Recommended Methods of Euthanasia: *Mice / Rats*

Species	Method	Description
Mice / Rats	Asphyxiation using CO2 followed by bilateral thoracotomy.	Using a non-precharged chamber, CO2 is dispensed from a commercial cylinder with fixed pressure regulator and inline restrictor controlling gas flow within 30%-70% of the chamber volume per minute to comply with 2020 AVMA Guidelines. CO2 flow will be maintained for > 60 seconds following respiratory arrest (which may take up to 5 minutes), followed by bilateral thoracotomy to assure euthanasia. *NOTE : Neonates < 7 days old should be euthanized by a physical method, such as sharp scissors.*
	Asphyxiation using CO2 followed by cervical dislocation.	Using a non-precharged chamber, CO2 is dispensed from a commercial cylinder with fixed pressure regulator and inline restrictor controlling gas flow within 30%-70% of the chamber volume per minute to comply with 2020 AVMA Guidelines. CO2 flow will be maintained for > 60 seconds following respiratory arrest (which may take up to 5 minutes), followed by cervical dislocation to assure euthanasia. *NOTE : Only acceptable in rats weighing < 200 grams. Neonates < 7 days old should be euthanized by a physical method, such as sharp scissors.*
	Asphyxiation using CO2 followed by decapitation.	Using a non-precharged chamber, CO2 is dispensed from a commercial cylinder with fixed pressure regulator and inline restrictor controlling gas flow within 30%-70% of the chamber volume per minute to comply with 2020 AVMA Guidelines. CO2 flow will be maintained for > 60 seconds following respiratory arrest (which may take up to 5 minutes), followed by decapitation using (*indicate equipment used*) to assure euthanasia. *NOTE : Neonates < 7 days old should be euthanized by a physical method, such as sharp scissors.*
	Asphyxiation using CO2 followed by exsanguination.	Using a non-precharged chamber, CO2 is dispensed from a commercial cylinder with fixed pressure regulator and inline restrictor controlling gas flow within 30%-70% of the chamber volume per minute to comply with 2020 AVMA Guidelines. CO2 flow will be maintained for > 60 seconds following respiratory arrest (which may take up to 5 minutes), followed by rapid exsanguination via (*indicate method or vascular incision points)* to assure euthanasia. *NOTE : Neonates < 7 days old should be euthanized by a physical method, such as sharp scissors.*
	Asphyxiation using CO2 followed by major organ harvest.	Using a non-precharged chamber, CO2 is dispensed from a commercial cylinder with fixed pressure regulator and inline restrictor controlling gas flow within 30%-70% of the chamber volume per minute to comply with 2020 AVMA Guidelines. CO2 flow will be maintained for > 60 seconds following respiratory arrest (which may take up to 5 minutes), followed by rapid harvest of (*indicate organs / tissues*) to assure euthanasia. *NOTE : Neonates < 7 days old should be euthanized by a physical method, such as sharp scissors.*
	Inhalant anesthetic overdose followed by bilateral thoracotomy.	Using a precision vaporizer with induction chamber and waste gas scavenger, (*indicate the gas anesthetic*) will be administered slowly up to [*indicate: > 4.5 % (for Isoflurane) or > 6.5 % (for Sevoflurane*)] in oxygen and continued until respiratory arrest occurs for > 60 seconds. The chamber is flushed with oxygen only, the animal is removed and bilateral thoracotomy is performed to assure euthanasia.

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Species	Method	Description
	Inhalant anesthetic overdose followed by cervical dislocation.	Using a precision vaporizer with induction chamber and waste gas scavenger, (*indicate the gas anesthetic*) will be administered slowly up to [*indicate: > 4.5 % (for Isoflurane) or > 6.5 % (for Sevoflurane*)] in oxygen and continued until respiratory arrest occurs for > 60 seconds. The chamber is flushed with oxygen only, the animal is removed and rapid cervical dislocation is performed to assure euthanasia. *NOTE : Only acceptable in rats weighing < 200 grams.*
	Inhalant anesthetic overdose followed by decapitation.	Using a precision vaporizer with induction chamber and waste gas scavenger, (*indicate the gas anesthetic*) will be administered slowly up to [*indicate: > 4.5 % (for Isoflurane) or > 6.5 % (for Sevoflurane*)] in oxygen and continued until respiratory arrest occurs for > 60 seconds. The chamber is flushed with oxygen only, the animal is removed and decapitated with *(indicate equipment used*) to assure euthanasia.
	Inhalant anesthetic overdose followed by exsanguination.	Using a precision vaporizer with induction chamber and waste gas scavenger, (*indicate the gas anesthetic*) will be administered slowly up to [*indicate: > 4.5 % (for Isoflurane) or > 6.5 % (for Sevoflurane*)] in oxygen and continued until respiratory arrest occurs for > 60 seconds. The chamber is flushed with oxygen only, the animal is removed and rapid exsanguination is performed by (*indicate method or vascular incision points*) to assure euthanasia.
	Inhalant anesthetic overdose followed by major organ harvest.	Using a precision vaporizer with induction chamber and waste gas scavenger, *(indicate the gas anesthetic*) will be administered slowly up to [*indicate: > 4.5 % (for Isoflurane) or > 6.5 % (for Sevoflurane*)] in oxygen and continued until respiratory arrest occurs for > 60 seconds. The chamber is flushed with oxygen only, the animal is removed and rapid removal of (*indicate tissues / organs*) is performed to assure euthanasia.
	Inhalant anesthetic overdose (dessicator jar) followed by bilateral thoracotomy.	Using a dessicator jar in a chemical fume hood, the animal is placed on a perforated platform that prevents direct contact with the liquid anesthetic (*indicate the gas anesthetic*). The lid is replaced and the animal monitored until lack of respiration for > 60 seconds is observed. The animal is removed from the jar and bilateral thoracotomy is performed to assure euthanasia.
	Inhalant anesthetic overdose (dessicator jar) followed by cervical dislocation.	Using a dessicator jar in a chemical fume hood, the animal is placed on a perforated platform that prevents direct contact with the liquid anesthetic (*indicate the gas anesthetic*). The lid is replaced and the animal monitored until lack of respiration for > 60 seconds is observed. The animal is removed from the jar and cervical dislocation is performed to assure euthanasia. *NOTE : Only acceptable in rats weighing < 200 grams.*
	Inhalant anesthetic overdose (via dessicator jar) followed by decapitation.	Using a dessicator jar in a chemical fume hood, the animal is placed on a perforated platform that prevents direct contact with the liquid anesthetic (*indicate the gas anesthetic*). The lid is replaced and the animal monitored until lack of respiration for > 60 seconds is observed. The animal is removed from the jar and decapitated with (*state equipment used*) to assure euthanasia.
	Inhalant anesthetic overdose (via dessicator jar) followed by exsanguination.	Using a dessicator jar in a chemical fume hood, the animal is placed on a perforated platform that prevents direct contact with the liquid anesthetic (*indicate the gas anesthetic*). The lid is replaced and the animal monitored until lack of respiration for > 60 seconds is observed. The animal is removed from the jar and exsanguination performed by (*indicate method or vascular incision points)* to assure euthanasia.
	Inhalant anesthetic overdose (via dessicator jar) followed by major organ harvest.	Using a dessicator jar in a chemical fume hood, the animal is placed on a perforated platform that prevents direct contact with the liquid anesthetic (*indicate the gas anesthetic*). The lid is replaced and the animal monitored until lack of respiration for > 60 seconds is observed. The animal is removed from the jar and (*state tissues / organs*) are harvested to assure euthanasia.

Recommended Methods of Euthanasia: *Mice / Rats*

Species	Method	Description
	Decapitation by Guillotine under sedation or anesthesia (Juveniles / Adults).	Post induction of anesthesia or sedation using *(indicate drug, dose in mg/kg, route & gauge needle),* the animal is placed head first into a decapicone and moved forward in the cone until secured. Holding the cone at the rear, the narrow end of the cone is inserted into a commercial guillotine to the level of the cervical vertebrae and the guillotine is activated.
	Decapitation by Guillotine without sedation or anesthesia (Juveniles / Adults).	Animals are placed head first into a decapicone and moved forward in the cone until secured. Holding the cone at the rear, the narrow end of the cone is inserted into a commercial guillotine to the level of the cervical vertebrae and the guillotine is activated. *NOTE: Scientific Justification is REQUIRED for the use of this method without sedation / anesthesia on juvenile or adult animals.*
	Decapitation by sharp scissors of neonates.	Neonates < 7 days of age: decapitation performed with sharp scissors without sedation or anesthesia.
	Cervical Dislocation without anesthesia / sedation.	The thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull with the animal laying on a table surface . With the other hand, the base of the tail is firmly and steadily pulled to cause separation of the cervical vertebrae and spinal cord from the skull. *NOTES : (1) Scientific Justification is REQUIRED for use of this method without sedation/ anesthesia. (2) Per the 2020 AVMA Guidelines, the IACUC should ascertain and document demonstrated technical competency by all staff conducting this procedure. If IACUC approved, a Vet Resources veterinarian will observe and document technical competency to ensure humane handling and euthanasia. (3) Only acceptable in rats weighing < 200 grams.*
	Vital perfusion under injectable anesthesia	(*Indicate drug, dose in mg/kg, route & gauge needle*) will be used to induce anesthesia. Surgical depth of anesthesia will be verified by lack of response to (*indicate stimulus*) stimulus. Vital perfusion will be performed using (*indicate name*) perfusate injected into the (*define point of vascular access or blood egress site*). Perfusion will be performed in a chemical fume hood if required by EHS. Perfusate waste will be disposed of by (*indicate method of disposal*).
	Injectable anesthetic overdose (Pentobarbital)	Administration of ≥ 100mg/kg of Pentobarbital (*state manufacturer*) intraperitoneal (IP) or intravascular (IV). Monitor animal until lack of heart beat is noted for ≥ 60 seconds prior to tissue harvest or carcass disposal.