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Date: 22.01.2021	Euthansia of mice by CO₂ CO₂ Euthanasia	LTK-TRT-14-EN Version: D

This SOP replaces:	Date: 30.04.19 Version: LTK-TRT-14-EN Version: C
Reason for Change:	New "Fachinformation" of federal office
Next Revision:	22.01.2024
Related SOPs:	None
Indication of Use:	Killing of mice by CO ₂
Aim of SOP:	Procedure to kill mice by CO ₂ in a humane fashion
Distribution:	1. Server
Attachments:	

Generated at: 22.01.2021	Checked and approved at: 22.01.2021
by: Thorsten Buch	by: Dr. Vanhecke

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

Principle of Method: CO₂ intoxication, cervical dislocation, pneumothorax, exsanguination

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Units and Formulas: None

Material to be used: Mice older than 14 days (younger mice require alternative method)
 Scissors
 Needles

Machine:

1. Laminar flow/changing station
2. CO₂ bottle/outlet with flow meter


Material:

1. Lid connected to CO₂ bottle by tube (should be equipped with dispensing plate)
2. Corpse bags

Reagents:
 CO₂

Safety:

1. Get an introduction on how to handle CO₂ bottle/valve/flow meter
2. CO₂ bottle needs to be safely attached to a wall
3. Never move bottle with valve system and without safety cap
4. Make sure CO₂ is turned off after finishing

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Method Description:


Refer also to description at respective CO₂ station.

All animals in a cage:

1. Examine system for defective flow meter, presence of sufficient CO₂, and leaks
2. Place animals within home cage under changing station/laminar flow
3. Remove lid and place "CO₂" lid on cage
4. Open CO₂ flow at 50% of cage volume per minute (0.012 m³/min = 12 cm³/min for Tecniplast 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 each animal of the group, make sure muscles have relaxed.
 - a. If above-described death signs (breathing, reflex, muscles) may be inconclusive (e.g. under anesthesia) apply a second euthanasia method such as
 - i. pneumothorax through opening with scissors
 - ii. exsanguination through a needle or by opening of the Vena jugularis, heart or aorta through scissors or scalpel
 - iii. cervical dislocation followed by organ removal or decapitation
 - iv. while remaining animals stay under CO₂
8. Place dead animals in cadaver bag and place into cadaver freezer

Single animal from a cage:

1. Examine system for defective flow meter, presence of sufficient CO₂, and leaks
2. Place animals within home cage on table or under changing station/laminar flow
3. Prepare second cage (without bedding!)
4. Transfer animal(s) to be euthanized into second cage
5. Place "CO₂" lid on cage
6. open CO₂ flow at 50% of cage volume per minute (0.012 m³/min = 12 cm³/min for Tecniplast 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, make sure muscles have relaxed.

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- a. If above-described death signs (breathing, reflex, muscles) may be inconclusive (e.g. under anesthesia) apply a second euthanasia method such as
- v. pneumothorax through opening with scissors
 - vi. exsanguination through a needle or by opening of the Vena jugularis, heart or aorta through scissors or scalpel
 - vii. cervical dislocation followed by organ removal or decapitation
 - viii. while remaining animals stay under CO₂
9. Remove dead animal, put into cadaver bag and place into cadaver freezer
10. Empty cage from CO₂ (invert) and clean by wiping with disinfectant (odor removal), use cage for next animals.

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


Factors influencing outcome:
 Flow rate too low will increase suffering time of the animals.
 Leaving animals for too short in CO₂ 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 CO₂ supply, obstructions, and/or leaks
 2. If problem persists contact group leader or Vet

Sample storage:
 Dead animals are stored in the cadaver freezer

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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-3.01-Fachgerechtes-tierschutzkonformes-Toeten-von-Versuchstieren-1.pdf 08.09.2020