

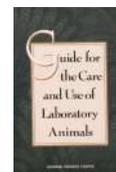
Euthanasia Review: Regulations and Methods



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Primary References

- AVMA Guidelines for the Euthanasia of Animals, 2013
- ACLAM Public Statement, 2005
- Animal Welfare Act, Regs, Policies
- Guide



Definitions: AVMA

The term euthanasia is derived from the Greek terms *eu* (good) and *thanatos* (death).

“Ending the life of an individual animal in a way that minimizes or eliminates pain and distress. A good death is tantamount to the humane termination of an animal’s life.”



Definitions: ACLAM Public Statement

“should be humane, minimizing pain and fear, delivered IAW current regulations, ensure rapid onset of unconsciousness followed by death, and avoid risk and aversion for animals and personnel.”



Definitions: AWA

- “...the humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death w/o evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death.”
- Methods of euth **must** be IAW this definition unless a deviation is justified, in writing, for scientific reasons



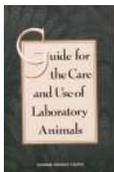
AWA, USDA Policy #3

- euthanasia **should** be consistent with the current AVMA Guidelines on Euthanasia
- the use of expired euthanasia drugs is considered inadequate veterinary care



Definition: Guide

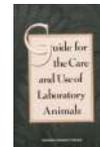
- the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods **should** be consistent with the *AVMA Guidelines on Euthanasia*



Guide

Considerations in evaluating appropriateness of method:

- ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety;
- reliability;
- irreversibility;
- time required to induce unconsciousness;
- appropriateness for the species and age of the animal;
- compatibility with research objectives;
- safety of and emotional effect on personnel.



Guide

- Training on euthanasia should be provided to personnel
- AV should provide guidance to all personnel on euthanasia
- Adequate veterinary care consists of effective management of euthanasia
- Emergency care: A veterinarian or the veterinarian's designee must be available to expeditiously assess the animal's condition, treat the animal, investigate an unexpected death, or advise on euthanasia.



Guide

- Generally, chemical agents (e.g., barbiturates, nonexplosive inhalant anesthetics) are preferable to physical methods (e.g., cervical dislocation, decapitation, use of a penetrating captive bolt); scientific considerations may preclude the use of chemical agents for some protocols
- The acceptability of CO₂ as a euthanasia agent for small rodents should be evaluated as new data become available.
- Neonates require longer exposure times—consider alternative methods
- Death **must be confirmed by personnel trained** to recognize cessation of vital signs in the species being euthanized. A secondary method of euthanasia (e.g., thoracotomy or exsanguination) can be also used to ensure death.

AVMA Guidelines for the Euthanasia of Animals 2013



- Designed for use by members of the veterinary profession who carry out or oversee the euthanasia of animals
- Panel on Euthanasia's objective is to provide guidance for veterinarians about how to prevent and/or relieve the pain and suffering of animals that are to be euthanized

AVMA Guidelines for the Euthanasia of Animals 2013

- "Animal issues are no longer socially invisible"
- Emphasis on Professional judgment
- "[Animal care and use] stakeholders look to veterinarians to provide leadership on how to care well for animals, including how to relieve unnecessary pain and suffering."



AVMA 2013 Guidelines

- Appendix 1, page 99. [Agents and methods of euthanasia by species](#). Cites section where information is presented: e.g., S3.2
- Appendix 2, page 100. [Some acceptable agents and methods of euthanasia](#) (including acceptable with conditions, and adjunctive methods).
- Appendix 3, page 102. [Some agents and methods that are unacceptable as primary methods of euthanasia](#).

AVMA 2013 Guideline Categories

Acceptable Methods (Appendix 1,2)

- Consistently produce a humane death when used as sole means

Acceptable with Conditions (Appendix 1,2)

- Greater potential for operator error or safety hazard
- May not consistently produce humane death
- Methods not well documented in literature, or
- May require secondary method to ensure death
- Equivalent to acceptable methods when all criteria for application of a method can be met.

Unacceptable Methods (Appendix 3)

- Methods deemed inhumane under any conditions or pose a substantial risk to the person applying

AVMA 2013 Guidelines



Euthanasia Criteria

- Ability to induce loss of consciousness and death with a minimum of pain and distress
- Time required to induce loss of consciousness
- Reliability
- Safety of personnel
- Irreversibility
- Compatibility with intended animal use and purpose

AVMA 2013 Guidelines



Euthanasia Criteria

- Documented emotional effect on observers or operators
- Compatibility with subsequent evaluation, examination, or use of tissue
- Drug availability of drug and human abuse potential
- Compatibility with species, age, and health status

AVMA 2013 Guidelines



Euthanasia Criteria

- Ability to maintain equipment in proper working order
- Safety for predators or scavengers should the animal's remains be consumed
- Legal requirements
- Environmental impacts of the method or disposition of the animal's remains

AVMA 2013 Guidelines

Human Behavior

- The human-animal bond positively impacts the quality of life of laboratory animals
- Euthanasia-related stress symptoms may manifest in personnel caring for research animals.
- Constant exposure may result in work dissatisfaction or alienation expressed as absenteeism, belligerence, or careless/callous animal handling
- Staff training to promote grief coping skills may counteract

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Mechanisms of Euthanasia Agents

- Hypoxia
- Direct depression of neurons needed for life function
- Physical disruption of brain activity & destruction of neurons needed for life

AVMA 2013 Guidelines

Use of Paralytic Agents

- e.g., succinylcholine, strychnine, curare, nicotine, potassium, or magnesium salts
- In vertebrates, these do not satisfy requirement that loss of consciousness precedes loss of voluntary muscle movement when used as sole agent
- Possible distress and conscious perception of pain prior to unconsciousness/death

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Confirmation of Death

- Death must be confirmed before disposal
- Criteria: Lack of pulse, breathing, corneal reflex and response to firm toe pinch, inability to hear respiratory sounds and heartbeat by use of a stethoscope, graying of the mucous membranes, and rigor mortis
- Percutaneous cardiac puncture with needle attached to syringe to check for cardiac muscle movement

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Disposal of Animal Remains

- Handle appropriately and IAW state and local laws
- Regulations apply to disposition of the remains (e.g., burial, incineration, rendering)
- Regulations apply to management of chemical residues (e.g., pharmaceuticals such as barbiturates, xylazine, antimicrobials, and other residues such as lead) that may adversely affect scavengers or result in the adulteration of rendered products used for animal feed
- Pentobarbital: legal responsibilities
- Zoonotic, FAD diseases: Safe handling, samples for testing, PPE, and biohazmat handling precautions

AVMA 2013 Guidelines

Types of Euthanasia Methods

- Inhalants
- Non-inhalant pharmaceuticals
- Physical methods
 - Adjunctive methods

AVMA 2013 Guidelines: Inhalant Agents



AVMA 2013 Guidelines: [Inhalant Agents](#)

General Considerations

- Inhaled agents can produce distress and aversion in people, therefore a concern for their use in animals
- Conditions (e.g., isolation or unfamiliar exposure chamber, handling stress) may have profound effects on animal response
- Time to unconsciousness is dependent on the displacement rate, container volume, and agent conc.
- Loss of consciousness is more rapid with initial exposure to a high concentration of the agent, but this can be aversive and distressing. Therefore, gradual exposure may be the most humane option.
- Inhaled agents must be supplied in purified form w/o contaminants or adulterants

AVMA 2013 Guidelines: [Inhalant Agents](#)

General Considerations

- A gentle death that takes longer is preferable to a rapid, but more distressing death
- Commercially supplied source, cylinder or tank allows an effective displacement rate and/or concentration to be readily quantified
- Equipment in good working order; compliant w/ regs
- Most inhaled agents are hazardous to animal workers
- Sick or depressed animals with decreased ventilation may become agitated. Excited animals may have increased cardiac output. Consider premedication or non-inhaled euthanasia method to offset the delay in alveolar gas concentration
- Hypoxia resistance in neonates = increased time to death; use with other adjunct methods or anticipate prolonged exposure

AVMA 2013 Guidelines: [Inhalant Agents](#)

General Considerations

- Reptiles, amphibians, and diving birds and mammals have great breath-holding capacity or anaerobic metabolism. Consider non-inhalant method; a secondary method is required to kill
- Rapid flow = noise and cold draft = fear; minimize these
- Clean, uncrowded chamber; only co-mingle same species
- Consider animal comfort.
- Ensure adequate gas mixing and that chambers/containers are leak free
- Verify death via individual exam or adherence to validated exposure process

AVMA 2013 Guidelines: [Inhalant Agents](#)

General Considerations

- gas displacement rate is critical to the humane application of inhaled methods
- an appropriate pressure-reducing regulator and flow meter combination or equivalent equipment with demonstrated capability for generating the recommended displacement rate for the size container being utilized is **absolutely necessary** when compressed gases are used for euthanasia.

AVMA 2013 Guidelines: [Inhalant Agents](#)

General Use

- Precision vaporizer
- Pressure-reducing regulator and flow meter combo
- Soaked gauze or cotton in closed receptacle
- Liquid state of most inhaled anesthetics is irritating - avoid direct contact of animal with liquid
- Some may be aversive and distressful. I, H, S in Rabbits: violent struggling, apnea, bradycardia*
- Sufficient air/O₂ during induction to avoid hypoxia



AVMA 2013 Guidelines: [Inhalant Agents](#)

Acceptable with conditions, for euthanasia of rodents/small mammals < 7kg, providing:

- Use in high [] to effect rapid loss of consciousness in non-averse species; gradual fill averse species
- Know the order of preference (I, H, S, E, M*, D). Do not use N₂O alone. Ether not acceptable. Methoxyflurane only if other agents/methods not available
- Acceptable but not generally used in larger animals due to cost and difficulty in administration
- Exposure of workers must comply with state/fed OHS regs

AVMA 2013 Guidelines: Inhalant Agents

Order of preference*:

- Isoflurane
- Halothane
- Sevoflurane
- Enflurane
- Methoxyflurane
- Desflurane
- Used with or without nitrous oxide



AVMA 2013 Guidelines: Inhalant Agents

Isoflurane

- Rapid induction
- Pungent odor = breath holding
- Nonflammable
- Occupational Health – properly scavenge; No exposure limit established by NIOSH for I, S, D



AVMA 2013 Guidelines: Inhalant Agents

Halothane

- Rapid induction
- Nonflammable
- Occupational Health – properly scavenge; keep levels <2ppm
- Not available in US



AVMA 2013 Guidelines: Inhalant Agents

Sevoflurane

- Less potent than I or H and has a lower vapor pressure
- Anesthetic [] can be achieved rapidly, but more is needed to kill animal
- Although reported to have a less objectionable odor than I, some species may struggle violently/apnea when administered by facemask or in chamber
- Like Enflurane, Sevoflurane induces epileptiform electrocortical activity

AVMA 2013 Guidelines: Inhalant Agents

Enflurane

- Rapid induction
- Nonflammable
- Seizure/convulsions in deeply anesthetized animals



AVMA 2013 Guidelines: Inhalant Agents

Methoxyflurane

- Unacceptably slow induction in some species
- Slow induction = agitation?
- Not available in US market, but can purchase internationally
- Acceptable with conditions in rodents and small mammals



AVMA 2013 Guidelines: Inhalant Agents

Desflurane

- Pungent/slow induction
- Volatile
- Special equipment
- Acceptable with conditions in rodents and small mammals



AVMA 2013 Guidelines: Inhalant Agents

Carbon Monoxide

- Forms carboxyhemoglobin
- Advantage
 - No pain, discomfort, insidious and rapid
- Disadvantages
 - Aversive in lab rodents
 - Exposure of personnel
 - Electrical equipment must be explosion-proof



AVMA 2013 Guidelines: Inhalant Agents

Carbon Monoxide

- Acceptable for dogs, cats, small mammals, etc...
- Use compressed CO
- Hazardous
- Toxic
- Difficult to detect—if used inside have CO monitors



AVMA 2013 Guidelines: Inhalant Agents

Nitrogen, Argon

- Colorless, odorless, non-flammable
- Readily available, highly effective
- They kill by displacing air, causing anoxia
- N₂ Aversive to rats, mice, mink
- N₂ and Ar are not aversive to chickens, turkeys, but hypoxia is
- Not directly aversive to pigs



AVMA 2013 Guidelines: Inhalant Agents

Nitrogen, Argon

- Hypoxemia from exposure to N₂ or Ar gas mixtures is acceptable with conditions for euthanasia of **chickens** and **turkeys**
- Use purified, regulated gases in prefilled chamber or rapid introduction
- Hypoxemia from exposure to Ar or N₂-CO₂ gas mixtures is acceptable with conditions for euthanasia of **pigs**, provided animal can be directly place into a <2% O₂ atmosphere and exposure times > 7 minutes
- N₂ and Ar are **unacceptable for other mammals**



AVMA 2013 Guidelines: Inhalant Agents

Carbon Dioxide – General info

- Odorless, compressed gas (only acceptable means of exposure)
- Higher concentrations = rapid anesthetic effect but may be distressful or aversive
- Heavier than air
- Acceptable in most species
- Supplemental O₂ prolongs time to death and delays unconsciousness
- Immature, reptiles, amphibians, burrowing, and diving animals may need high [] and prolonged exposure



AVMA 2013 Guidelines: [Inhalant Agents](#)

Carbon Dioxide – Advantages

- Rapid induction, analgesic & anesthetic effects
- Minimal occupational health hazards
- Minimal biologic and physiologic effects

AVMA 2013 Guidelines: [Inhalant Agents](#)
Carbon Dioxide – Disadvantages

- Higher concentrations = rapid, deep anesthetic effect but may be distressful or aversive
- Neonatal, diving/burrowing species tolerance
- Reptiles and amphibians may breathe too slowly for the use of CO₂
- Incomplete chamber filling may allow animals to escape effects
- Induction at <[80]% may -> pulmonary and upper respiratory tract lesions



AVMA 2013 Guidelines: [Inhalant Agents](#)

Carbon Dioxide - Recommendations

- CO₂ is acceptable with conditions in species where aversion or distress can be minimized
- CO₂ **must be precisely regulated** and in purified form
- **Displace chamber at 10-30% volume/min**
- **Immersion in 100% CO₂ is unacceptable unless uncons.**
- Maintain flow for at least one minute past apparent death
- No benefits of adding O₂; may prolong time to death
- Longer exposure and higher [] needed in some species
- Requires regulator and flow meter

AVMA 2013 Guidelines: [Inhalant Agents](#)

Nitrous Oxide

- Least potent. Not acceptable as sole agent: used with other inhalants to speed onset of anesthesia
- Human abuse potential
- Occupational health risks – keep levels <25ppm
- Creates hypoxic environment prior to loss of consciousness and supports combustion



AVMA 2013 Guidelines: [Inhalant Agents](#)

Diethyl Ether

- Highly soluble w/ slow induction
- Irritating to mucous membranes
- Highly flammable and **explosive**
- Used to create models of stress
- **Not acceptable**



AVMA 2013 Guidelines:
Noninhaled Agents



AVMA 2013 Guidelines: [Noninhaled Agents](#)

General Characteristics

- Primary routes are parenteral, topical application, immersion
- Use of injectable euthanasia agents is rapid, reliable
- Usually the most desirable method when performed w/o causing fear/distress in the animal
- Sedation may be necessary if aggressive/fearful
- Use of paralytic agents is unacceptable as sole means
- IP, intracoelomic admin of non-irritating barbiturate is acceptable
- IM*, SC, intracardiac, intrathoracic, intrapulmonary, intrathecal, intra-hepatic, etc., not acceptable routes of administration for injectable euth agents in awake animals
- *OK to do IM ultrapotent opioids and some anesthetics



AVMA 2013 Guidelines: [Noninhaled Agents](#)

Immersion

- Immersion of aquatic animals in water containing euthanasia agents may be the best way to minimize P/D
- Agent absorbed via gills, skin, ingestion
- Ideally:
 - non-irritating to skin, eyes, and oral/respiratory tissues
 - rapid loss of consciousness
 - minimal signs of distress or avoidance behavior
- Currently, no FDA-approved drugs for the euthanasia of aquatic animals

AVMA 2013 Guidelines: [Noninhaled Agents](#)

Topical

- Slow and variable absorption of topically applied agents make them unacceptable as EAs in most animals.
- Exceptions include animals with highly permeable skin to which a nonirritating, rapidly absorbed agent is applied (e.g., amphibians euthanized with benzocaine gel).
- There are currently no topical EAs approved by the FDA for any species

AVMA 2013 Guidelines: [Noninhaled Agents](#)

Barbituric Acid Derivatives

- Depress CNS > Respiratory > Cardiac
- All that are acceptable for anesthesia are acceptable for euth when given IV
- IP or intracoelomic can be used if IV would be distressful; other routes acceptable only if animal is unconscious or anesthetized
 - The section on lab animals recognizes that IP may be performed in rodents, rabbits, and that degree of pain is not yet defined
- Controlled substances
- May lead to tissue artifacts (e.g., splenomegaly)
- Preferred for dogs, cats, small animals, horses and acceptable for all other species

AVMA 2013 Guidelines: [Noninhaled Agents](#)

Pentobarbital Combos

- Usually sodium pentobarbital combined with local anesthetics, other CNS depressants (eg, phenytoin, ethanol), or agents that metabolize to pentobarbital
- Not acceptable to add NMBAs
- Controlled substance but lower schedule (III)
- Combo agent recommendations are the same as for pure barbituric acid derivatives



AVMA 2013 Guidelines: [Noninhaled Agents](#)

T-61

- Injectable nonbarbiturate, nonnarcotic combo
- Schedule III
- Acceptable given by slow IV by trained personnel
- May cause dysphoria prior to loss of consciousness
- Not available in the US

AVMA 2013 Guidelines: [Noninhaled Agents](#)

Potassium Chloride

- Acceptable only in anesthetized or unconscious animals
 - Loss of reflex muscle response, loss of response to noxious stimuli
 - In unconscious animals, other methods must not be available or feasible
- Rapid onset of cardiac arrest
- Not a controlled substance

AVMA 2013 Guidelines: [Noninhaled Agents](#)

Tricaine Methane Sulfonate (MS 222, TMS)



- FDA approved for temp. immobility (not E) of finfish, amphibians and other cold-blooded, aquatic animals
- Benzoic acid derivative; not controlled
- Formulation
 - Buffer to pH 7.0-7.5 w/ Sodium Bicarb
 - Store in dark bottle and chill
 - Works in salt or fresh water
- Acceptable for finfish, some amphibians, and reptiles
- When used for large finfish and some amphibians, such as *Xenopus*, should use a secondary method to ensure death
- Intracoelomic injxn of MS222 alone is not acceptable for *Xenopus* and other amphibs
- Retinal toxicity in humans

AVMA 2013 Guidelines: [Noninhaled Agents](#)

Benzocaine HCl

- Benzocaine base is not water soluble, prepare in acetone or ethanol
- Benzocaine HCl is water soluble, must buffer
- Not controlled
- Acts by depression of the nervous and CV systems
- Applicable to the ventral abdomen of amphibians is effective means of anesthesia and euthanasia for some species
- Breaks down in water w/in 4 hours
- Fast and effective topical agent for anesthesia and euthanasia in finfish and amphibians

AVMA 2013 Guidelines: [Noninhaled Agents](#)

Other agents in Guidelines:

- Ultrapotent Opioids: Etorphine, carfentanil
- Dissociative agents and α_2 -adrenergic receptor agonists (e.g., K/X) acceptable when doses and routes have been established
- Alcohols – EtOH in specific situations for mice; triobromoethanol accept. for rodents when IACUC-approved
- Clove oil, isoeugenol, and eugenol acceptable for E in finfish
- Phenoxyethanol & Quinaldine: Not controlled; acceptable for finfish under certain circumstances, not accept. when fish will be food
- Metomidate – Very effective in some species, but illegal to use as EA
- Bleach (1-10%) in unhatched and hatched zebrafish up to 7 days after fertilization

AVMA 2013 Guidelines: [Appendix 3](#)

Unacceptable Agents

- Strychnine
- Nicotine
- Caffeine
- Cleaning agents, solvents
- Disinfectants
- Chloral hydrate, a-chloralose
- Formaldehyde (has exceptions-sponges)
- NM Blocking Agents, KCl, $MgSO_4$ may be used for euthanasia of anesthetized or unconscious animals, but not as a sole agent



AVMA 2013 Guidelines: [Physical Methods](#)

AVMA 2013 Guidelines: [Physical Methods](#)

- Captive bolt
 - Gunshot
 - Cervical dislocation
 - Decapitation
 - Electrocutation
 - Microwave irradiation
 - Freezing
- 
- Thoracic compression
 - Kill traps
 - Exsanguination*
 - Maceration
 - Stunning*
 - Pithing*

* Exsanguination, stunning and pithing are not recommended as a sole means of euthanasia, but may be considered adjuncts to other agents/methods.

AVMA 2013 Guidelines: [Physical Methods](#)

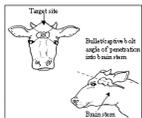
General Characteristics

- Aesthetically objectionable
- Can be more rapid, painless, humane
- Requires highly trained/experienced personnel w/ serviced equipment
- Most considered conditionally acceptable

AVMA 2013 Guidelines: [Physical Methods](#)

Penetrating Captive Bolt

- 2 types: regular and air injection
- Lack of maintenance is a major cause of captive bolt gun failure
- Acceptable with conditions in ruminants, horses, swine
 - Use adjunct method (exsang, pithing) unless PCB is built for euthanasia. Don't use pithing for food animals
 - Must have adequate restraint
- Destructive to brain so consider impact on postmortem exams



AVMA 2013 Guidelines: [Physical Methods](#)

Nonpenetrating Captive Bolt

- Stun only and should not be used as a sole method of euthanasia (exception below)
 - Correct positioning important; not effective in bulls, large swine, or cattle with long hair
 - Exception: Purpose-built pneumatic NCB guns for suckling pigs, neonatal ruminants, and turkeys.
- May make animal unsuitable for postmortem of brain

AVMA 2013 Guidelines: [Physical Methods](#)

Manually Applied Blunt Force Trauma to the Head

- Can be a humane method of euthanasia for neonatal animals with thin craniums via single sharp blow to central skull bones. Rapid loss of consciousness when properly performed.
 - Small laboratory animals with thin craniums; young piglets
 - Repeated performance can lead to user fatigue, loss of efficacy and humane concerns
 - AVMA encourages an active search for alternate approaches
- Unacceptable in neonatal calves

AVMA 2013 Guidelines: [Physical Methods](#)

Gunshot

- Preferred target should be the brain without the bullet exiting contralateral side of head
 - Highly skilled personnel, legal and safe use
 - Muzzle energy: animals <400 lb is >300 ft-lb; > 400 lb is 1000 ft-lb
 - Scientific info on bullet and firearm selection are lacking
 - .22 should never be used on aged bulls, boars, or rams
 - Acceptable with conditions when other methods can't be used
 - Ideally: muzzle 1-2' from forehead, perpendicular to skull, aim for foramen magnum
- 

AVMA 2013 Guidelines: [Physical Methods](#)

Cervical Dislocation

- Acceptable with conditions
 - Poultry & other small birds (<200g), mice, immature rats (<200g) and rabbits
 - Individuals with high degree of technical proficiency
- Rapid & no tissue residues
- If not technically competent: train on anesthetized or dead animals



AVMA 2013 Guidelines: [Physical Methods](#)

Decapitation

- Acceptable with conditions
 - When required by the experimental design and approved by the IACUC
 - Lab rodents; small rabbits; poultry; birds (<200g); some finfish, reptiles, and amphibians
- Exsanguination assists rapid loss of consciousness
- No tissue contaminates, brain intact
- Commercial guillotines; *sharp blades for neonates*
- Proper personnel training; monitoring
- Maintain equipment
- Plastic cones may reduce distress, improve positioning of animal, and minimize injury risk



AVMA 2013 Guidelines: [Physical Methods](#)

Focused Beam Microwave Irradiation

- Acceptable in mice & rats
- Most effective method for brain tissue fixation *in vivo* for assay of enzymatically labile chemicals
- Microwaves directed toward head; heat brain
- Unconsciousness <100 msec; death <1 sec
- Only equipment designed for this use



AVMA 2013 Guidelines: [Physical Methods](#)

Maceration

- Poultry up to 72 hours old, embryonated eggs
- Acceptable
 - FASS, Ag Canada, EU, OIE
- Instant death/minimal pain & distress
- Need special equipment
- Chicks delivered carefully to avoid injury or distress before maceration



AVMA 2013 Guidelines: [Physical Methods](#)

Thoracic Compression

Potential for substantial pain and distress – Not acceptable unless deeply anesthetized or insentient

Kill Traps

Controversial; do not always render a rapid or stress-free death consistent with the POE criteria; humane killing

Live traps followed by another method of euthanasia is preferred

Electrocution - acceptable with conditions

It is imperative that animals be unconscious and insensible to pain before being electrocuted

Three approaches – all must induce a grand mal epileptic seizure. Head-only approach does not cause cardiac fibrillation and must be followed by a secondary method

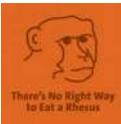
AVMA 2013 Guidelines: [Adjunctive Methods](#)

Those methods that should not be used as a sole method of euthanasia, but that can be used in conjunction with other methods to euthanize

AVMA 2013 Guidelines: [Adjunctive Methods](#)

Exsanguination

- **NOT a sole means**
 - Anxiety w/hypovolemia
- **Obtain large terminal blood sample or blood products**
- **Animals should first be sedated, stunned, or anesthetized**



AVMA 2013 Guidelines: [Adjunctive Methods](#)

• Pithing

- Adjunctive procedure used on an unconscious animal to ensure death
- Pithing tools destroy brainstem and spinal cord tissue
- Pithing of frogs and other amphibians is strongly discouraged, unless the patient is anesthetized first



AVMA 2013 Guidelines: [By Species and Environment](#)

- Fetuses and Neonates
 - Mammalian embryos and fetuses are in a state of unconsciousness throughout pregnancy and birth
 - Euthanize dam by injection of barbiturate or barbituric acid derivative (avoid IP)
 - Leave fetuses in uterus for 15-20 mins undisturbed after death of dam
 - Late stage fetuses when uterus opened may require individual euthanasia

AVMA 2013 Guidelines: [By Species and Environment](#)

- Small Lab Rodents, Inhaled Agents
 - use of inhaled anesthetics for preanesthesia removes the necessity for slow filling of the chamber with CO₂
 - Death may be confirmed by physical examination, ensured by adjunctive physical method, or obviated by validation of euthanasia chambers and process
 - Pre-filled chambers are unacceptable
 - Clean and empty chamber between uses

AVMA 2013 Guidelines: [By Species and Environment](#)

- Lab Rodents: treat precocial young as adults
- Neonatal mice may take up to 50 mins to die from CO₂
- Gradual cooling of fetuses and altricial neonates (< 7 days old) is acceptable with conditions
- 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 Nitrogen

AVMA 2013 Guidelines: [By Species and Environment](#)

Finfish in the research facility

Acceptable methods:

- Immersion in anesthetic solution for > 10 mins after opercular cessation (1 step w/ any of the below)
 - Benzocaine, quinaldine, MS 222, 2-phenoxyethanol
- Rapid chilling of zebrafish and Australian river gizzard shad, see next slide

Acceptable w/ conditions:

- Immersion in CO₂-saturated water, eugenol, isoeugenol, or clove oil
- Decap then pith (2 step)
- Blunt force trauma then pith (2 step)
- Maceration
- Rapid chilling for fish which lower lethal temp is > 4C

AVMA 2013 Guidelines: By Species and Environment

Finfish, Danio rerio:

- May euthanize by rapid chilling and subsequent holding in icewater (2 to 4°C) until loss of orientation and operculum movements
 - >10 mins for adults
 - >20 mins for fry 4 to 7 dpf
- Rapid chilling and MS 222 alone are ineffective alone for embryos < 3 dpf, need adjunct
- Use of a dilute sodium hypochlorite or calcium hypochlorite solution may be an adjunctive method for early life stages, including embryos and larvae

AVMA 2013 Guidelines: By Species and Environment

X. laevis and *Rana* spp.

- Best euthanized by a physical method while fully anesthetized



AVMA 2013 Guidelines: By Species and Environment

- Lab Farm Animals, Dogs, Cats, Ferrets, NHPs, Rabbits: When fully anesthetized, may use bilateral thoracotomy, exsanguination, perfusion, and IV or intracardiac injection of potassium chloride to euthanize
- Rabbits: hold their breath when confronted with unpleasant odors; CO₂ as sole agent is distressful; premedicate or anesth first for inhalants; CD in rabbits is accept. w/ conditions, mature rabbits require technical competency



AVMA 2013 Guidelines: By Species and Environment

Birds

- Lack diaphragm, have single coelomic cavity, use caution to avoid injections into air sacs
- Pneumatic bones communicate with resp. sys. so do not administer EA IO into humerus or femur
- Suggests render unconscious by inhalant then give IV EA
- CO₂ concentrations required to achieve euthanasia of newly hatched chicks may be much higher (as much as 80% to 90%) than those for adults of the same species

AVMA 2013 Guidelines: By Species and Environment

Birds

- Bird embryos that have attained > 50% incubation have developed a neural tube sufficient for pain perception & should be euthanized by methods used in avian neonates such as anesthetic overdose, decapitation, or prolonged (> 20 minutes) exposure to CO₂
- Eggs at < 50% incubation may be destroyed by prolonged exposure (> 20 minutes) to CO₂, cooling (< 4°C for 4 hours), or freezing
- Egg addling can be used to destroy embryos

Summary

- In accordance with the *Guide* and the AWA, euthanasia *should* comply with the AVMA Guidelines
- Remember Scientific justification and IACUC approval
- Appendices are very useful!

Questions?

