SUBJECT: Euthanasia Guideline

DATE: January 11, 2024

Euthanasia Guideline

The *Guide for the Care and Use of Laboratory Animals* (the Guide, NRC 2011) states: "Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the *AVMA Guidelines on Euthanasia* (AVMA 2007 or later editions)." (p. 123)

The *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition* states: "Euthanasia methods are classified in the Guidelines as acceptable, acceptable with conditions, and unacceptable. Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia. Methods acceptable with conditions are those techniques that may require certain conditions to be met to consistently produce humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure death. Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method can be met." (p. 9)

Rodent euthanasia - important information: Activities that contribute to distress in rodents include transport, handling (in animals not accustomed to it), disruption of compatible groups, bright lighting, and elimination of established scent marks. While eliminating all sources of distress may not be practical or possible, the selected method of euthanizing rodents should minimize these sources of potential distress.

- Euthanasia may cause distress vocalizations or pheromones that could influence other animals in the room. Euthanasia may be best performed in a separate location, if transportation distress can be minimized.
- Light intensity during euthanasia should be the same or darker than the rodents' housing room.
- Euthanasia by injectable or inhaled agents must be followed by a secondary method of euthanasia to assure death.
    - **Acceptable secondary methods** are cervical dislocation for mice, voles, and rats <200g, bilateral pneumothorax, exsanguination, decapitation, removal of vital organs, and perfusion.

Mice	
Acceptable Method(s)	○ Injected barbiturates and barbiturate combinations followed by a secondary method ○ Injected ketamine combinations followed by a secondary method ○ Adjunctive method under anesthesia (exsanguination, bilateral pneumothorax, perfusion with fixative)
Acceptable Method(s) ***With Conditions****	○ Inhaled anesthetics followed by a secondary method ○ CO_2 followed by a secondary method ○ Cervical dislocation ○ Decapitation ○ Intraperitoneal ethanol followed by a secondary method See conditions below
Rats and Voles	

Acceptable Method(s)	○ Injected barbiturates and barbiturate combinations followed by a secondary method ○ Injected ketamine combinations followed by a secondary method ○ Adjunctive method under anesthesia (exsanguination, bilateral pneumothorax, perfusion with fixative)
Acceptable Method(s) ***With Conditions****	○ Inhaled anesthetics followed by a secondary method ○ CO_2 followed by a secondary method ○ Cervical dislocation (<200g only) ○ Decapitation See conditions below
Neonatal rodents	
Acceptable Method(s)	Up to 10 days of age: ○ Injected barbiturates and barbiturate combinations followed by a secondary method ○ Injected dissociative agent combinations followed by a secondary method ○ Decapitation with surgical scissors ○ Cervical dislocation ○ Altricial neonates less than 5 days of age and fetuses may be euthanized through rapid freezing in liquid nitrogen.

Method	Conditions
Inhaled anesthetics	• May be used to anesthetize an animal prior to an adjunctive method of euthanasia (exsanguination, bilateral pneumothorax, perfusion with fixative) • Animals may need to be exposed for prolonged time periods when used as a sole euthanasia agent delivered via vaporizer. • Open-drop technique may be used for a more rapid death but ensure that the rodent does not come into direct contact with the anesthetic.
CO_2	• It is unacceptable to place a conscious animal directly into a container prefilled with 100% CO_2. • Rodents should be kept in their home cage with familiar cage-mates during CO_2 administration. • Carbon dioxide must be provided from a commercially supplied cylinder or tank. • An appropriate pressure-reducing regulator and flow meter is required. • Use a displacement rate of 30% to 70% of the chamber volume/min. • CO_2 flow should be maintained for at least 1 minute after respiratory arrest. • If an induction chamber or shared empty cage is used, it should be emptied and cleaned between uses.
Cervical dislocation	• Cervical dislocation is acceptable with conditions for mice and rats < 200 g when performed by individuals with a demonstrated high degree of technical proficiency, or while the animal is anesthetized. • Those responsible for the use of this method must ensure that personnel who perform cervical dislocation have been properly trained to do so and consistently apply it humanely and effectively. • Death must be confirmed by physical exam or other **acceptable secondary methods**.
Decapitation	• Decapitation is acceptable with conditions for mice and rats. • It may be used in research settings when its use is required by the experimental design and approved by the IACUC. • Specialized rodent guillotines are available and must be kept clean, in good condition with sharp blades. • The use of plastic cones to restrain animals is recommended to provide animal restraint, minimize distress, and improve positioning. • Those responsible for the use of this method must ensure that personnel who perform decapitation have been properly trained to do so and are monitored for competence. • See IACUC Guillotine and Scissor Maintenance and Testing SOP.
Intraperitoneal Ethanol	• IP 70 -100% ethanol is acceptable with conditions as a method for euthanasia of laboratory mice when approved by the IACUC and prepared, stored, and administered at the appropriate dosage. • It may be used for mice >35 days of age.
Zebrafish	

Acceptable Method(s)	○ Immersion in buffered MS 222 for at least 30 minutes; it is recommended that this is followed by an adjunctive method (decapitation, pithing, exsanguination, freezing). ○ Adult: Rapid chilling (2-4°C) for a minimum of 10 additional minutes after loss of operculum movements. ○ Fry 4-14 days post-fertilization: Rapid chilling (2-4°C) followed by an adjunctive method or rapid chilling for a minimum of 20 additional minutes after loss of operculum movements. ○ Embryos up to 7 dpf: immersion in diluted sodium or calcium hypochlorite solution, or rapid chilling followed by immersion in diluted sodium or calcium hypochlorite solution. See conditions below
Acceptable Method(s) ***With Conditions****	○ Decapitation followed by pithing See conditions below
Method	**Conditions**
Rapid chilling	• Rapidly transition from housing tank to 2-4°C with minimal transfer of housing tank water volume, ideally using a net.

Zebrafish	
	• Fish should not be in direct contact with ice in the water. • Insulated containers help maintain water at 2-4°C. A probe thermometer can be used to confirm water temperature.
Buffered MS-222	• See the IACUC Guidlines For The Use Of Tricaine Methanesulfonate (MS-222) In Fish for detailed information.
Decapitation followed by pithing	• Perform with proper equipment. • Training by personnel who are regularly monitored for proficiency.

Rabbits	
Acceptable Method(s)	○ Intravenous or intraperitoneal barbiturates and barbiturate combinations ○ Adjunctive method under anesthesia (exsanguination, bilateral pneumothorax, perfusion with fixative)
Acceptable Method(s) ***With Conditions****	○ Barbiturates (alternate routes of administration): Intracardiac injections must only be used if the animal is anesthetized.

For all other species, contact the Attending Veterinarian.

***** The IACUC requires that if the use of a method of euthanasia that is *acceptable with conditions* is proposed in an IACUC protocol application the Principal Investigator must review the conditions that must be met and assure the IACUC that they will be followed.