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Standard Procedures for Euthanasia of Mice and Rats

1. Animals should only be euthanized in a quiet, clean environment, and preferably away from other animals. In some cases, release of pheromones occurs during induction of unconsciousness. For that reason, other animals should not be present when euthanasia is performed. Do not euthanize rodents in a Procedure Room at the same time in which it is being used for animal procedures.
2. When euthanizing animals, watch over them and be in the room until the last animal is euthanized and removed from the euthanasia chamber into a biohazard disposal bag.
3. Mice and rats must not be brought into the Procedure Room at the same time. If you need to euthanize two species, complete euthanizing one species first before bringing the second species into the Procedure Room.
4. To reduce social aggression and distress, minimize regrouping animals of the same species from different cages. If regrouping of animals is to be performed, the time during which mixed groups of animals are together in the same cages must be minimized. Rats and mice must not be regrouped into the same cage.
5. When regrouping, do not overcrowd cages. All animals must be able to stand with all 4 feet on the cage floor and must have sufficient space to turn around comfortably. The BRC standards are:
 - a) For mice:
 - i) ITS slim cages (1145T) - max of 30 weaners (15g BW)
- max of 15 adult mice (30g BW)
 - ii) ITS cages (1285L) - max of 40 weaners (15g BW)
- max of 20 adult mice (30g BW)
 - iii) Greenline (GM500) - max of 40 weaners (15g BW)
- max of 20 adult mice (30g BW)
 - b) For rats:
 - i) ITS cages (1500) - max of 30 rats (up to 200g BW)
- max of 15 rats (200 – 400g BW)
- max of 5 rats (> 400g BW)
 - ii) Greenline (GR900) - max of 20 rats (< 200g BW)
- max of 10 rats (> 200g BW)
- max of 6 rats (> 300g BW)
- max of 3 rats (> 400g BW)

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- Euthanasia by CO₂ is only recommended for mice and rats which are at least 10 days' old. The duration of exposure to CO₂ varies with neonate age and strain. For euthanasia of fetuses and neonates less than 10 days' old, decapitation using a sharp scissors, or cervical dislocation is recommended (see Para. 26).

Euthanasia of Rodents (Neonates 10 days of age and older)

- Animals must not be euthanized in any animal holding room. Bring IVC cage(s) with animals of the same species to be euthanized to the Procedure Room. IVC cages in the Procedure Room should either be inside the euthanasia chamber, or left outside the Procedure Room. Live animals should not be present when euthanasia is performed.
- A clear perspex chamber with lid is used as the euthanasia chamber. Ensure that the lid was left open before starting. If not, open and air the chamber for at least 10 minutes.
- Check to ensure that the rubber gasket mounted into the perimeter of the chamber lid is in place and not cracked. This rubber gasket reduces the leakage of carbon dioxide from the euthanasia chamber into the procedure room.
- Check to ensure that the euthanasia chamber is clean and free of excreta and debris. If required, use a damp paper towel to clean using Clidox or ethanol. Alternatively, place an underpad on the floor of the chamber and dispose after all animals have been euthanized.
- Check to ensure that the tubing from CO₂ supply to euthanasia chamber is attached and secured.
- Remove the water bottle and cage lid of the IVC cage containing the animals. Do not remove the wire mesh from these cages.
- Place the IVC cage(s) into the euthanasia chamber and close the lid gently.
- Follow instructions on the CO₂ control module to release CO₂ into the euthanasia chamber.
- For systems with set flow rates, CO₂ tap should be turned on fully—Follow the instructions displayed at the euthanasia chambers.
- For systems without set flow rates, the flowrate is calculated as a 10-30% displacement rate of chamber air with CO₂ gas/minute. (e.g. if the volume of chamber is 10L, flowrate is 3L/min)
- Observe the animals. Once they appear to relax and are no longer moving around, continue to run the CO₂ until the animals no longer show any respiratory or movements. Gas flows should

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be maintained for at least 1 minute after the animals no longer show any respiratory and other purposeful movements.

12. Then turn off the CO₂ by following instructions on the CO₂ control module. Depending on the number of animals in the chamber, it may take 5 minutes (for mice) and 10 minutes (for rats) before the animals are sufficiently immobilized to turn off the CO₂.
13. Now open the lid of the euthanasia chamber. Remove one animal and palpate its chest to ensure there is no heart beat to confirm death. Pinch the webbing between its toes and ensure there is no withdrawal reflex. If any sign of respiratory or other movements occur, you must return the animal into the chamber and repeat steps 8 to 13.
14. At this point, you must also cervically dislocate the animal, or perform a bilateral thoracotomy to ensure death. Repeat this for all animals in the euthanasia chamber.
15. It is important to verify that an animal is dead before removing it from the chamber. Place the dead animals into its assigned bag (purple for cytotoxic carcasses, yellow biohazard bag for all others, and label it correctly. There must be no disposal of carcasses until death is confirmed.
16. Remove the IVC cage(s) from the euthanasia chamber and clean the chamber with paper towels using a disinfectant if required. Once a week, BRC facility staff will clean the euthanasia chambers with Clidox.
17. Before leaving the Procedure Room, ensure the lid of the euthanasia chamber is kept open, the chamber is dry and clean ready for re-use by the next person.
18. Live animals must not be placed with dead animals at the same time in the euthanasia chamber.

Euthanasia of Fetuses up to 15 days' gestation

For mouse and rat fetuses up to 15 days' gestation, euthanasia of the mother or removal of the fetuses should ensure rapid death of the fetus due to loss of blood supply and non-viability of fetuses at this stage of development.

Euthanasia of Fetuses over 15 days' gestation and Neonates up to 10 days' of age

For mouse and rat fetuses over 15 days' gestation and neonates up to 10 days' of age, decapitation with a sharp pair of scissors or cervical dislocation is recommended.