

HKSTP IACUC

11 – Euthanasia Guidelines

Version History

Version	Effective Date
1	28/02/2023

1. Purpose

The guidelines aim to provide a reference to the minimum standard required when euthanizing laboratory animals. Please note that requirements for euthanasia of animals for specific species may differ according to the species' physical structure.

Euthanasia is derived from the Greek terms *eu* meaning good and *thanatos* meaning death. The term is usually used to describe ending the life of an individual animal in a way that minimizes or eliminates pain and distress. A good death is tantamount to the humane termination of an animal's life

2. Methods for Euthanasia [Rodent Specific]

2.1 Acceptable methods for euthanasia of laboratory animals (adults)

- i. **Barbiturates** (e.g. Pentobarbital) – administered via intraperitoneal injection (IP) at 3 times the anaesthetic dose.
- ii. **Dissociative agent combinations** (e.g. Dissociative agents and alpha2-adrenergic receptor agonist) – administered via IP injection at 5 times the anaesthetic dose.

Example of combinations: Ketamine and xylazine receptor
Ketamine and benzodiazepines (e.g. diazepam)

2.2 Conditionally acceptable methods for euthanasia of laboratory animals (adults)

- i. **Inhaled agents**
 - a. **Halogenated anaesthetic** (e.g. isoflurane) – Halogenated anaesthetic may be used where physical restraint is difficult or impractical, however, disadvantages include requiring prolonged exposure to ensure death. Care should be taken to ensure animals do not come in direct contact with anaesthetic. **Research facility is required to be equipped with active waste gas scavenging system and local exhaust when using Halogenated anaesthetic.**
 - b. **Carbon dioxide (CO₂)** – Gas flow must be precisely regulated. An optimal flow rate for CO₂ euthanasia systems should displace 30% to 70% of the chamber cage volume/min, with the understanding that there is potential for increased distress due to dyspnoea at lower flow rates or mucous membrane pain associated with flow rates

at high ends of this range. Home cage euthanasia shall be used whenever possible. In situations where home cage euthanasia is inappropriate, induction chambers should be emptied and cleaned between uses. Prefilled chambers are not recommended. **CO₂ should only be used in well ventilated area and research facility is required to be equipped with CO₂ monitor to monitor the CO₂ in the premise during Euthanasia.**

ii. **Non-inhaled agents**

- a. **Tribromoethanol** – This should not be used as a sole agent for euthanasia, it is recommended as an anaesthetic before the application of an approved secondary method. Tribromoethanol should be prepared, stored properly and administered at the appropriate dosage.

iii. **Physical methods**

- a. **Cervical dislocation** – This method is acceptable for mice and rats <200g. Personnel should be trained on anaesthetized and/or dead animals to demonstrate proficiency.

2.3 Acceptable methods for euthanasia of laboratory animals (fetuses and neonates)

- i. **Euthanasia of the dam and mammalian fetuses** – removal of fetuses for euthanasia is not necessary as euthanasia of the dam leads to unconsciousness of the rodent fetuses in utero and hypoxia does not evoke a response.

ii. **Non-inhaled agents**

- a. **Injectable barbiturates** (e.g. pentobarbital) – dissociative agents combined with alpha2-adrenergic receptor agonist or benzodiazepines. Examples: ketamine + xylazine, ketamine + diazepam
- b. **Carbon dioxide (CO₂)** – Neonatal mice and rats may take up to 50 and 35 minutes to die from CO₂ exposure respectively. It should be ensured that adequate exposure time is provided. If adequate exposure time cannot be provided, an adjunctive method (e.g. cervical dislocation) should be performed after a neonate is nonresponsive to painful stimuli.

iii. **Physical methods**

- a. **Hypothermia** – This method is only acceptable for fetuses and neonates of rats and mice at or below 10 days of age with the condition that there is no direct contact between the animal and the cooling material (e.g. ice, precooled surfaces). Gradual cooling should be followed by a secondary method following loss of movement.
- b. **Rapid freezing** – This method is only acceptable for fetuses and neonates of rats and mice at or below 5 days of age. Animal shall be killed quickly by rapid freezing in liquid nitrogen.

- c. **Cervical dislocation** – This method is acceptable for mice and rats <200g. Personnel should be trained on anaesthetized and/or dead animals to demonstrate proficiency.

References

Artwohl J., Brown P., Corning B., Stein S., ACLAM Task Force. (2006). Report of the ACLAM Task Force on Rodent Euthanasia.

AVMA Guidelines for Euthanasia of Animals 2020. <https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>