HOW TO USE THE CARBON DIOXIDE (CO\textsubscript{2}) EUTHANASIA CHAMBER

1. Place the Euthanex stainless steel lid over the plastic cage. The lid should be connected to a CO\textsubscript{2} tank via a plastic hose.
   a. 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\textsubscript{2}.
   b. Make sure the plastic cage does not have an automatic watering opening.

2. Remove each animal from the housing chamber and place into the euthanasia chamber. Never place the housing chamber into the euthanasia chamber. Do not place different animal species in the chamber at the same time. Do not overcrowd the chamber. Each animal should have enough floor space available to lie down.

3. Turn on the valve located on top of the CO\textsubscript{2} tank. Next, set the flow meter by adjusting the regulator valve on the left side of the flow meter (see photo on the next page):
   a. Standard DLAM Shoebox style RAT cage: 8 liters per minute (lpm)
   b. Standard DLAM Shoebox style MOUSE cage: 1.8 liters per minute (lpm)
   c. Other CO\textsubscript{2} Chambers: Use the following formula to calculate the appropriate flow rate:
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      \text{flow rate} = \frac{\text{height} \times \text{width} \times \text{length}}{61}\text{ liters} \times 0.20 = \text{flow rate/minute}
      \]

4. Continue to allow CO\textsubscript{2} to flow into the chamber for one minute after breathing stops (approximately 6 minutes for mice and 8 minutes for rats). Young animals, certain strains of mice, and sick animals may require more time to become deeply anesthetized.

5. 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.

6. 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.

7. Place dead animals into a non-PVC containing bag. DLAM provides these bags in a variety of sizes. Label the bag with the ACAP ID#. Seal the bag securely. Place the bag with dead animal(s) into the DLAM carcass freezer available in each animal facility. Please see the Policy on Rodent Carcass Disposal for more information.

8. Disinfect the euthanasia chamber bottom after each use.

*This procedure can be adapted for use in investigator laboratories