

Approved euthanasia methods

IC = intracoelomic
IV = intravascular
IP = intraperitoneal

Important: Confirm death prior to carcass disposal by creating a pneumothorax, confirming rigor mortis, or confirming cardiac arrest

Important Safety Consideration: Because of associated human health risks, the use of inhalant anesthetics is not permitted on open bench tops. Waste gases from precision vaporizers must be properly scavenged. Procedures involving the use of inhalant anesthetics without precision vaporizers must be performed in a certified ducted hood, biosafety cabinet or portable ductless hood equipped with a charcoal filter. OEHS approval is required for all other hood options.

1-DEA licenses required.

Amphibian

Chemical	Notes
Tricaine methane sulfonate	Immersion in 1% solution. Must be buffered to pH 7.0-7.5
Pentobarbital sodium ¹	150 mg/kg IC.
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	2.2 ml/kg IC.
Physical	
Decapitation	Must be immediately followed by pithing the brain. Instruments must be sharp and well maintained.
Pithing	Both spinal cord and brain must be pithed

Bird

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	2.2 ml/kg IV.
Pentobarbital sodium ¹	150 mg/kg IV.
Carbon dioxide	Bottled gas only see http://iacuc.yale.edu/policies/CO2euthanasia.html

Cat

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	0.4 ml/kg IV.
Pentobarbital sodium ¹	150 mg/kg IV.

Dog

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	0.22 ml/kg IV.
Pentobarbital sodium ¹	150 mg/kg IV.

Ferret

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	0.4 ml/kg IV.
Pentobarbital sodium ¹	150 mg/kg IV.

Fish

Chemical	Notes
Tricaine methane sulfonate	Immersion in 1% solution. Must be buffered to pH 7.0-7.5
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	2.2 ml/kg IC.
Pentobarbital sodium ¹	150 mg/kg IC.
Physical	
Decapitation	Only appropriate in species large enough to pith Must be immediately followed by pithing the brain. Instruments must be sharp and well maintained.
Cold Shock: Only appropriate for tropical species using the following or similar method	Place a small volume of water in a container big enough to allow the fish to freely swim about. Add ice at four times the volume of water. Immerse the fish for a minimum of 20 minutes then remove and freeze or keep in ice water in a cold room or refrigerator overnight.

Gerbil

Chemical	Notes
Carbon dioxide	Bottled gas only see http://iacuc.yale.edu/policies/CO2euthanasia.html
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	5.0 ml/kg IV or IP.
Pentobarbital sodium ¹	150 mg/kg IV or IP.

Goat

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	0.22 ml/kg IV.
Pentobarbital sodium ¹	150 mg/kg IV.

Guinea pig

Please refer to **Guidelines (p.8)** for euthanasia of feti and neonates.

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	2.2 ml/kg IV or IP.
Pentobarbital sodium ¹	150 mg/kg IV or IP.
Inhalant anesthetic overdose (halothane, isoflurane, metofane)	Inhalant anesthetic use within an animal facility is restricted to biosafety cabinets.
Physical	
Exsanguination	Must be done under general anesthesia

Hamster

Please refer to **Guidelines (p.8)** for euthanasia of feti and neonates.

Chemical	Notes
Carbon dioxide	Bottled gas only, see http://iacuc.yale.edu/policies/CO2euthanasia.html
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	2.2 ml/kg IV or IP.
Pentobarbital sodium ¹	150 mg/kg IV or IP.
Inhalant anesthetic overdose (halothane, isoflurane, metofane)	Inhalant anesthetic use within an animal facility is restricted to biosafety cabinets.

Physical	
Decapitation	Requires training and general anesthesia. With scientific justification may be done without general anesthesia. Instruments must be sharp and well maintained.
Exsanguination	Must be done under general anesthesia

Mastomys

Chemical	Notes
Carbon dioxide	Bottled gas only, see http://iacuc.yale.edu/policies/CO2euthanasia.html
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	2.2 ml/kg IV or IP.
Pentobarbital sodium ¹	150 mg/kg IV or IP.
Inhalant anesthetic overdose (halothane, isoflurane, metofane)	Inhalant anesthetic use within an animal facility is restricted to biosafety cabinets.
Physical	
Decapitation	Requires training and will likely require general anesthesia due to animals temperament. Instruments must be sharp and well maintained.
Exsanguination	Must be done under general anesthesia

Mouse

Please refer to Guidelines (p.8) for euthanasia of feti and neonates.

Chemical	Notes
Carbon dioxide	Bottled gas only, see http://iacuc.yale.edu/policies/CO2euthanasia.html
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	22 ml/kg IV or IP.
Pentobarbital sodium ¹	150 mg/kg IV or IP.
Inhalant anesthetic overdose (halothane, isoflurane, metofane)	Inhalant anesthetic use within an animal facility is restricted to biosafety cabinets.
Physical	
Cervical dislocation	Requires training and general anesthesia. With scientific justification and certification by Regulatory Services may be done without general anesthesia.
Decapitation	Requires training and general anesthesia. With scientific justification may be done without general anesthesia. Instruments must be sharp and well maintained.
Exsanguination	Requires general anesthesia

Microwave irradiation	Microwave ovens are not acceptable , requires euthanasia specific irradiator.
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Nonhuman Primate

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	0.22 ml/kg IV.
Pentobarbital sodium ¹	150 mg/kg IV.
Physical	
Exsanguination	Must be done under general anesthesia.

Peromyscus

Please refer to **Guidelines (p.8)** for euthanasia of feti/neonates.

Chemical	Notes
Carbon dioxide	Bottled gas only, see http://iacuc.yale.edu/policies/CO2euthanasia.html
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	22 ml/kg IV or IP.
Pentobarbital sodium ¹	150 mg/kg IV or IP.
Inhalant anesthetic overdose (halothane, isoflurane, metofane)	Inhalant anesthetic use within an animal facility is restricted to biosafety cabinets.
Physical	
Cervical dislocation	Requires training and general anesthesia. With scientific justification and certification by Regulatory Services may be done without general anesthesia.
Decapitation	Requires training and general anesthesia. With scientific justification may be done without general anesthesia. Instruments must be sharp and well maintained.
Exsanguination	Requires general anesthesia

Pig

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	0.22 ml/kg IV.
Pentobarbital sodium ¹	150 mg/kg IV.

Rabbit

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	0.4 ml/kg IV.
Pentobarbital sodium ¹	150 mg/kg IV.
Inhalant anesthetic overdose (halothane, isoflurane)	Inhalant anesthetic use within an animal facility is restricted to biosafety cabinets.
Physical	
Cervical dislocation	Rabbit must weigh <1kg , requires training, and general anesthesia. With scientific justification and certification by Regulatory Services may be done without general anesthesia.
Decapitation	Rabbit must weigh <1kg , requires training and general anesthesia. With scientific justification may be done without general anesthesia. Instruments must be sharp and well maintained.
Exsanguination	Requires general anesthesia

Rat

Please refer to **Guidelines (p.8)** for euthanasia of feti and neonates.

Chemical	Notes
Carbon dioxide	Bottled gas only see http://iacuc.yale.edu/policies/CO2euthanasia.html
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	5 ml/kg IV or IP.
Pentobarbital sodium ¹	150 mg/kg IV or IP.
Inhalant anesthetic overdose (halothane, isoflurane, metofane)	Inhalant anesthetic use within an animal facility is restricted to biosafety cabinets.
Physical	
Cervical dislocation	Rat must weigh <200g , requires training, and general anesthesia. With scientific justification and certification by Regulatory Services may be done without general anesthesia.
Decapitation	Requires training and general anesthesia. With scientific justification may be done without general anesthesia. Instruments must be sharp and well maintained.
Exsanguination	Requires general anesthesia
Microwave irradiation	Microwave ovens are not acceptable , requires euthanasia specific irradiator.

Reptile

Chemical	Notes
Carbon dioxide	Appropriate for lizards and snakes but will require 30-minute exposure time. Bottled gas only, see http://iacuc.yale.edu/policies/CO2euthanasia.html
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	5.0 ml/kg IV or IC.
Pentobarbital sodium ¹	150 mg/kg IV or IC.
Physical	
Decapitation	Requires training, general anesthesia and must be immediately followed by pithing the brain.

Sheep

Chemical	Notes
Euthanasia solutions ¹ (eg: Euthasol®, Beuthanasia®-D)	0.22 ml/kg IV.
Pentobarbital sodium ¹	150 mg/kg IV.

Field Methods of Euthanasia

In addition to the previously listed methods, the following may be considered for field euthanasia

Chemical Methods	Species	Comments
Benzocaine 20% gel	Amphibian	Apply to ventral abdomen. Available OTC worldwide
Chlorobutanol	Amphibian, fish	Administer by immersion: 300 mg/l
Quinaldine	Amphibian, fish	Administer by immersion: 100 mg/l
Ethanol 10%	Amphibian, fish	Administer by immersion. Only use ethyl alcohol
Physical Methods	Species	Comments
Penetrating captive bolt	Acceptable for most species	Requires specialized gun, skill, proper placement, and restraint. Animal must be large enough for the device.
Concussion (Consult with VCS for methods)	Amphibian, bird, fish, rodents, reptile	Death must be ensured by subsequent destruction of the brain or injection of euthanasia solution
Cervical dislocation	Birds	Appropriate for small to poultry sized (3kg) birds.
Gun shot (shotgun, handgun, rifle)	Birds, mammals, large reptiles	Firearm and ammunition must be appropriate for the species. Investigator must be trained in safe use and strictly adhere to applicable laws.
Thoracic compression	Birds	Only acceptable for field euthanasia of small (<50g) birds.

Guidelines for the Euthanasia of Rodent Feti and Neonates

When pre-term feti are removed euthanasia should be accomplished as soon as possible after removal from the dam. Neonatal animals are relatively resistant to hypoxia thus require additional steps to ensure euthanasia when inhalent agents are employed.

1. **Mouse, Rat and Hamster feti up to 15 days and Guinea Pig feti up to 34 days' gestation:** euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus at this stage of development
2. **Mouse, Rat and Hamster Feti 15 days gestation to birth and Guinea Pig Feti 35 days gestation to birth:**
 - a. At this stage of gestation euthanasia of individual feti is indicated. Euthanasia may be by injection of chemical euthanasia agents (e.g., pentobarbital), decapitation, cervical dislocation or specialized microwave.
 - b. Anesthesia is required prior to rapid freezing, or chemical fixation of the whole fetus. When adequately justified, hypothermia may be used to induce anesthesia in pups \leq six day sold.
 - c. Feti at this age are resistant to hypoxia and require extended exposure to inhalant anesthetics or CO₂. 60 minutes of exposure to lethal concentrations of the gas are required.
3. **Atricial mouse, rat and hamster neonates:**
 - a. Acceptable methods for euthanasia include: injection of chemical euthanasia agents (e.g., pentobarbital), decapitation, cervical dislocation or specialized microwave.
 - b. For inhalants see 2c
4. **Guinea Pig Neonates:** Follow guidelines for adults.
5. **Haired Mouse, Rat and Hamster Neonates:** Follow guidelines for adults

ENDORSED BY THE IACUC: 10/17/07

References

2007 AVMA Guidelines on Euthanasia
[Guidelines for the use of Wild Mammals in Research Mammal PDF](#)
[Guidelines to the Use of Wild Birds in Research Bird PDF](#)
[Guidelines for use of Live Amphibians and Reptiles in Field and Laboratory Research Amphibian, Reptile PDF](#)
[Guidelines for the Use of Fishes in Research Fish guidelines PDF](#)
[Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small Laboratory Animals. \[http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html\]](http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html)