water (2-3°C) which requires a 5-8 minute induction time. (2-3 minutes to unconsciousness and 3-5 minutes to complete blockage of neural transmission).
- Alternatively, pups may be placed in a paper-lined tube and packed in crushed ice which may require up to 15 minutes to obtain a

surgical plane of anesthesia.

- Do NOT place the animals directly on the cooling medium. Provide a cloth, paper, or other barrier material as cold surfaces can cause tissue damage and presumably pain. Simply placing conscious animals in a cold room or on an ice pack is unacceptable as induction may take 30-45 minutes.
- Once animals are fully anesthetized, immediately perform a physical method of euthanasia (i.e., cervical dislocation, thoracotomy, major organ harvest, or decapitation) to confirm death.

Inhalant Anesthetics – May be administered by vaporizers via nose cone or induction chambers or by drop method in various types of chambers.

- Induction chambers must allow animals appropriate floor space without being too large. Chambers that are too large require increased volumes of the anesthetic agent and may result in slow induction time.
- The lid should fit securely, and the chamber **must be used in a fume hood, a ducted biosafety cabinet, or with a properly functioning active scavenging system.**
- Pre-charge the anesthetic chamber by opening the vaporizer or placing two to three pieces of absorbent material on the bottom of the chamber. Add approximately 3-5 mls of isoflurane liquid to the absorbent material (amount determined by the size of the chamber). Close the lid and wait five (5) minutes for the liquid to form a volatile gas within the chamber.
- Remove the lid of the chamber, quickly place the animals in the chamber, **ensure the absorbent material is not in direct contact with the animal**, and immediately close the lid.
- The animals should be anesthetized in 2-5 minutes. Neonates require a longer time to anesthetize and should remain in the chamber for at least five (5) minutes.
- When animals are completely recumbent and deeply anesthetized, remove them from the chamber. (If the chamber is opened outside of a chemical fume hood, contact EHS to have the Waste Anesthetic Gas measured/assessed.)
- **Immediately** perform a physical method of euthanasia. Isoflurane is highly volatile, and animals will quickly regain consciousness once removed from the chamber. Therefore, it is imperative that physical euthanasia be performed immediately.

SOP Gaseous Carbon Dioxide (CO_2) (Non-automated)

- Remove each animal from the housing chamber and place into the euthanasia chamber.
    - Never place the housing chamber into the euthanasia chamber.
    - Never pre-charge the chamber.

    - Only place animals of the same species in the chamber at the same time.
    - Each animal should have enough floor space available to lie down.
- Place the stainless-steel lid over the plastic cage. The lid should be connected to a CO_2 tank via a plastic hose.
    - Make sure the two holes on the top of the lid are not blocked, as these holes allow air to be pushed out by the heavier CO_2.
    - Make sure the euthanasia chamber does not have an automatic watering opening.
- Turn on the valve located on top of the CO_2 tank. Next, check the settings of the regulator to ensure the flow settings are set to the DCM standards below and adjust the regulator valve on the left side of the flow meter if needed.
    - Standard DCM Shoebox style RAT cage: 12.0 liters per minute (lpm)
    - Standard DCM Shoebox style MOUSE cage: 2.8 liters per minute (lpm)
    - Other CO_2 Chambers: Use the following formula to calculate the appropriate flow rate: (Height x width x length) / 61 = liters x .45 = flow rate/minute (units = inches)
- Administer CO_2 approximately six (6) minutes for mice and eight (8) minutes for rats. Young animals, certain strains of mice, and sick animals may require more time to become deeply anesthetized.
- Continue to allow CO_2 to flow into the chamber for one (1) minute after breathing stops.
- Once animals are fully anesthetized, immediately perform a physical method of euthanasia (i.e., cervical dislocation, thoracotomy, major organ harvest, or decapitation) to confirm death.
  Note: If a terminal procedure (i.e., cardiac puncture, tissue collection) must be performed before the secondary physical method, ensure that animals remain deeply anesthetized and that a physical method of euthanasia is performed following the terminal procedure.
- Place dead animals into a non-PVC containing bag. DCM provides these bags in a variety of sizes. Label the bag with the IACUC protocol #. Seal the bag securely.
- Place the bag with dead animal(s) into the DCM carcass freezer available in each animal facility. Please see the Disposal of Rodent Carcasses Standard for more information.
- Disinfect the euthanasia chamber after each use.

SOP for Automatic Carbon Dioxide (CO_2) Systems

Precautions

• Always wear the required Personal Protective Equipment when working with the Euthanex Smartbox Auto CO2 System.

• This system is equipped with a touch screen for operation. While the chamber door is open, there will be no presence of a "START" button on the touch screen.

• There are **three pressure lock knobs that need to be fully engaged in a clockwise position to seal the door** prior to using the machine. These knobs are numbered 1-3 from right to left and should be engaged for use in numerical order**. If any of the pressure lock knobs are not fully engaged, the system may not be sealed properly and will not be able to function at optimum efficiency**. Improper use may result in the animals not being exposed to the proper gas concentration.

• Red "Emergency Stop" button on the touch screen may be pressed at any time to stop the euthanasia cycle. **Gas will immediately stop flowing.**

• The chamber door cannot be opened while the system is operating.

• In the event of loss of power to the system, the system will immediately begin the evacuation process of CO2.
• Report any issues you encounter while operating the machine to the Facility Manager/Supervisor **IMMEDIATELY**.

Required Personal Protective Equipment (PPE)

All personnel working with the Euthanex Smartbox Auto CO2 Systems are required to wear:

- Disposable Gown
- Gloves

ADDITIONAL PPE MAY BE REQUIRED IN CERTAIN AREAS. PLEASE SEE ROOM SIGNS OR FACILITY MANAGER/SUPERVISOR FOR FURTHER INFORMATION.

CO2 Tank Set Up

Tank set up is completed at initial set up of machine and replaced as necessary depending on usage.

1. Attach the regulator to the CO2 tank. Use a wrench to tighten mounted regulator.
2. Plug the regulator into an AC outlet. The regulator is thermostatically controlled to automatically turn on and off heat as needed to maintain CO2 temperature.
3. Attach the tubing between the CO2 regulator output hose to the "CO2 IN" on top of the smartbox fixed flow controller.
4. Open the gas flow from the CO2 tank by turning the tank valve knob counterclockwise.
5. **Set the regulator flow rate between 150-175, and ideally at 162.5 CFPH (Figure 2).** Exceeding this flow rate may introduce stress to the animals, while not reaching the appropriate flow rate may not allow the euthanasia process to be carried out as designed.

6. There is an exhaust hose attached to the system where waste gas will be expelled. In some facilities, the hose can be attached to the ceiling exhaust and in other facilities the hose will be dropped to the floor and exhausted into the room.
    a. If a ducted Biological Safety Cabinet (BSC) or hood is located in the room (indicating the BSC/hood is the sole source of ventilation) it **MUST be turned on with air circulating** when the system is in use in a room where the waste gas is exhausted to the floor!

Operation of Unit

The system works with preset timings that assure humane and efficient levels of CO2. Gas flow rate is set at the source (fixed flow), while the timings are controlled by the automated controller unit.

1. If not already on, press the "On" switch on the back of the Euthanex Smartbox Auto CO2 system.
2. Verify the CO2 tank is turned on and the gauge reads > 150 psi, indicating the amount of CO2 present in the tank **(Figure 1)** □ If tank is ≤ 150 psi, it must be replaced with a new tank prior to operating the system
3. Turn the three pressure lock knobs on the front of the chamber door counterclockwise to open. The knobs must be in a fully horizontal position in order to open.
4. Load the chamber with cages.
    - **Do not overcrowd cages!** See the IACUC Standards on Cage Density for Mice or Rats.
    - **If you are only euthanizing a single layer of cages:** Individual cage lids (filter tops) should remain on the cages, but must be unlatched (front and rear clasps) to break the seal and allow for the CO2 to better enter the cage. Alternatively, filters can be removed from the cage lids instead of unlatching the front and rear clasps. Cages can then be loaded into the chamber.
    - **If you are euthanizing more than a single layer of cages (only in Genetic Medicine Upper Basement):** A shelf divider is in place inside each of 3 chambers, so removal of filter tops and wire bar lids is necessary in order for the cages to fit inside the chambers.
        i. Remove filter tops and wire bar lids from the first layer of cages and place under the divider panel in the chamber.
        ii. Remove filter tops and wire bar lids from the second layer of cages and place in the chamber on top of the divider panel.
    - Total number of cages accommodated per use will vary depending on actual cage size.
5. After loading the chamber, close the door and **turn all three pressure lock knobs (in numerical order) fully clockwise to seal**. **If the knobs are not fully turned the system will not operate.**
6. Engage the touch screen by tapping it with your finger. Then, press "START".
7. Type in your assigned password and press "Next".
8. Select the proper cycle to run depending on which species and the age of the animal you will be euthanizing.

9. Press “START” again. A picture of the chamber will appear on the touch screen and will flash green to indicate that the cycle has started and a countdown will begin.
10. Ensure the CFPH gauge is reading between 150-175 and as close to 162.5 as possible **(Figure 2)** and if the reading is above or below the required level, turn the flow knob on the side of the Euthanex regulator **(Figure 3)** until the needle reaches the proper level.
11. The system will cycle through three stages:
    - Charge: Gas flows through the chamber, fully charging the chamber with CO2.
    - Dwell: Gas flow stops and the chamber remains fully charged with CO2.
    - Exhaust: Exhaust blower turns on and purges the chamber, fully evacuating CO2.
12. After the blower automatically switches off, the pressure lock knobs will release, and the animals may be safely removed from the chamber.
13. Remove all cages from the chamber.
14. Place all animals in plastic carcass bags prior to placing in designated box for carcass incineration or donation.
    - All plastic bags with carcasses should be labeled with the protocol number, date, and either “Euthanex Smartbox” or abbreviate with “ESB” to indicate that the animal was euthanized via the automatic CO2 system, therefore secondary euthanasia is not necessary.
15. Wipe down chamber using approved disinfectant.
16. Fill out Incinerator Box Tracking Sheet with all pertinent information. You MUST circle “Yes” indicating that the animals were euthanized using the automated CO2 system.