
 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 1 of 5
Date: 30.01.2020	Euthansia of rats by CO₂ CO₂ Euthanasia rat	LTK-RES-68-EN Version: A

This SOP replaces: None	
Reason for Change: None	
Related SOPs: None	
Indication of Use: Killing of rats by CO ₂	
Aim of SOP: It is the aim of this procedure to kill rats by CO ₂ in a humane fashion	
Distribution: 1. Original: Thorsten Buch 2. Intranet	
Attachments:	
Generated at: 30.01.2020	Checked and approved at: 30.01.2020
by: Thorsten Buch	by: Philippe Bugnon

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

Principle of Method: CO₂ intoxication, pneumothorax, exsanguination

 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 2 of 5
Date: 30.01.2020	Euthansia of rats by CO₂ CO₂ Euthanasia rat	LTK-RES-68-EN Version: A

Units and Formulas: None

Material to be used: Rats older than 14 days
 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

 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 3 of 5
Date: 30.01.2020	Euthanasia of rats by CO₂ CO₂ Euthanasia rat	LTK-RES-68-EN Version: A


Method Description:

All animals in a cage:

1. Examine system for defective flow meter, absence of 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 Tecniplas green line IVC cage)
5. Observe animals until stop of movement and breathing
6. If the animal(s) start/s to jump, reduce the CO₂ flow
7. Wait further 20 s
8. Open lid and check pedal withdrawal reflex by pinching of one animal of each group with forceps in the interdigital skin
9. 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 while remaining animals stay under CO₂
10. Place dead animals in cadaver bag and place into cadaver freezer

Single animal(s) from a cage:

11. Examine system for defective flow meter, absence of CO₂, and leaks
12. Place animals within home cage under changing station/laminar flow
13. Prepare second cage (without bedding!)
14. Transfer animal(s) to be euthanized into second cage
15. Place "CO₂ lid" on cage
16. open CO₂ flow at 50% of cage volume per minute (0.012 m³/min = 12 cm³/min for Tecniplast green line IVC cage)
17. Observe animal(s) until stop of movement and breathing
 - a. If the animal(s) start/s to jump, reduce the CO₂ flow
18. Wait further 20 s
19. Open lid and check pedal withdrawal reflex by pinching of one animal of each group
20. 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
21. Remove dead animal, place into cadaver bag and place into cadaver freezer
22. Empty cage from CO₂ (invert) and clean by wiping with disinfectant (odor removal), use cage for next animals.

 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 4 of 5
Date: 30.01.2020	Euthanasia of rats by CO₂ CO₂ Euthanasia rat	LTK-RES-68-EN Version: A

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.
 Flow rate too high provokes suffocation before unconsciousness and should be avoided.
 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, and/or leaks
2. If problem persists, contact group leader or vet

Sample storage:

Dead animals are stored in the cadaver freezer

 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 5 of 5
Date: 30.01.2020	Euthanasia of rats by CO₂ CO₂ Euthanasia rat	LTK-RES-68-EN Version: A

Literatur:

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

2: Wong D, Makowska IJ, Weary DM. Rat aversion to isoflurane versus carbon dioxide. *Biol Lett.* 2012 Dec 19;9(1):20121000. doi: 10.1098/rsbl.2012.1000. Print 2013 Feb 23. PubMed PMID: 23256183; PubMed Central PMCID: PMC3565521.

3: Hickman DL. Home Cage Compared with Induction Chamber for Euthanasia of Laboratory Rats. *J Am Assoc Lab Anim Sci.* 2018 Oct 10. doi: 10.30802/AALAS-JAALAS-17-000160. [Epub ahead of print] PubMed PMID: 30305198; PubMed Central PMCID: PMC6241385.

4: Boivin GP, Hickman DL, Creamer-Hente MA, Pritchett-Corning KR, Bratcher NA. Review of CO₂ as a Euthanasia Agent for Laboratory Rats and Mice. *J Am Assoc Lab Anim Sci.* 2017 Sep 1;56(5):491-499. Review. PubMed PMID: 28903819; PubMed Central PMCID: PMC5605172.