

University Animal Care Committee Standard Operating Procedure		
Document No: 10.5.2	Subject: Humane Killing of Rats using CO ₂	
Date Issued: August 21 st , 2012	Revision: 4	Page No: 1

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the procedure for the humane killing of adult rodents using CO₂ asphyxiation.

1. Introduction and Definitions: As per the UACC policy on humane killing of animals used in science, CO₂ asphyxiation is a *conditionally acceptable* method of humane killing for rodents when conducted properly (current Canadian Council on Animal Care guidelines). Principal Investigators will be required to justify its use (within their animal use protocol). Following humane killing with CO₂ asphyxiation, a secondary method must be used to confirm death.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI**, subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP**, intramuscular **IM**, per os **PO**, per rectum **PR**

2. Materials:

- CO₂ source (facility outlet, or tank and regulator)
- Flow meter
- Appropriately sized lid for the rodent cage (custom lids for CO₂ humane killing)
- Instruments (for secondary method of humane killing)
- Body bags

3. Procedures:

- To minimize stress, animals should be humanely killed in their home cage with a maximum of:
 - Three adult rats or one litter per cage (do not pool rats from different cages).
 - Neonatal rats (up to 10 days of age) are resistant to the hypoxia induced by CO₂; therefore, alternative methods are recommended (e.g., decapitation using sharp blades). See SOP 10.5.5 Euthanasia Method for Rodent Neonates and Fetuses.
 - Place the appropriately sized CO₂ lid on the animal cage (remove cage lid first). Lid holes must be unobstructed and over the body of the cage.
-

University Animal Care Committee Standard Operating Procedure		
Document No: 10.5.2	Subject: Humane Killing of Rats using CO ₂	
Date Issued: August 21 st , 2012	Revision: 4	Page No: 2

- Ensure the regulator hose is connected to the lid fitting.
 - Do not pre-charge the chamber.
 - Open the CO₂ tank valve (if using).
 - Set the regulator to the appropriate setting (35% Displacement):
 - Standard rat cage (7.5" x 13.5" x 14.75"): 8.6 LPM (liters per minute)
 - Double decker rat cage (10" x 18" x 14.5"): 15.0 LPM (liters per minute)
 - Cages of different dimensions: contact Veterinarians.
 - After the animals have become unconscious, the flow rate can be increased to reduce the time to death. The time required for humane killing can be several minutes.
 - Maintain the CO₂ flow until the animal has stopped breathing.
 - Close the flow meter, or the valve on the tank (if using tank system). Leave the animals in contact with loaded CO₂ cage for a minimum of 2 additional minutes.
 - To check for death, verify: no chest movement, poor mucous membrane colour, and colour change or opacity of the eyes.
 - To confirm death, verify: no palpable heartbeat and no response to toe pinch.
 - Once the cage is empty and the session is complete, ensure the flow is off, and flush the line (if using this system).
 - A secondary method of humane killing such as cervical dislocation or bilateral pneumothorax is required for your animals (unless justified in the animal use protocol) before disposal to ensure death.
-

University Animal Care Committee Standard Operating Procedure		
Document No: 10.5.2	Subject: Humane Killing of Rats using CO ₂	
Date Issued: August 21 st , 2012	Revision: 4	Page No: 3

References:

[Journal of the American Association for Laboratory Animal Science](#), Volume 45, Number 1, January 2006, pp. 98-105(8) James Artwohl, Patricia Brown, Brian Corning and Susan Stein Report of the ACLAM Task Force on Rodent Euthanasia

<https://www.ccac.ca/Documents/Standards/Guidelines/Euthanasia.pdf>

SOP Revision History:

Date	New Version
August 21 st , 2012	SOP Created
February 28 th , 2019	Triennial Update
October 8 th , 2020	Update
May 27 th , 2021	Update
January 22 nd , 2025	Triennial Update - updated language and removed neonate and fetus section. Removed mice and updated to Humane Killing.