





Date: 04.01.2022

**Euthanasia of mice by CO<sub>2</sub>**  
**CO<sub>2</sub> Euthanasia**

**LTK-TRT-14-EN**  
**Version: E**

**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. CO<sub>2</sub> bottle/outlet with flow meter

**Material:**

1. Lid connected to CO<sub>2</sub> bottle by tube
2. Corpse bags

**Reagents:**

CO<sub>2</sub>

**Safety:**

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



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**Method Description:**

**Refer also to description at respective CO<sub>2</sub> station.**

All animals in a cage:

1. Examine system for any defects or leaks at the flow meter/tubing, ensure presence of sufficient CO<sub>2</sub>.
2. Place animals within home cage under changing station/laminar flow
3. Remove lid and place "CO<sub>2</sub>" lid on cage
4. Open CO<sub>2</sub> flow at 50% of cage volume per minute (0.012 m<sup>3</sup>/min = 12000cm<sup>3</sup>/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 while remaining animals stay under CO<sub>2</sub>
8. Transfer dead animals in cadaver bag and place into cadaver freezer

Single animal from a cage:

1. Examine system for any defects or leaks at the flow meter/tubing, ensure presence of sufficient CO<sub>2</sub>.
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 "CO<sub>2</sub>" lid on cage
6. open CO<sub>2</sub> flow at 50% of cage volume per minute (0.012 m<sup>3</sup>/min = 12000 cm<sup>3</sup>/min for Tecniplast green line IVC cage)
7. Observe animal(s) until stop of movement and breathing
8. Wait further 2 min



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9. Open lid and check pedal withdrawal reflex by pinching of one animal of each 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
    - iv. pneumothorax through opening with scissors
    - v. exsanguination through a needle or by opening of the Vena jugularis, heart or aorta through scissors or scalpel
    - vi. cervical dislocation followed by organ removal or decapitation
    - vii. while remaining animals stay under CO<sub>2</sub>
9. Transfer dead animal into cadaver bag and place into cadaver freezer
10. Empty cage from CO<sub>2</sub> (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 CO<sub>2</sub> 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<sub>2</sub> 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 CO<sub>2</sub> 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.
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- 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.
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