Document Number: ANP005	Title: EUTHANASIA OF RATS AND MICE	Effective Date: NOVEMBER 2023
Section: Animal Research		Supersedes Date: NOVEMBER 2015
Subsection: Procedure	***CONFIDENTIAL INFORMATION*** **MOLECULAR MEDICINE RESEARCH INSTITUTE.**	Page: 1 of 3

1.0 OBJECTIVE

1.1 The objective of this procedure is to describe the AVMA approved procedures used at MMRI, Inc. for euthanizing rats and mice.

2.0 SCOPE

2.1 This procedure applies to rats and mice from the MMRI animal care facility.

3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to ensure rats and mice are euthanized in a humane manner using accepted and approved methods.

4.0 RESPONSIBILITIES

4.1 It is the responsibility of Manager of Animal Research or designated alternate to implement this procedure and revise it when necessary.

5.0 REFERENCES

5.1 American Veterinary Medical Association Panel on Euthanasia

5.2 Animal Carcass Disposal, ANP003

5.3 AVMA Panel Recommendation on Depopulation

5.4 Disaster Contigency

6.0 PROCEDURE

6.1 Euthanasia methods

6.1.1 Carbon Dioxide

6.1.1.1 Make sure that the euthanasia chamber is free of CO2 before placing animal inside. Place animal either in smaller chamber labeled as Mouse or bigger chamber labeled as Rat and then turn up clockwise the knob of regulator up to sign marked as Mouse (3L/min) or Rat (8L/min), corresponding to the chamber being used. {Since, oxygen tank flow regulator is being used, the 3L/min flow rate is actually 1.5L/min of CO2 and 8L/min flow rate is 4L/min of CO2, which provides 25% displacement of chamber volume in one minute as recommended in current AVMA Guidelines for the Euthanasia of Animals}

6.1.1.2 Continue flow of CO2 for approximately 3-5 minutes. CO2 flow should be maintained for at least one minute after respiratory arrest

6.1.1.3 Remove animal from the chamber. Verify death using the criteria listed in section 6.2 of this SOP.

6.1.1.4 Perform cervical dislocation or a bilateral thoracotomy on the animal after exposure to CO2.

6.1.2 Cervical Dislocation

6.1.2.1 Cervical Dislocation is to be used to euthanize conscious mice and rats less than 150 grams. Rats over 150 grams should be anesthetized before cervical dislocation.

6.1.2.2 To dislocate the cervical vertebrate, place the thumb and index finger on either side of the neck at the base of the skull. Alternatively, a rod can be pressed at the base of the skull.

6.1.2.3 With the other hand, quickly pull the base of the tail, using a slight upwards motion. This should separate the cervical vertebrae.

6.2 Verify death by two or more of the following methods prior to disposal of carcass.

6.2.1 Absence of heartbeat or pulse

6.2.2 Dilated pupils unresponsive to light

6.2.3 Opaque Cornea

6.2.4 Absence of respiration

6.2.5 Mucous membranes of a bluish color

6.2.6 Absence of toe pinch reflex (pinch toes using tweezers)

6.2.7 Rigor Mortis

6.3 On each animal's card record the date of euthanasia, stamp or write "euthanized" with an explanation in comments section and initial

6.4 Place carcasses in a red plastic bag and dispose off according to SOP number ANP003, Animal Carcass Disposal