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Direction des services vétérinaires

Standard Operating Procedure

Re: Rodent euthanasia	Number: EU-1
Scope: A directive from the Direction des services vétérinaires to users and staff of Université Laval animal facilities (campus and affiliated research centres).	
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Purpose: Describe acceptable euthanasia procedures for rodents	Version 6

General considerations

- Animal euthanasia is used to put an animal to death in a humane manner that minimizes pain and distress.
- The chosen method must result in rapid loss of consciousness followed by cardiac and respiratory arrest and, lastly, brain death. It must be compatible with experimental objectives.
- Euthanasia procedures must be performed by appropriately trained personnel.
- Always perform the euthanasia of one or more animals in an unoccupied room to limit stress.
- Never perform euthanasia in the same room where animals are housed. In exceptional cases, emergency euthanasia may be performed within a separate ventilated enclosure inside an animal housing room if the other animals are in ventilated cages.
- Barbiturate overdose euthanasia is the method of choice.
- Animals must be fully anesthetized whenever additional procedures are required (perfusion or exsanguination).
- Dispose of carcasses as described in the approved SOP.
- For carbon dioxide euthanasia, keep animals in their home cages to reduce the stress created by pooling cages.
- For all methods, a secondary means of euthanasia is recommended to ensure the animals' death (absence of auscultation sounds).

Procedures

Acceptable methods

Barbiturate overdose

- Weigh the animal and check its identification.
- Administer a 120 mg/kg dose of sodium pentobarbital (Euthanyl®, Euthansol®) intravenously or intraperitoneally.
- Place the animal in a clean cage or in its home cage in a quiet area to minimize excitation until euthanasia is complete.
- Confirm the animal's death by verifying the absence of respiratory movements and the withdrawal reflex.
- Perform a physical method of euthanasia (cervical dislocation, decapitation, or opening the chest cavity).
- Fill out the appropriate register for controlled drugs.

Injectable anesthetic overdose

- Weigh the animal and check its identification.
- Administer a dose 2 to 3 times greater than the anesthetic dose intravenously or intraperitoneally
- Place the animal in a clean cage or in its home cage in a quiet area to minimize excitation until euthanasia is complete.
- Confirm the animal's death by verifying the absence of respiratory movements and the withdrawal reflex.
- Perform a physical method of euthanasia (cervical dislocation, decapitation, or opening the chest cavity).
- Fill out the appropriate controlled drug register.

Asphyxiation by carbon dioxide (CO₂) overdose under general anesthesia

- Check the animal's identification.
- Place the animal in an induction chamber connected to an anesthesia machine. Alternatively, a lid designed for this purpose can be placed directly over the home cage, or the home cage can be placed within a larger induction chamber.
- Anesthetize the animal with isoflurane according to the procedure in *SOP A-1 Analgesia and anesthesia in rodents (PNF A-1 Analgésie et anesthésie des rongeurs)*.
- After the animal loses consciousness, turn off the anesthesia machine and fill the chamber with CO₂. Continue to administer CO₂ for at least 1 minute after breathing has ceased.

- Close the CO₂ valve and leave the animal in the chamber for an additional minute.
- Confirm the animal's death by verifying the absence of respiratory movements and the withdrawal reflex.
- Perform a physical method of euthanasia (cervical dislocation, decapitation, or opening the chest cavity).
- If the chamber will be reused for other animals, wait until the air inside refreshes. CO₂ is denser than air and may concentrate at the bottom of the chamber, where it can cause pain and distress to animals.
- Clean the chamber thoroughly before placing other animals inside.

Cervical dislocation under general anesthesia

*Do not use this method for rodents over 200 g.

- Check the animal's identification.
- Anesthetize the animal according to the procedure in *SOP A-1 Analgesia and anesthesia in rodents* and check for the absence of the withdrawal reflex.
- Grasp the base of the skull with your thumb and forefinger, or with a long flat instrument for larger rodents.
- With the other hand, either grasp the base of the tail and pull sharply to separate the cervical vertebrae from the skull or press the thumb against the index finger so they touch once the vertebrae have separated.
- Check for successful dislocation by palpating the cervical vertebrae.
- If the dislocation is unsuccessful, immediately euthanize by decapitation or by a barbiturate injection/injectable anesthetic overdose.

Decapitation under general anesthesia

- Check the condition of the blades; make sure they are clean and sharp.
- Check the animal's identification.
- Anesthetize the animal according to the procedure in *SOP A-1 Analgesia and anesthesia in rodents* and check for the absence of the withdrawal reflex.
- Place the animal's neck in the guillotine or between the scissor blades (for small species). Quickly and firmly depress the guillotine or close the scissors.
- Thoroughly clean the guillotine or scissors before the next animal.

Exsanguination

- Check the animal's identification.
- Anesthetize the animal according to the procedure in *SOP A-1 Analgesia and anesthesia in rodents* and check for the absence of the withdrawal reflex.
- Withdraw as much blood as possible by cardiac puncture or via collection from the abdominal aorta (refer to the approved SOPs on blood collection).
- Confirm the animal's death by verifying the absence of respiratory movements and the withdrawal reflex.
- Performance of a physical method of euthanasia is recommended to ensure the animal's death, e.g., cervical dislocation, decapitation, or opening of the thoracic cavity.

Perfusion

- Weigh the animal and check its identification.
- Anesthetize the animal according to the procedure in *SOP A-1 Analgesia and anesthesia in rodents* and check for the absence of the withdrawal reflex.
- Perform the perfusion as described in the protocol. Use a chemical fume hood or extraction arm when perfusing with formaldehyde.
- After perfusing a single organ (not the whole body), performance of a physical method of euthanasia is recommended to ensure the animal's death, e.g., cervical dislocation, decapitation, or opening of the thoracic cavity.

Euthanasia of fetuses and gestating females

- Before two-thirds of gestation has passed, euthanize gestating females using the methods described above. Simple removal of fetuses from the uterus will also ensure rapid fetal death.
- Once two-thirds of gestation has passed, euthanize gestating females with a method that causes rapid fetal cerebral anoxia (e.g., anesthesia followed by exposure to CO₂ or cervical dislocation). If the gestating female is merely anesthetized, euthanize fetuses by
 - overdose of injectable anesthetics, via a route appropriate to their size and developmental stage,
 - decapitation with sharp, well-maintained scissors, or
 - cervical dislocation.

Euthanasia of neonatal rodents (0 to 10 days)

- Euthanize neonates using the methods described above for adult animals, with the exception of asphyxiation by carbon dioxide overdose, which is ineffective in such cases.
- If using a physical method (decapitation or cervical dislocation), anesthetize the animals first.
- If using hypothermia on ice, perform a physical method of euthanasia (cervical dislocation, decapitation, or opening the chest cavity) to ensure the animal is dead.

Choice of euthanasia method

- Depending on the situation, some methods are preferred over others (see the table below for a partial list). For example, CO₂ euthanasia is not the method of choice during experimental protocols, given its expected side effects.

Recommended methods of euthanasia for different situations

Situation	Plan A	Plan B	Plan C
Euthanasia of a large number of animals in the colony	CO ₂ under anesthesia	-	-
Euthanasia during anesthesia	Dislocation under anesthesia (mouse or rat under 200 g)	Exsanguination	Overdose of anesthetic or barbiturates
End of experimentation	Any method listed in this SOP that meets the experimental requirements		
Limit points reached	Overdose of anesthetic or barbiturates	CO ₂ under anesthesia	Dislocation under anesthesia

References

AAZV, *Guidelines for Euthanasia of Nondomestic Animals*, 2006.

AVMA *Guidelines for the Euthanasia of Animals*, 2013.

CCAC *guidelines on: euthanasia of animals used in science*, 2010.

SOP Revision History		
Version 2	August 9, 2012	Modified euthanasia of fetuses after two-thirds of gestation.
Version 3	February 8, 2013	Added clarifications to the general considerations. Added clarifications on anesthesia of neonatal rodents.
Version 4	June 7, 2013	Clarified accepted methods and methods requiring scientific justification. Removed lidocaine during sodium phenobarbital injection. Added link and section on <i>Choice of euthanasia</i> method. Added table on recommended methods of euthanasia.
Version 5	September 10, 2013	Added perfusion. Added clarifications on euthanasia of fetuses and gestating females.
Version 6	March 9, 2016	Added clarifications on euthanasia permitted in the animal housing room. Added requirement to perform a physical method after a chemical procedure. Added the home cage for carbon dioxide euthanasia.