

CO₂ EUTHANASIA OF MICE IN-PERSON TRAINING
UNIVERSITY OF SOUTH FLORIDA
COMPARATIVE MEDICINE

Attendee Name: _____
Print Sign Date

Discuss/Review:

- ___ Carbon Dioxide (CO₂) gas cylinders are **dark gray** in color (oxygen gas cylinders, used to deliver isoflurane anesthesia to mice, are green in color).
- ___ Compressed gas tank cylinders are the only acceptable CO₂ source for euthanasia of mice.
- ___ Neonatal mice are naturally resistant to this method of euthanasia.
- ___ Prefilling a chamber with CO₂ is not acceptable.

Demonstrate/Assess:

- ___ Compressed gas tank cylinders have a valve to which a **pressure-reducing regulator** is attached.
- ___ The **flow meter** ensures an appropriate displacement rate of air inside the enclosure, displacing 10-30% of the chamber volume per minute.
- ___ Euthanasia of more than one mouse at a time must be performed in cohorts of only live animals.
- ___ Mice should be euthanized in their **home cage**, whenever possible.
- ___ CO₂ can be delivered directly into a primary enclosure.

Primary Enclosure

- ___ **Blue** line by inserting the tubing into the plastic lid's air supply port.
- ___ **Green** line using a stainless steel lid with tubing attached.
- ___ First **ensure the flow meter is "off"** by turning it fully clockwise.
- ___ Open the cylinder valve by turning it counter-clockwise.
- ___ Refer to the table to **determine flow rate** in L/min.

Euthanex Smartbox

- ___ Ensure that the green flow meter for individual cage euthanasia is turned off.
- ___ Confirm that the **white "ready" light is on**.
- ___ Turn the three black knobs on the front of the chamber to open the door to a horizontal position.
- ___ The **red "fault" light** will turn on, indicating the **door is open**.
- ___ Load the chamber with home cages, each covered with a wire bar lid, **offsetting** cages so no cage is directly stacked on top of another.
- ___ Close the door and **seal** it by turning the three black knobs clockwise.
- ___ **Select** either "adults" or "neonates".
- ___ Press **Start**.
- ___ The **white "ready" light** and the **green "run" light** will remain on **until the cycle completes**.
- ___ At the **completion** of a cycle, **both** the white "ready" light and the green "run" light **will turn off**.
- ___ The red "stop" button can be used at any time to stop a cycle in an emergency.
- ___ Disinfect the interior of the chamber after each use with Oxivir.
- ___ Death must be **verified** by employing a **secondary physical method** of euthanasia such as cervical dislocation, decapitation, or bilateral thoracotomy, or by observation at room air for at least 10 minutes.
- ___ CO₂ exposure should not be used as the sole means of euthanizing neonatal mice, and must be followed by a secondary physical method of euthanasia.

Training & assessment provided by: _____
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