



Institutional Animal Care and Use Committee (IACUC) Directive 15.0: Euthanasia of Rodents Using Carbon Dioxide (CO₂)

1.0 Directive Purpose

The purpose of this directive is to ensure proper and humane procedures are employed when research personnel euthanize rodents using carbon dioxide (CO₂).

2.0 To Whom the Directive Applies

This directive applies to all individuals using rodents at Brown University under an approved IACUC animal use protocol.

3.0 Directive Statement

This directive outlines the specific procedures to be used when euthanizing rodents with CO₂ as a primary euthanasia method. It also establishes the IACUC's requirement that a secondary method of euthanasia be used when using CO₂ as a primary euthanasia method.

3.1 Background

This directive is derived from and follows regulatory requirements set forth by the Public Health Service (PHS) Office of Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH), and the American Veterinary Medical Association (AVMA).

In accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition (the *Guide*) and the NIH, personnel administering CO₂ to rodents must be properly trained in this technique and adhere to the corresponding IACUC-approved animal use protocol and institutional policies.

CO₂ inhalation is a common method of euthanasia for rodents and must be included in a researcher's IACUC-approved animal use protocol. CO₂ is an anesthetic and its effects are reversible; therefore, upon completion of CO₂ euthanasia, death must be confirmed for each animal by one or more of the secondary methods listed below.

Disposal of an animal that has not been properly euthanized is a serious animal welfare concern and a significant breach of the conditions of Brown University's approved PHS Animal Welfare Assurance, and may result in a reportable non-compliance and other IACUC actions.

3.2 CO₂ Euthanasia for Other Species and Neonates

Any rodent used for research may be euthanized by CO₂ in accordance with the guidance below. This includes, but is not limited to, mice of the genus *Mus*, rats of the genus *Rattus*, hamsters, gerbils, and other laboratory rodents. Researchers must consult Center for Animal Resources and Education (CARE) veterinary staff prior to submitting a protocol requesting to use CO₂ for

any species other than rodents, and for assistance with CO₂ euthanasia for neonatal (up to 21 days of age) rodents.

3.3 Procedures

Only compressed CO₂ from a cylinder affixed with a flowmeter regulator may be used for CO₂ euthanasia. All other sources of CO₂ are prohibited (*e.g.*, dry ice, fire extinguishers, or chemical means) for this purpose.

Animals should be euthanized in their home cage whenever possible. Animals in the euthanasia chamber must be readily visible and research personnel must remain present for the entire euthanasia procedure. Animals must not be overcrowded in the chamber by combining multiple cages of animals, and must also not be mixed with unfamiliar or incompatible animals (*e.g.*, animals from different cages must not be mixed in the chamber during euthanasia). All animals in the chamber must be able to make normal postural adjustments. If euthanizing in an OptiMice cage, attach the OptiMice inhalation nozzle and cap so the delivery hose can be attached properly to the cage.

The euthanasia chamber must not be pre-filled with CO₂ prior to placing rodents in the chamber. Sudden exposure of conscious animals to CO₂ concentrations of 70% or greater has been shown to be distressful and potentially painful. CO₂ is heavier than room air; therefore, always empty the chamber by turning it over and clean the chamber with dilute chlorhexidine between uses to remove residual CO₂ and avoid a pre-fill situation.

After placing rodents in the chamber, start with room air then slowly fill the chamber with CO₂ over several minutes. To ensure that conscious rodents are exposed to an appropriate level of CO₂, the flow rate should be set to displace 30-70% of chamber volume, as follows:

- 2L/minute when using the **small** chamber or a **split Thoren mouse cage**;
- 5L/minute when using the **large** chamber or a **solid Thoren mouse or rat cage**; and
- 2L/minute when using the OptiMice cage.

CO₂ exposure first anesthetizes the animal, then results in CO₂ narcosis following adequate exposure time. Animals should be left in the euthanasia chamber for at least one minute after spontaneous movements have ceased, with CO₂ continuing to flow at the same rate. Animals must be continuously monitored for adverse reactions for the duration of the euthanasia procedure. It is important to contact CARE staff promptly if the procedure appears to produce excessive agitation or other complications.

Neonatal rodents are resistant to CO₂-induced hypoxia resulting in a prolonged time to unconsciousness (up to 60 minutes); therefore, it is recommended that neonatal rodents be placed into the CO₂ chamber for 10-15 minutes until fully anesthetized, which is evidenced by no observable movement and no response to a toe pinch, after which they may be euthanized by decapitation with a sharp blade or scissors.

Minimum Time in 100% CO ₂ for Mice and Rats		
Age	Mice	Rats
Non-haired pups (0-6 days old)	60 minutes	40 minutes
Haired pups, eyes closed (7-13 days old)	20 minutes	20 minutes

Haired pups, eyes open, preweaning (14-20 days old)	10 minutes	10 minutes
Weanlings and adults (21+ days old)	5 minutes	5 minutes

3.4 Confirmation of Death Via a Secondary Method

Death of the animal must be confirmed via a secondary method prior to disposal of the rodent carcass. CO₂ is an anesthetic and the effects are reversible. Failure of the animal to move or show a reflex response is not sufficient to confirm death. One of the secondary methods below must be used to ensure that the rodent will not recover:

- Bilateral thoracotomy
- Exsanguination
- Cervical dislocation
- Decapitation.

3.5 Non-Compliance

Failure to confirm death of a euthanized rodent is a significant noncompliance that may be reported to the appropriate regulatory and accrediting agencies, and will require corrective actions (*e.g.*, re-training) and possible additional steps (*e.g.*, prohibition of certain personnel from performing CO₂ euthanasia) depending on the specific circumstances of the noncompliance.

4.0 Definitions: N/A

5.0 Responsibilities

All individuals to whom this directive applies are responsible for becoming familiar with it and following this. Animal research program stakeholders (IACUC, CARE, ARC) are responsible for promoting the understanding of this document and for taking appropriate steps to help ensure adherence to it.

6.0 Consequences for Violating this Document

Violation of this document may be considered a serious event of noncompliance that is reportable to the IACUC, funding and accrediting agencies, as well as other regulatory agencies. Violations of this document are a serious matter that may adversely affect both the ability to perform animal work and acquire funding sources.

7.0 Related Information

Brown University is a community in which employees are encouraged to share workplace concerns with University leadership. Additionally, [Brown's Anonymous Reporting Hotline](#) allows anonymous and confidential reporting on matters of concern online or by phone (877-318-9184).

The following information complements and supplements this document. The information is intended to help explain this document and is not an all-inclusive list of policies, procedures, laws and requirements.

7.1 Related Policies/Directives/SOPs:

- IACUC Maintenance of Blades

7.2 Related Procedures: N/A

7.3 Related Forms: N/A

7.4 Frequently Asked Questions (FAQs): N/A

7.5 Other Related Information:

- AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. AVMA: Schaumburg, IL.
- Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals, 8th Edition. National Academies Press, 2020.
- ILAR. 1991. ILAR News 33(4):68-70. Issues for Institutional Animal Care and Use Committee.
- National Institutes of Health (U.S). Office of Laboratory Animal Welfare., United States. Public Health Service. 2019. [Public Health Service Policy on Human Care and Use of Laboratory Animals.](#)
- [PHS Policy on Humane Care and Use of Laboratory Animals: Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small Laboratory Animals.](#)

8.0 Document Owner and Contact

8.1 Owner: IACUC

8.2 Approved by: IACUC

8.3 Subject Matter Contact: Brown University Animal Research Compliance (ARC)

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9.0 Document History

9.1 Effective Date: February 5, 2016

9.2 Last Reviewed: June 2, 2023

9.3 Update/Review Summary: This document is not new; it was converted to the directive format and reviewed by the IACUC at its June 2, 2023 meeting.

- This policy is not new; it was converted to the University's new policy template and re-reviewed by the IACUC at its convened meeting on August 7, 2020. Prior policy version:
- IACUC Policy on Euthanasia of Mice and Rats using Carbon Dioxide (CO₂), date of IACUC review and approval: May 31, 2019.