SAN DIEGO STATE UNIVERSITY

Office of Laboratory Animal Care

Standard Operating Procedures

Use of Carbon Dioxide (CO2) Euthanasia Chamber

Date Implemented: 10/13/14
Date(s) Revised: 03-06-20 RS, 03-08-20 LS. Final 4-7-2020

The PHS Policy requires that euthanasia be conducted in a manner consistent with the American Veterinarian Medical Association (AVMA) Guidelines for Euthanasia: 2020 Edition, as well as the National Research Council's Guide for the Care and Use of Laboratory Animals (Eighth Edition) (OLAW). It is the responsibility of all animal users to ensure compliance the SDSU IACUC Guidelines on Euthanasia for Animals used in Research and Teaching.

1.0 SCOPE

This Standard Operating Procedure (SOP) is to ensure the proper use of the CO2 euthanasia chamber as a means of euthanasia for rodents. This document is relevant to all investigators, technicians, and animal care staff using the CO2 euthanasia chamber for euthanasia of their research animals.

2.0 DEFINITIONS

2.1 CO2 – Carbon Dioxide. CO2 is a gas that is heavier than air and nearly odorless. The only acceptable source of carbon dioxide is from compressed CO2 gas cylinders. Carbon dioxide generated from other sources such as dry ice, fire extinguishers or chemical means (antacids) is unacceptable.

2.2 Euthanasia – The following is excerpted from the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition: Euthanasia is derived from the Greek terms *eu* meaning good and *thanatos* meaning death. The term is usually used to describe ending the life of an individual animal in a way that minimizes or eliminates pain and distress. A good death is tantamount to the humane termination of an animal's life.

3.0 MATERIALS

3.1 CO2 Chamber; solid, clean and with lid.

3.2 Gas Cylinders

3.2.1 Tank containing 100% CO2

3.2.2 2 stage regulator which displays tank pressure and line pressure

3.3 CO2 Flow meter and connector hose

3.4 Rat or mouse cage with wire bar and lid

3.5 Biohazard bag.

4.0 PROCEDURE – Preparing the chamber

4.1 Connect the hose from gas cylinder to the adapter on the chamber top.

4.2 Open the main valve on the CO2 gas cylinder by turning the valve ½ turn counter clockwise. Adjust the regulator controlling the line pressure to the flow meter to 20psi.

5.0 PROCEDURE – Addition of animals into the chamber

5.1 Animals to be euthanized must be transported in a manner that avoids overcrowding, physical trauma and fighting. If fighting occurs separate animals immediately.

5.2 Mice should be euthanized in their home cages whenever possible, however a clean empty mouse or rat cage placed inside the euthanasia chamber may also be used. Lids should be removed from cages and wire bar feeders left in place.

5.3 When placed into the chamber all animals must have ample floor space and be able to make normal postural adjustments.

5.4 Only one species at a time should be placed in the chamber.

5.5 Euthanasia should always be done in cohorts. Never place live animals in the chamber with dead animals.

6.0 PROCEDURE – Euthanasia

6.1 Once animals have been placed in the chamber, close the lid and observe the animals closely. Using the table below, turn the flow meter to the appropriate setting to achieve a fill rate into the chamber of 30-70% volume/minute.

OLAC Euthanasia Station Location	Chamber Volume (cm)	Flow Rate (for 30% - 70% fill rate)
NLS 17E	56 x 38 x 33	21-49 L/min
NLS21	48 x 25 x 20	7-17 L/min
Alvarado	48 x 25 x 20	7-17 L/min

Important Note: These flow rates are calculated for specific chambers in OLAC Euthanasia Stations. CO2 euthanasia stations set up by labs should use the following example formula to calculate the appropriate flow rate range for the chambers they use.

Euthanasia Formula: (per AVMA Guidelines on Euthanasia)

1. Calculate the volume of the euthanasia chamber. (e.g., Super Mouse 750TM)

Inside Height x length x width = volume .263 ft3 = 7,447.33 cm3= 7.44733 liters

2. Calculate the volume of CO2 that needs to be delivered. You need to deliver 30-70% of the chamber's volume per minute (average 30-70%/min).

7.44733 liters x 0.30 = 2.234 liters
7.44733 liters X 0.70= 5.213 liters

Thus, you would need to deliver 2.234 - 5.213 liters of CO2 per minute.

6.2 The animals should be unconscious within approximately 30 seconds. If animals do not lose consciousness within 30 seconds, continue the flow of the CO2 until animals lose consciousness.

6.3 After the animals become unconscious, maintain the flow of CO2 into the chamber for approximately 5 minutes for mice 3 weeks of age or older.

6.3.1 After 5 minutes, turn the flow meter and main cylinder valves off by turning it clockwise.

6.3.2 Death must be confirmed when animals are removed from the chamber by use of a secondary method. Active secondary methods include: cervical dislocation (rodents less than 200g), decapitation, bilateral thoracotomy, or major organ collection. If no active secondary method is to be used, the carcasses must be left in the chamber for 10 minutes to assure death. Ensure loss of breathing and heartbeat before putting the animals in a biohazard bag and placing it in the designated carcass freezer.

6.4 Neonates (less than 3 weeks) are resistant to the effects of hypoxia and require up to 30 minutes or more of exposure to CO2 before they expire. For neonates <10 days old decapitation is the recommended method of euthanasia.

7.0 PROCEDURE – Cleaning the chamber

7.1 No new animals may be added to the chamber until the previous group has been removed and the chamber appropriately cleaned.

7.1.1 Wipe out any debris in the chamber, clean thoroughly with an appropriate disinfectant, and allow to dry.

7.1.2 Ensure that the CO2 chamber is emptied of residual CO2 before adding new animals to the chamber. In most cases this can be done by turning the chamber over and "dumping" the CO2 since it is heavier than oxygen.

8.0 PROCEDURE – Managing the gas cylinders

8.1 When the chamber is no longer needed be sure the main cylinder valve is completely off (clockwise). Make sure all gauges read zero or gas will continue to leak and tanks will become empty.

8.2 When the pressure gauge for the cylinders reaches 500 psi notify the OLAC Manager so that the tanks can be replaced.

9.0 PROCEDURE – Quick Directions for Posting at Station

9.1 Hook up hose to chamber lid.

9.2 Open CO2 main valve.

9.3 Place animals in the chamber, close the lid and observe.
9.4 Using the table below, turn the flow meter to the appropriate setting to achieve a fill rate into the chamber of 30-70% volume/minute.

Euthanasia Station Location	Chamber Volume (cm)	Flow Rate (for 30% - 70% fill rate)
NLS 17E	56 x 38 x 33	21-49 L/min
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9.5 When animals are unconscious, continue to administer the CO2 for 5 minutes. For neonates (<3wks of age) continue to administer the CO2 for 30 minutes.
9.6 Turn off flow meter of 100% CO2 cylinder and leave carcasses in the chamber for 10 minutes (20 minutes for neonates).
9.7 Check for loss of breathing and heartbeat prior to disposing of carcasses.
9.8 When finished check to make sure main cylinder valve is off. Make sure all gauges read zero.
9.9 Clean the chamber prior to next group of animals.

10.0 References

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. American Veterinary Medical Association.
https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf