AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

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LABORATORY ANIMALS

Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method can be met.

GENERAL CONSIDERATIONS

General comments about companion animals, farm animals, poikilotherms, and birds are provided elsewhere in the Guidelines, and usually apply to these species in the laboratory setting. Some other commonly used laboratory animal species are addressed later in the text. Most laboratory mammals currently used in biomedical research are small rodents that are maintained in large numbers. Venous access is typically difficult and injectable agents are usually delivered via the IP route.

In addition to humane outcomes, an important consideration in the choice of method for euthanasia of laboratory animals is the research objectives for the animals being euthanized. Euthanasia methods can lead to metabolic and histologic artifacts that may affect research outcomes. For example, isoflurane may artificially elevate blood glucose concentrations, while IP injection of barbiturates can create artifacts in intestinal tissues

and/or result in alterations in reproductive hormones.436–438 Euthanasia by inhalation of CO_2 elevates serum potassium concentrations.439 Time elapsed between euthanasia and tissue collection can also be a critical factor affecting choice of euthanasia method.440 Research needs may also require the use of an adjunctive method (eg, bilateral thoracotomy, exsanguination, perfusion with fixatives, injection of potassium chloride). The application of such adjunctive methods is acceptable when the animal is fully anesthetized. Animals used in infectious disease studies may require special handling for animal and human health and safety.

SMALL LABORATORY AND WILD-CAUGHT RODENTS (MICE, RATS, HAMSTERS, GUINEA PIGS, GERBILS, DEGUS, COTTON RATS)

All activities related to the euthanasia of rodents deserve consideration equivalent to the euthanasia method itself, and may factor into the choice of method. Laboratory rodents to be euthanized are often removed from the home room and/or home cage, placed in unfamiliar groups, and then held for a period of time before euthanasia. Activities that contribute to distress in rodents include transport, handling (in animals not accustomed to it), disruption of compatible groups, and elimination of established scent marks.441–451 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. Methods of euthanasia likely to elicit distress vocalizations or pheromones that other animals in the room could hear or smell may be best performed in another location, if transportation distress can be minimized. Similarly, wild-caught animals should be handled and euthanized in the manner least stressful to the animals.

Acceptable Methods

Noninhaled Agents

Barbiturates and barbituric acid derivatives—Injectable barbiturates act quickly and smoothly to render rodents unconscious. If there is vascular access, IV administration is preferred. The IP route is, however, most practical. Pain may be associated with injections given via the IP route,426,427 but the degree of pain and the methods for controlling pain have yet to be defined. The euthanasia dose is typically three times the anesthetic dose. Pentobarbital is the most commonly used barbiturate for laboratory rodents because of its long shelf life and rapidity of action.

Injectable barbiturate combinations—Injectable barbiturates are often used in combination with local anesthetics and anticonvulsants. An adequate dose of barbiturate is the most important component in these combinations.

Dissociative agent combinations—Lethal doses of dissociative agents such as ketamine are commonly used in laboratory settings. In some species, ketamine alone can result in stimulatory activity prior to sedation and loss of consciousness. In conscious rodents, ketamine and similar dissociative agents should be used in combination with an a2-adrenergic receptor agonist such as xylazine or benzodiazepines such as diazepam.452

Acceptable With Conditions Methods

Inhaled Agents

Inhaled anesthetics—Halothane, isoflurane, sevoflurane, or desflurane, with or without N2O, are acceptable with conditions for euthanasia of laboratory rodents. Nitrous oxide should not be used alone for euthanasia. These agents may be useful in cases where physical restraint is difficult or impractical. When used as a sole euthanasia agent delivered via vaporizer or anesthetic chamber (open-drop technique), animals may need to be exposed for prolonged time periods to ensure death.453 All other caveats as discussed in this and other sections should be followed, including recommended flow rates, maintaining compatible groups, and chamber maintenance. The use of inhaled anesthetics for preanesthesia removes the necessity for slow filling of the chamber with CO2; however, it is important to verify that an animal is dead when inhaled agents are used for euthanasia. Death may be confirmed by physical examination, ensured by adjunctive physical method, or obviated by validation of euthanasia chambers and process.147

Carbon dioxide—Carbon dioxide, with or without premedication with inhaled anesthetics, is accepted by AVMA Guidelines for the Euthanasia of Animals: 2013 Edition 49 able with conditions for euthanasia of small rodents. Compressed CO2 gas in cylinders is the recommended source of CO2 because gas inflow to the chamber can be precisely regulated. An optimal flow rate for CO2 euthanasia systems should displace 10% to 30% of the chamber or cage volume/min.152,238 Prefilled chambers are unacceptable. If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses. It is important to verify that an animal is dead after exposure to CO2.147 Death may be confirmed by physical examination, ensured by an adjunctive physical method, or obviated by calibration and validation of the euthanasia chamber and process. If an animal is not dead, CO2 narcosis must be followed with another method of euthanasia. Addition of O2 to CO2 will prolong the time to death and may complicate determination of consciousness. There appears to be no advantage to combining O2 with CO2 for euthanasia.238,427

Carbon monoxide—Although not commonly used in a laboratory animal setting, CO administration is acceptable with conditions as a method of rodent euthanasia when the conditions for effective and safe use can be met (see Inhaled Agents).

Non inhaled Agents

Tribromoethanol—Although unavailable as a commercial or pharmaceutical-grade (United States Pharmacopeia/National Formulary/British Pharmacopeia) product, tribromoethanol is a commonly used rodent anesthetic. Its use is controversial due to its reported adverse effects (peritonitis and death).324 However, many biomedical IACUC have approved its use in rodents. Tribromoethanol is acceptable with conditions as a method for euthanasia when prepared, stored, and administered at the appropriate dosage.

Ethanol—It has been suggested that IP injections of 70% ethanol might be an appropriate method of euthanasia for mice when physical methods are not desired or other euthanasia agents are unavailable.454 Mice injected with 0.5 mL of 70% ethanol demonstrated gradual loss of muscle control, coma, and death in 2 to 4 minutes.307 While ethanol is acceptable with conditions for certain applications (antibody production

in mice), other methods discussed as being acceptable and acceptable with conditions in the laboratory setting are much preferred. Its use in larger species is unacceptable.

Physical Methods

Cervical dislocation—Cervical dislocation is used in laboratory settings. Cervical dislocation requires neither special equipment nor transport of the animal and yields tissues uncontaminated by chemical agents. Loss of cortical function following cervical dislocation is rapid and occurs within 5 to 10 seconds as measured by a significant reduction in amplitude recordings of visual evoked responses and EEG.51,58 Cervical dislocation is acceptable with conditions for mice and rats < 200 g. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency.

Decapitation—Decapitation is used in laboratory settings because it yields tissues uncontaminated by chemical agents. Loss of cortical function following decapitation is rapid and occurs within 5 to 30 seconds as measured by a significant reduction in amplitude recordings of visual evoked responses and EEG changes.51,58,59 Specialized rodent guillotines are available and must be kept clean, in good condition with sharp blades. If handled correctly, rats do not show evidence of hypothalamic-pituitary-adrenal axis activation from decapitation, or from being present when other rats are decapitated.455 Decapitation is acceptable with conditions for mice and rats. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency.

Focused beam microwave irradiation—Focused beam microwave irradiation, using a machine professionally designed for animal euthanasia (see Physical Methods), is acceptable with conditions for euthanizing mice and rats. It is the preferred method when immediate fixation of brain metabolites is required for research purposes.

Unacceptable Methods

Inhaled Agents

Nitrogen and argon—Administration of N_2 or Ar is only acceptable in anesthetized mammals, as a coexisting O_2 concentration of < 2% is necessary to achieve unconsciousness and death. Achieving that condition is difficult. In addition, Ar has been shown to be highly aversive to rats.195 With heavy sedation or anesthesia, it should be recognized that death may be delayed. Although N_2 and Ar are effective, other methods of euthanasia are preferable.

Non inhaled Agents

Potassium chloride—Intravenous or intracardiac administration of potassium chloride is not acceptable as a sole approach to euthanasia.
Neuromuscular blocking agents—Paralytic agents are unacceptable for use as sole euthanasia agents.

Injectable barbiturates and neuromuscular blocking agents—Combining injectable barbiturates and neuromuscular blocking agents in the same syringe for administration

is unacceptable because the neuromuscular blocking agents may take effect before the animal is anesthetized.
Opioids—Opioids are unacceptable for euthanasia of laboratory animals as they are not rapidly acting, require high doses, and are not true anesthetic agents.

Urethane—Urethane is a human carcinogen and has a slow onset of action. It is unacceptable as a sole euthanasia agent.

a *Chloralose*—a Chloralose is unacceptable as a sole agent of euthanasia.50 AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

Fetuses and Neonates

Rodents with altricial young, such as mice and rats, must be differentiated from rodents with precocial young, such as guinea pigs. Precocial young should be treated as adults.

Acceptable Methods

Euthanasia of the dam and fetuses—Rodent fetuses along with other mammals are unconscious in utero and hypoxia does not evoke a response.456 Therefore, it is unnecessary to remove fetuses for euthanasia after the dam is euthanized.

No inhaled Agents

Injectable barbiturates alone and in combination with local anesthetics and anticonvulsants; dissociative agents combined with a*2-adrenergic receptor agonist or benzodiazepines*—These agents are acceptable for use in fetuses or neonates. See discussion on the use of these agents in adult rodents.

Acceptable With Conditions Methods

Inhaled agents

Inhaled anesthetics—Nonflammable volatile anesthetic agents are effective for both in utero fetuses and neonatal rodents. Neonatal mice may take up to 50 minutes to die from CO2 exposure.273 Adequate exposure time should be provided, or an adjunctive method (eg, cervical dislocation, or decapitation) should be performed after a neonate is nonresponsive to painful stimuli.

Physical Methods

Hypothermia—The gradual cooling of fetuses and altricial neonates is acceptable with conditions. As cold surfaces can cause tissue damage and presumably pain, the animals should not come in direct contact with ice or pre-cooled surfaces. Hypothermia for anesthesia is not recommended after approximately 7 days of age.457 Therefore, it is also an unacceptable euthanasia method in animals older than this age.458 Fetuses that are believed to be unconscious and altricial neonates < 5 days of age that do not have sufficient nervous system development to perceive pain may be quickly killed by rapidly freezing in liquid N2.432,459

Decapitation—Decapitation using scissors or sharp blades is acceptable with conditions for altricial neonates (< 7 days of age). Some rodent neonates, whether atricial or precocial, may have a tissue mass that is too large for some scissors. Consideration should be given to the potential of pain from tissue crushing as well as to personnel safety. When appropriate, another method should be selected or an adult decapitator used.

Cervical dislocation—Cervical dislocation by pinching and disrupting the spinal cord in the high cervical region is acceptable with conditions for fetal and neonatal mice and rats.

LABORATORY RABBITS
General Considerations

Rabbits will struggle and breath-hold when confronted with any unpleasant or unfamiliar odors. This makes most inhaled methods difficult to use in rabbits without premedication. Wild-caught animals should be handled and euthanized in the manner least stressful to the animals.

Acceptable Methods

No inhaled Agents

Barbiturates and barbituric acid derivatives—If rabbits are used to handling, venous access may be obtained via the ear. In the case of fractious rabbits, sedation may be necessary to gain venous access for administration of an injectable barbiturate or injectable barbiturate combination. Barbiturates may also be administered IP. As indicated previously, pain may be associated with injections given via the IP route426,427; however, the degree of pain and methods to control it have yet to be defined. These approaches are acceptable for companion rabbits as well.

Acceptable With Conditions Methods

Inhaled Agents

Inhaled anesthetics—Although rabbits breath-hold when confronted with unpleasant odors,156,298,460 AVMA Guidelines for the Euthanasia of Animals: 2013 Edition 51

Animals already under anesthesia may be euthanized by an overdose of anesthetic.

Carbon dioxide—While CO2 is an effective method of euthanasia, its use as the sole agent in rabbits results in apparent distress to the rabbit. Premedication with sedative agents will allow for the administration of CO2 for euthanasia.

Physical Methods

Cervical dislocation—Cervical dislocation is acceptable with conditions for rabbits when performed by individuals with a demonstrated high degree of technical proficiency. The

need for technical competency is great in heavy or mature rabbits in which the large muscle mass in the cervical region makes manual cervical dislocation more difficult. Commercial devices designed to aid in rabbit cervical dislocation are available and should be evaluated for their effectiveness.

Penetrating captive bolt—The use of rabbit-sized penetrating captive bolts to euthanize rabbits in laboratory or production facilities is acceptable with conditions. The captive bolt must be maintained in clean working order, positioned correctly, and operated safely by trained personnel.

Special Cases

When rabbits to be euthanized are in a surgical plane of anesthesia, adjunctive methods such as delivery of potassium chloride, exsanguination, or bilateral thoracotomy are acceptable.

LABORATORY FINFISH, AQUATIC INVERTEBRATES, AMPHIBIANS, AND REPTILES

Recommending euthanasia methods for finfish, aquatic invertebrates, amphibians, and reptiles used in biomedical research is challenging due to the enormous number of species and variations in biological and physiologic characteristics. Methods for euthanizing species commonly used in research are discussed in detail in the relevant sections of the Guidelines. See these sections for additional information.

As described in the aquatics section it is acceptable for zebrafish (*Danio rerio*) to be euthanized by rapid chilling (2° to 4°C) until loss of orientation and operculum movements and subsequent holding times in ice-chilled water, specific to finfish size and age.316,461,462 Adult zebrafish should be exposed for a minimum of 10 minutes and fry 4 to 7 days after fertilization (dpf) for at least 20 minutes following loss of operculum movement. Rapid chilling (as well as MS 222) has been shown to be an unreliable euthanasia method for embryos < 3 dpf. To ensure embryonic lethality these methods should be followed with another agent such as diluted sodium or calcium hypochlorite solution.462 If necessary to ensure death of other life stages, rapid chilling may be followed by either an approved adjunctive euthanasia method or a humane killing method. Until further research is conducted, rapid chilling is acceptable with conditions for other small-bodied tropical and subtropical stenothermic species.

Amphibian species commonly used in research include the African clawed frog (*X laevis*) and leopard and bull (*Rana* spp) frogs. These species are best euthanized via a physical method while fully anesthetized.

Agents and methods of euthanasia by species recap

Rabbits	Intravenous barbiturates	Inhaled anesthetic overdose, CO_2, cervical dislocation (as anatomically appropriate), penetrating captive bolt
Reptiles	As appropriate by species—Injected barbiturates, dissociative agents and anesthetics as specified	As appropriate by species—Inhaled anesthetics as specified, CO_2, penetrating captive bolt or firearm, manually applied blunt force trauma to the head, rapid freezing for animals < 4 g
Rodents	Injected barbiturates and barbiturate combinations, dissociative agent combinations	Inhaled anesthetics, CO_2, CO, tribromoethanol, ethanol, cervical dislocation, decapitation, focused beam microwave irradiation