

University Animal Care Committee Standard Operating Procedure		
Document No: 7.5.6	Subject: Humane Killing of Adult Rodents with CO <sub>2</sub> Asphyxiation under Isoflurane Anesthesia (Mice)	
Date Issued: November 27 <sup>th</sup> , 2024	Revision: 1	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 Isoflurane followed by CO<sub>2</sub> asphyxiation.

**1. Introduction and Definitions:** As per the UACC policy on humane killing of animals used in science, CO<sub>2</sub> humane killing is a *conditionally acceptable* method. Anesthetizing rodents with isoflurane prior to exposure to CO<sub>2</sub> will minimize pain and distress, this method of humane killing is considered an acceptable method. This method is for adult rodents only. Neonatal mice (up to 10 days of age) are resistant to the hypoxia induced by CO<sub>2</sub>; therefore, alternative methods are recommended (e.g. decapitation using sharp blades. See SOP 7.5.5. Euthanasia Method for Rodent Neonates and Fetuses).

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

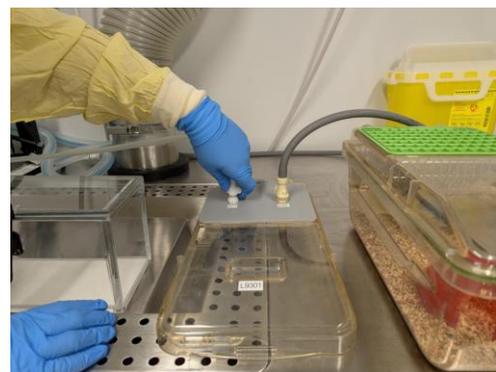
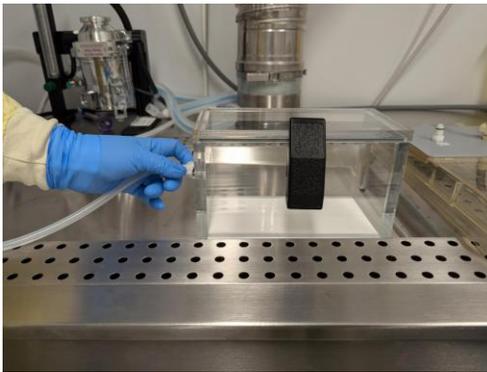
## 2. Materials:

- Isoflurane anesthetic machine (serviced within the last 12 months)
  - Oxygen
  - CO<sub>2</sub> Source with flow meter (facility outlet, or tank and regulator)
  - Appropriately sized rodent cage lid with integrated connections for Isoflurane and CO<sub>2</sub>
  - Instruments (for secondary method of humane killing). See SOP 7.5.4 Humane Killing of Rodents via Physical Methods
  - Body bags
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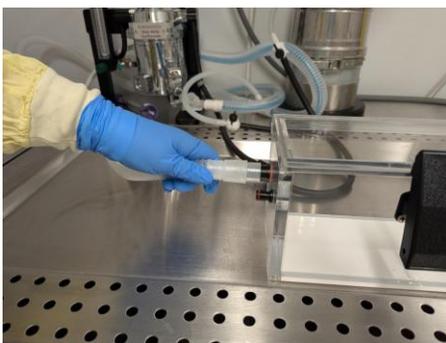
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### 3. Procedures:

- To minimize stress, animals should be humanely killed in their home cage with a maximum of five adult mice or one litter per cage (do not pool mice from different cages).
- Remove the lid, food hopper and igloo, place the appropriately sized custom lid on the rodent cage.
- Disconnect the isoflurane anesthetic supply line from the induction chamber and connect it to the white quick connect on the lid.

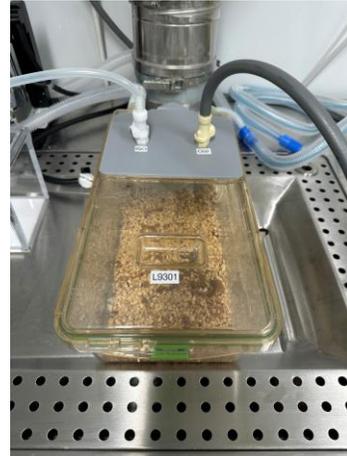


- Connect the grey CO<sub>2</sub> supply line to the beige quick connect on the lid.
- Disconnect the scavenger hose from the induction chamber.



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- Connect the scavenger hose to the adapter on the lid.



- Turn on the active scavenger to 15L/m.



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- Turn on the oxygen flowmeter to 1L/min.



- Set the isoflurane vaporizer to 5%.



- Once the animal has lost consciousness (in lateral recumbency, breathing slower) while leaving the active scavenge on, turn off the isoflurane vaporizer and the oxygen flowmeter and promptly switch over to the CO<sub>2</sub> adaptor, setting the flowmeter to the maximum flow rate of 12 L/min.
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- Maintain the CO<sub>2</sub> flow until the animal has stopped breathing, this process can take several minutes.
- Turn off the CO<sub>2</sub> flow meter and active scavenge and leave the animals in contact with loaded CO<sub>2</sub> cage for a minimum of two additional minutes.
- To confirm death, monitor animals for the following signs: no chest movement, no palpable heartbeat, poor mucous membrane colour, no response to toe pinch, colour change or opacity of the eyes.
- Once death is confirmed, increase the active scavenge to full flow
- Leave active scavenge on while removing dead rodent from cage
- Once the cage is empty and the session is complete, ensure the active scavenge is off.

#### 4. Secondary Means of Humane Killing:

- To ensure death a physical method of humane killing is required unless justified in the Animal Use Protocol. See SOP: 7.5.4 Humane Killing of Rodents via Physical Methods.

#### 5. Disposal of Animal Carcass:

- Place carcass in body bag and then in fridge for disposal
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**References:**

**The Canadian Council on Animal Care [www.CCAC.ca](http://www.CCAC.ca)**  
[https://ccac.ca/Documents/Standards/Guidelines/Implementation\\_of\\_the CCAC Revised Guidance on Euthanasia Using Carbon Dioxide.pdf](https://ccac.ca/Documents/Standards/Guidelines/Implementation_of_the_CCAC_Revised_Guidance_on_Euthanasia_Using_Carbon_Dioxide.pdf)

**SOP Revision History:**

Date	New Version
11/27/2024	SOP Created
4/8/2025	Photos added