This SOP replaces:	Date: 11.12.18 Version: LTK-TRT-14-EN Version: B
Reason for Change:	Request by cantonal veterinary office to ensure death after cervical dislocation and to perform procedure from age of 14 days on only.
Related SOPs:	None
Indication of Use:	Killing of mice by CO_2
Aim of SOP:	It is the aim of this procedure to kill mice by CO_2 in a humane fashion

Distribution:

1. Original: Thorsten Buch
2. Copy: Animal rooms
3. Intranet

Attachments:

Generated at: 30.4.19	Checked and approved at: 30.4.19
by: Thorsten Buch	by: Dr. Beffinger

Responsible Persons: Animal caretakers and scientists, registered at VETA Zürich

Principle of Method: CO_2 intoxication, cervical dislocation, pneumothorax, exsanguination

Units and Formulas: None

Material to be used:
Mice older than 14 days
Scissors
Needles

Machine:
1. Laminar flow/changing station
2. CO_2 bottle/outlet with flow meter

Material:
1. Lid connected to CO_2 bottle by tube (should be equipped with dispensing plate)
2. Corpse bags

Reagents:
CO_2

Safety:
1. Get an introduction on how to handle CO_2 bottle/valve/flow meter
2. CO_2 bottle needs to be safely attached to a wall
3. Never move bottle with valve system and without safety cap
4. Make sure CO_2 is turned off after finishing

Method Description:

All animals in a cage:

1. Examine system for defective flow meter, absence of CO_2, and leaks
2. Place animals within home cage under changing station/laminar flow
3. Remove lid and place "CO_2" lid on cage
4. Open CO_2 flow at 50% of cage volume per minute (0.012 m^3/min = 12 cm^3/min for Tecniplas green line IVC cage)
5. Observe animals until stop of movement and breathing
6. Wait further 2 min
7. Open lid and check pedal withdrawal reflex by pinching of one animal of each group
8. Apply a second euthanasia method such as
   a) pneumothorax through opening with scissors
   b) exsanguination through a needle or by opening of the Vena jugularis, heart or aorta through scissors or scalpel
   c) cervical dislocation followed by organ removal or decapitation
   while remaining animals stay under CO_2
9. Place dead animals in cadaver bag and place into cadaver freezer

Single animal(s) from a cage:

1. Examine system for defective flow meter, absence of CO_2, and leaks
2. Place animals within home cage under changing station/laminar flow
3. Prepare second cage (without bedding!)
4. Transfer animal(s) to be euthanized into second cage
5. Place "CO_2" lid on cage
6. open CO_2 flow at 50% of cage volume per minute (0.012 m3/min = 12 cm3/min for Tecniplas green line IVC cage)
7. Observe animal(s) until stop of movement and breathing
8. Wait further 2 min
9. Open lid and check pedal withdrawal reflex by pinching of one animal of each group
10. Apply a second euthanasia method such as:
    a) pneumothorax through opening with scissors or scalpel
    b) exsanguination through a needle or by opening of the Vena jugularis, heart or aorta by use of scissors
    c) cervical dislocation followed by organ removal or decapitation
11. Remove dead animal, put into cadaver bag and place into cadaver freezer
12. Empty cage from CO_2 (invert) and clean by wiping with disinfectant (odor removal), use cage for next animals.

Controls:
Observe carefully that animals are dead (movement, breathing, heartbeat, pedal withdrawal reflex)

Factors influencing outcome:
Flow rate too low will increase suffering time of the animals.
Leaving animals for too short in CO_2 after last movement/breathing and they may recover.

Criteria for approving outcome:
Humane death

Documentation:
The killing of the animals has to be documented in iRATs

Problem management:

1. If unconsciousness has not yet occurred within 2 to 3 minutes, the chamber fill rate should be checked. The system should also be examined for a defective flow meter, absence of CO_2 supply, and/or leaks
2. If problem persists contact group leader or Vet

Sample storage:
Dead animals are stored in the cadaver freezer

Literatur:

1: Moody CM, Chua B, Weary DM. The effect of carbon dioxide flow rate on the euthanasia of laboratory mice. Lab Anim. 2014 Oct;48(4):298-304. doi: 10.1177/0023677214546509. Epub 2014 Aug 5. PubMed PMID: 25097256.

2: Makowska J, Golledge H, Marquardt N, Weary DM. Sedation or inhalant anesthesia before euthanasia with CO2 does not reduce behavioral or physiologic signs of pain and stress in mice. J Am Assoc Lab Anim Sci. 2012 Jul;51(4):396-7; author reply 397-9. PubMed PMID: 23043800; PubMed Central PMCID: PMC3400683.

3: Valentine H, Williams WO, Maurer KJ. Sedation or inhalant anesthesia before euthanasia with CO2 does not reduce behavioral or physiologic signs of pain and stress in mice. J Am Assoc Lab Anim Sci. 2012 Jan;51(1):50-7. PubMed PMID: 22330868; PubMed Central PMCID: PMC3276966.

4: Conlee KM, Stephens ML, Rowan AN, King LA. Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. Lab Anim. 2005 Apr;39(2):137-61. Review. PubMed PMID: 15901358.

5: Fachinformation 3.04: Fachgerechte und tierschutzkonforme Antikörperproduktion in Kaninchen, Hühnern und Labornagetieren, Bundesamt für Lebensmittelsicherheit und Veterinärwesen.