Presented by the following members of the AVMA Panel on Euthanasia:

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- Cheryl B. Greenacre, DVM, DABVP-Avian, DABVP-Exotic Companion Mammals; University of Tennessee
- Stephen L. Leary, DVM, DACLAM; Washington University, St. Louis
- Robert Meyer, DVM, DACVA; Mississippi State University
- David S. Miller, DVM, PhD, DACZM; Loveland Colorado

With:
- John Bradfield, DVM, PhD, DACLAM; AAALAC, International
- Patricia A. Brown, VMD, MS, DACLAM; NIH, OLAW
- Carol Clarke, DVM, DACLAM; USDA, APHIS, AC
- Axel Wolff, DVM, MS; NIH, OLAW


Sam Cartner, DVM, PhD, DACLAM
University of Alabama at Birmingham

AVMA Guidelines

Oversight Organization Position

- OLAW: Implementation by PHS Assured institutions no later than September 1, 2013.
- USDA: Comment to be provided
- AAALAC: The 2013 AVMA Guidelines on Euthanasia are currently under review by the AAALAC International Council on Accreditation as consideration for their potential adoption as an AAALAC reference resource. AAALAC International reference resources are intended as guidance documents for accredited institutions and site visit teams during the site visit. Additionally, reference resources may be used during Council deliberations when discussing issues identified during site visits.
Presentation Goals

- Review history of the Report on Euthanasia
- Review major changes in the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition
- Emphasize changes to laboratory animal methods of euthanasia
- Address questions and issues of interest and concern

1963 Panel on Euthanasia

- Directed to study methods in use for unwanted small animals
- Review literature
- Observe field activities
- Consult others
- Evaluate findings
- Make recommendations
- 8 page report

History

AVMA Guidelines on Euthanasia

<table>
<thead>
<tr>
<th>Year</th>
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<tr>
<td>1963</td>
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1972 and 1978 Reports

- **1972**
  - Added laboratory animals (CO₂ and decapitation recommended)

- **1978**
  - Added cervical dislocation (mice and poultry)
  - Added statement about food animals
  - Warren submitted a letter to the editor drawing attention to the 1975 Mikeska / Klemm paper that described persisting EEG after decapitation

1986 Report

- CO₂ minimal flow rate 20% displacement volume/minute (Hornett 1984)
- Decapitation
  - "should be used only after animal has been sedated or lightly anesthetized, unless the head will be immediately frozen in liquid nitrogen subsequent to severing."
- Cervical Dislocation
  - Weight limits
    - <200 g rodents;
    - <1 kg rabbits;
    - preferable to lightly anesthetize.

1993 Report

- IACUC was formally introduced in amendments to AWA (1985) and PHS Policy (1986)
- 1993
  - CO₂ - no change
  - Cervical dislocation - scientifically justified and approved by the IACUC
  - Decapitation - scientifically justified and approved by the IACUC
  - Added special considerations - equine, food animal, zoo, wildlife, aquatics
2000 Report
- First use of acceptable, conditionally acceptable
- CO\textsubscript{2} - acceptable - eliminated dry ice as source
- Cervical dislocation - scientifically justified and approved by the IACUC
- Decapitation - conditionally acceptable..."when its use is required by the experimental design and approved by the IACUC"

2007 Guidelines
- Changed name from Report to Guidelines
- Maceration - acceptable for newly hatched poultry
- Caution Statement

Caution Statement
“A combination of pentobarbital with a neuromuscular blocking agent is not an acceptable euthanasia agent”

Caution – The AVMA Guidelines on Euthanasia (formerly the 2000 Report of the AVMA Panel on Euthanasia) have been widely misinterpreted
- The guidelines are in no way intended to be used for human lethal injection
- The application of a barbiturate, paralyzing agent, and potassium chloride delivered in separate syringes or states (the common method used for human lethal injection) is not cited in the report
- The report never mentions pancuronium bromide or Pavulon, the paralyzing agent used in human lethal injection
Panel on Euthanasia 2013

- 14 panel members
- 11 working groups
- 3 methods
- 8 species and environment
- 102 pages

Changes

- Introduction emphasizes processes prior to and after euthanasia (ethics, carcass disposal, etc.)
  - “end of life decisions” and “life worth living”
- Diagrams and specific guidance on some techniques
- Glossary
  - (e.g. unconsciousness = loss of righting reflex)

Separate Guidelines

- Depopulation and slaughter
- Euthanasia is defined as:
  "ending the life of an individual animal in a way that minimizes or eliminates pain and distress"
Acceptable with Conditions

- Methods acceptable with conditions are:
  
  **considered to be equivalent to acceptable methods when criteria for application of a method can be met.**

- Dependence on IACUC to approve any method as appropriate, as necessary, regardless of category.
- No reference to “scientific justification” in 2011 Edition

Acceptable with Conditions (continued)

- Conditions met to consistently produce humane death
- May have greater potential for operator error or safety hazard
- Not well documented in the scientific literature
- May require a secondary method to ensure death

Acceptable with conditions methods are considered to be equivalent to acceptable methods when specific criteria for application of a method can be met.

Changes (continued)

- Cervical dislocation of poultry (turkeys)
  - “Appropriate size”
- Thoracic compression
  - Unacceptable
- Captive invertebrates
  - Spiders, insects
Changes to Laboratory Animals Guidelines

- Separate section for laboratory animals
- Focus on rodents, rabbits and aquatics
- Other species referred to other sections

Rodents

- **Acceptable** — IP or IV barbiturate
  - Momentary pain may be associated with IP injections (Svendsen, 2007; Ambrose et al. 2000), but the degree of pain and the methods to control have yet to be defined.
- **Acceptable with conditions**
  - Inhalant anesthetics (open drop), CO₂, cervical dislocation, decapitation, microwave irradiation
  - CO₂ - Home cage best, *gradual displacement rate of 10-30%* (Hornett, 1984; Smith 1997)
  - Tribromoethanol

Neonatal Rodents

- Precocial young (guinea pigs) treated as adults
- **Acceptable** — IP barbiturate derivatives
- **Acceptable with conditions**
  - Gaseous anesthetics or CO₂ (>50 mins)
    - Must be confirmed by physical examination, adjunctive physical method, or validation of the euthanasia chamber and process
  - Rapid freezing (<5 d), hypothermia (< 7d, prevent contact with cold surfaces), decapitation, cervical dislocation
Rabbits

- Acceptable
  - Small numbers of rabbits are best euthanized using the same techniques as used in the private practice setting +/- sedation with IV barbiturate

- Acceptable with conditions
  - Inhalant anesthetic, carbon dioxide (with sedation), captive bolt designed for rabbits (best for large numbers in production setting), cervical dislocation (requires demonstrated proficiency)

Zebrafish

- Acceptable
  - Tricaine methanesulfonate (MS222) followed by physical adjunctive method or immersion in 5% sodium/calcium hypochlorite

- Acceptable
  - Rapid chilling (2 - 4°C) until loss of orientation and operculum movements followed by appropriate holding times (10 mins adults, 20 mins fry) or an approved physical adjunctive method or immersion in 5% sodium hypochlorite

Rapid Chilling, Maceration, Clorox

50:50 mixture of ice water

Clorox for Embryos

Maceration
Frogs

- Acceptable
  - MS222 (5g/L) immersion
    - May be injected in lymph sacs or coelomic cavity
    - May require prolonged emersion
    - Follow with physical adjunctive method (decapitation, pithing)
  - Benzocaine hydrochloride (250 mg/L) also available as benzocaine gel (20% concentration)

Living Document

- From 2013 Edition forward, the Panel on Euthanasia continues to exist as an AVMA entity (rather than being sunset upon submission of its report), allowing important changes to be made as needed.
- Animal Welfare Forum 2014 — Animal Euthanasia, Slaughter and Depopulation

Questions and Issues

- Avian — Cheryl Greenacre, DVM, DABVP; University of Tennessee
- Inhalants — Robert Meyer, DVM, DACVA; Mississippi State University
- Captive and Free-Ranging Nondomestic Animals — David Miller, DVM, PhD, DACZM; Loveland, CO
Faster CO₂ Flow Rates?

- **Pre fill** — we know it causes severe pain and distress prior to loss of consciousness
- **Gradual fill** — 10 to 30% displacement rate/min seems to be best welfare compromise between speed of onset and nociception
- **Faster fill?** — Limited data; Valentine’s 2012 study saw more agitation and dyspnea with 100% displacement rate in rats (1t = 1 min)

AVMA Recommends CO₂ Inflow Rate 10-30% of Chamber vol/min

- Gradual displacement less likely to cause nociceptor pain prior to loss of consciousness
- 20% inflow produces a CO₂ concentration of >30% within 2.5 min and 63% within 5 min
- Relationship holds for any size leak-free container

Fig 1, Niel and Weary, Appl An Behavioral Sci 2006
20 L box, 3.5 L/min inflow; "t" = 5.7 min

Valentine Video Analysis

Carbon Dioxide
- CO₂ anesthesia due to ↓pHi
- Reduces both basal and evoked neural activities
- Produces unconsciousness and death over wide range of concentrations
- Does not rely on induction of hypoxia

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Thoracic Compression

- **What it is:**
  - Application of pressure to an animal’s chest to prevent respiration and/or cardiac movement
  - Used for small mammals and birds by some field biologists
- **Why it has been used:**
  - Tradition
  - No equipment or materials required
  - Perceptions of unaltered anatomical or biological samples for research or archiving

Thoracic Compression Compliance with POE Criteria for Methods

- Minimal pain and distress - compression = pain
- Time until consciousness - undocumented
- Reliability - undocumented
- Irreversible - undocumented (no training guidelines)
- Compatibility with intended use and purpose - poorly documented
- Compatibility with post-mortem exam or tissue use - undocumented

Summary

- Substantial animal welfare concerns: pain, distress, asphyxiation
- No published documentation supporting efficacy
- No performance standards for proficiency and method
- Practical alternatives (injectables, portable anesthetic machines, “drop method,” etc.) are available and supported by AAWV, AAV, etc.
- Convenience (not wanting training and/or taking equipment into the field) is not adequate justification
- Does not meet criteria for euthanasia
Thoracic Compression
Alternative — TC may be justified as humane killing, under a few select circumstances where alternative options are inferior and training / performance standards can be established
  • Humane killing = recognition that there is a need to end animal's lives as humanely as possible when strict adherence to euthanasia standards is not possible
  • Field work is hard

  AVMA backgrounder: https://www.avma.org/KB/Resources/Backgrounders/Pages/Welfare-Implications-of-Thoracic-Compression.aspx

Pain
Defined by IASP as “a conscious experience”
  • Unpleasant sensory or emotional experience assoc w/actual or potential tissue damage
  • Activity induced in nociceptor and nociceptor pathways by a noxious stimulus is not pain, which is always a psychological state

Unconsciousness
  • Loss of individual awareness
    • Occurs when brain’s ability to integrate information is blocked or disrupted
    • All inhaled methods have potential to cause distress
    • loss of consciousness is not instantaneous
    • In animals, loss of consciousness occurs with loss of righting reflex (LORR; also called Loss of Position)
    • Memory and awareness in humans and animals suppressed at anes conc <50% of those needed to abolish movement
    • Actions following LORR not consciously perceived
Agent Purity and Euthanasia

- Pharmaceutical-grade substances, when available, must be used to avoid toxicity or side effects that may threaten the health and welfare of vertebrate animals or interfere with the interpretation of research results (OLAW, USDA)
  - However, the decision to use non-pharmaceutical-grade substances rests with the IACUC
  - If no equivalent veterinary or human drug is available for experimental use, OLAW, USDA, and AAALAC agree the highest-grade equivalent chemical reagent should be used

Industrial or Food Grade Gas May Be Similar or Higher Purity than Medical Grade

- BB-C-101D defines federal specs for CO₂
  - Grade A gases manufactured under certified Good Manufacturing Practices (cGMP) as defined by federal statute 21 CFR 211.84 and meet the applicable specifications of the USP/NF, which include a certificate of analysis, validated analytical procedures, lot number assignment, traceability and recall procedures
  - Grade B lacks cGMP compliance, but purity is to be no less than 99.5%