



Date: 20.10.2015

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

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

**This SOP replaces:** Date: None  
 Version: None

**Reason for Change:** None

**Related SOPs:** None

**Indication of Use:** Killing of mice by CO<sub>2</sub>

**Aim of SOP:** It is the aim of this procedure to kill mice by CO<sub>2</sub> in a humane fashion

**Distribution:**

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

**Attachments:**

Generated at: 9.12.14

Checked and approved at: date

by: Thorsten Buch

by: Philippe Bugnon

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

**Method:** CO<sub>2</sub> intoxication



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**Principle of Method:** CO<sub>2</sub> intoxication

**Units and Formulas:** None

**Material to be used:** Mice older than 6 days

**Min/Max amount:** -

**Material acquisition:** -

**Calibration:** -

**Storage of Material:** -

**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 (should be equipped with dispensing plate)
2. corpse bags

**Reagents:**

CO<sub>2</sub>



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**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 and without safety cap
4. Make sure CO<sub>2</sub> is turned off after finishing

**Method Description:**

All animals in a cage:

1. place animals within home cage under changing station/laminar flow
2. remove lid and place "CO<sub>2</sub>" lid on cage
3. open CO<sub>2</sub> flow at 50% of cage volume per minute (0.012 m<sup>3</sup>/min = 12 cm<sup>3</sup>/min for Tecniplas green line IVC cage)
4. observe animals until stop of movement and breathing
5. wait further 2 min
6. open lid and check pedal withdrawal reflex by pinching of one animal of each group
7. -place dead animals in cadaver bag and place into cadaver freezer

Single animal(s) from a cage:

1. place animals within home cage under changing station/laminar flow
2. prepare second cage
3. transfer animal(s) to be euthanized into second cage
4. place "CO<sub>2</sub>" lid on cage
5. open CO<sub>2</sub> flow at 50% of cage volume per minute (0.012 m<sup>3</sup>/min = 12 cm<sup>3</sup>/min for Tecniplas green line IVC cage)
6. observe animal(s) until stop of movement and breathing
7. wait further 2 min
8. open lid and check pedal withdrawal reflex by pinching of one animal of each group
9. remove dead animal, put 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.



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**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<sub>2</sub> after last movement/breathing and they may recover

**Criteria for approving outcome:**

humane death

**Analysis:**

-

**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, and/or leaks
2. if problem persists contact group leader or Vet

**Sample storage:**

Dead animals are stored in the cadaver freezer

**Method validation:**

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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.

3: Valentine H, Williams WO, Maurer KJ. 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 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.