OLAW Online Seminar


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

Samuel C. Cartner, DVM, PhD, DACLAM; U. Alabama at Birmingham
Cheryl B. Greenacre, DVM, DABVP-Avian, DABVP-Exotic Companion Mammals; University of Tennessee
Steven L. Leary, DVM, DACLAM; Washington University, St. Louis
Robert Meyer, DVM, DACVAA; Mississippi State University
David S. Miller, DVM, PhD, DACZM; Loveland Colorado
Emily Patterson-Kane, PhD; AVMA

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
AVMA Guidelines Adoption Status

- **OLAW:** Implementation by PHS Assured institutions no later than September 1, 2013.

- **USDA:**
  - The US Department of Agriculture endorses the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition
  - The AVMA Guidelines are in accordance with the definition of Euthanasia as found in the Animal Welfare Act Regulations [§1.1 Definitions]
AVMA Guidelines Adoption Status

**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.

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

5 Veterinarians

Directed to study methods in use for unwanted small animals

Review literature

Observe field activities

Consult others

Evaluate findings

Make recommendations

8 page report

Members of the Panel on Euthanasia

Steven Leary, DVM, DACLAM (Chair), Washington University, St. Louis, Missouri
Wendy Underwood, DVM (Vice Chair), Eli Lilly and Company, Indianapolis, Indiana
Raymond Anthony, PhD (Ethics); University of Alaska Anchorage, Anchorage, Alaska
Samuel Carver, DVM, MPH, PhD, DACLAM (Lead, Laboratory Animals Working Group); University of Alabama at Birmingham, Birmingham, Alabama
Douglas Pugh, DVM (Lead, Equine Working Group); Associated Veterinary Clinic, Walls, Walls, Washington
Temple Grandin, PhD (Lead, Physical Methods Working Group); Colorado State University, Fort Collins, Colorado
Cheryl Green, DVM, DABVP (Lead, Avian Working Group); University of Tennessee, Knoxville, Tennessee
Sharon Guelin, DVM, PhD, DABVT, DABT (Lead, Noninvasive Agents Working Group); ASPCA Poison Control Center, Urbana, Illinois
Mary Ann McCracken, DVM, PhD, DACVE, Lead, Companion Animals Working Group); Virginia Polytechnic Institute and State University, Blacksburg, Virginia
Robert Meyer, DVM, DAVCO (Lead, Inhaled Agents Working Group);
Mississippi State University, Mississippi State, Mississippi
David Miller, DVM, PhD, DACPS (Lead, Reptiles, Zoo and Wildlife Working Group); Loveland, Colorado
Jan Shearer, DVM, MS, DACW (Lead, Animals Farmed for Food and Fiber Working Group);
Iowa State University, Ames, Iowa
Roy Yang, VMD (Lead, Aquatic Working Group); University of Florida, Gainesville, Florida

AVMA Staff Consultants

Gail C. Golds, PhD, DVM, MANVSc, DACW, Director, Animal Welfare Division
Emily Patterson-Kuur, PhD, Animal Welfare Scientist, Animal Welfare Division

The following individuals continued as members through their participation in the Panels Working Groups and their assistance is sincerely appreciated:

Inhaled Agents—Scott Holm, DVM, DABVP; Lisa Hul, PhD, DVM, MS
Noninvasive Agents—Virginia Harr, DVM, PhD, DACVCP; Dan Sepker, DVM, PhD, DACO, DABVP
Physical Methods—Richey Glacier, DVM; Ted Hill, PhD, Jennifer Woods, DVM
Aquatics—Craig Harms, DVM, PhD, DACW; Helen Robson, DVM, Rick Satter-Thompson, DVM, Michael Steen, DVM, PhD, DACW
Avian—Coulson, DVM, MPH; Llewellyn, DVM, DABVP; Karen Foss, DVM, DABVP; Bob Marshall, DVM, DABVP; James Morgan, DVM, PhD, DABVP
Companion Animals—Kathleen Cross, MS, DACW; Stacy Pfitzner, DVM; Julie May, DVM, PhD, DACW
Other—Patrick Ruel, DVM, MBA, DACW; David Sailer, DVM, DACW; Penelope Leib, DVM, DACW, Thomas A. Lucas, DVM, MS, DABT
Necropsy—Kathleen Mount, DVM, DABVP; Gregory Pepper, DVM, St. Louis, Missouri, DACW
Food and Fiber Animals—Brian Scott, PhD, C. Joseph Daniels, DVM, MS, John Dyer, DVM, PhD, DABVP, DABO, DAVCO
Robert Press, PhD, DVM, DACW; Jeremy Gerber, DVM, MS, Tania Grills, DVM, MS, Carlisle Goodell, DVM, G. John Johnson, DVM, FASCP
Richard Reynolds, PhD, James Reynolds, DVM, MAPP, DACW; Bruce Walker, PhD
Laboratory Animals—James Avila, MS, DVM, DACLAM, Larry Geyken, DVM, FEL, FEL, Geyken, Paul Blackwell, PhD, DAC, MRCVS, FLD, BCBACP, DABCP, DABSN, DABT, DABCP, FASCP, DABCP, FEL, DABT
Veterinary Pathologists—James Cross, DVM, DACLAM, MRCVS
Reptiles, Amphibians, and Wild Animals—Gitanjali Kanwar, DVM, DAAPM, Marcia Driscoll, DVM, MS, DACC, Julie Golden, DVM, Barry Hargreaves, DVM, MS, Gregory Latta, PhD, DVM, DAC, Douglas R. James, MS, DABVP, FASCP, David A. Merwin, DVM, DACW

AVMA Guidelines on Euthanasia

1963
1972
1978
1986
1993
2000
2007
2013
1972 and 1978 Reports

- **1972**
  - Added laboratory animals (CO$_2$ 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

- CO$_2$ 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; and
    - preferable to lightly anesthetize.
1993 Report

- IACUC was formally introduced in amendments to AWA (1985) and PHS Policy (1986)

1993
- \( \text{CO}_2 \) - 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$_2$ - 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)
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 CO$_2$ (>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)
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

- Inhalants — Robert Meyer, DVM, DACVAA; Mississippi State University
- Captive and Free-Ranging Nondomestic Animals — David Miller, DVM, PhD, DACZM; Loveland, CO
- Avian — Cheryl Greenacre, DVM, DABVP; University of Tennessee
Question 1

- Why do the AVMA Guidelines recommend low flow CO₂ euthanasia?
  - Low flow CO₂ euthanasia takes longer. Would it be more humane for the animals to die more quickly?
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
CO$_2$ and Distress

1. Pain due to formation of carbonic acid on respiratory and ocular membranes

2. “Air hunger”; breathlessness

3. Direct stimulation of acid-sensing ion channels within the amygdala associated with fear response
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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Faster CO$_2$ 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 CO$_2$ 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 CO$_2$ 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; 1t = 5.7 min
Quality Control with CO$_2$

- Accurate chamber volume and flow rate?
-Leaks?
-Do observed behaviors occur following loss of righting/consciousness?
-Inhaled anesthetics prior to CO$_2$?
-Nasal bleeding?
Question 2

- Why does the Panel consider thoracic compression unacceptable?
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
Thoracic Compression Compliance with POE Criteria for Methods

• 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
Questions 3 and 4

- Is it acceptable for an IACUC to decide that terminating the lives of wild animals in a field setting is humane killing rather than euthanasia?

- Do the AVMA Guidelines apply to field research conducted by a PHS funded investigator who has traveled to a foreign country to conduct that research?
Question 5

- What was the Panel’s rationale for the acceptability of cervical dislocation?
2013: Cervical Dislocation

- Acceptable with conditions - personnel should be trained... demonstrate proficiency
- No requirement for scientific justification
1978 Report: Cervical Dislocation and Decapitation

- Disarticulation of the skull and cervical vertebrae is a method of producing euthanasia in mice and poultry
- Guillotine devices have been used for decapitating smaller laboratory animals, especially rats... it is rapid, inexpensive, and when properly done, produces instant death

1979 Warren, JAVMA, Letter to Editor
Decapitation of Laboratory Animals and Euthanasia
Oct 4, 1978

Dear Sir:

In the report of the AVMA Panel on Euthanasia, (JAVMA, July 1, 1978, p 60), decapitation is said to produce instant death. I doubt this because of an experiment that was conducted by Mikeska and Klemm¹.
EEG EVALUATION OF HUMANENESS OF ASPHYXIA AND DECAPITATION EUTHANASIA OF THE LABORATORY RAT1.2.3

J.A. MIKESKA AND W.R. KLEMM

SUMMARY - The relative humaneness of asphyxia and decapitation was objectively evaluated in rats by EEG monitoring. EEG activation (low voltage, fast activity) was considered to indicate discomfort, pain, and affective responses to euthanasia. Such activation was present 37.3 ± 7.5 sec after asphyxia and 13.6 ± 4.6 sec after decapitation. Decapitation was also characterized by an immediate, large, and relatively long-lasting, ultra-slow voltage, detected by non-polarizable scalp electrodes. Isoelectric activity (death) occurred 69.4 ± 9.9 sec after onset of asphyxia and 27.2 ± 4.4 sec after decapitation.
1986 Report: Decapitation

- Decapitation – “until additional information is available… the technique should be used only after the animal has been sedated or lightly anesthetized, unless the head will be immediately frozen in liquid nitrogen.”
1986 Report: Cervical Dislocation

- “…humane techniques to euthanatize poultry, mice, and rats…<200 gm… rabbits <1kg”
- “Because unconsciousness may not occur immediately, it is preferable to lightly anesthetize or sedate…”
- “IACUCs… must determine…personnel… have been properly trained.”
Decapitation Debate

- Vanderwolf (1988) concluded EEG did not resemble EEG in response to pain
- Derr (1991) reported $O_2$ tension too low to support consciousness with 2.7 secs
1993 Report: Cervical Dislocation and Decapitation

“Until additional information is available… should only be used in research settings when scientifically justified by the user and approved by the IACUC.”
2000 Report: Decapitation

- EEG activity does not infer ability to perceive pain and ....loss of consciousness develops rapidly

- “is conditionally acceptable ... and should be used in research settings when its use is required by scientific design..”
2000 Report: Cervical Dislocation

- Humane technique for birds and small rodents when performed by trained personnel

- In lieu of demonstrated competency animals must be sedated/anesthetized

- “In research settings, this technique should be used only when scientifically justified by the user and approved by the IACUC.”
Loss of Cortical Function in Mice After Decapitation, Cervical Dislocation, Potassium Chloride Injection, and CO₂ Inhalation

Samuel C Cartner,¹,⁎ Shayne C Barlow,¹,† and Timothy J Ness²

Electroencephalograms (EEG) and visual evoked potentials (VEP) in mice were recorded to evaluate loss of cortical function during the first 30 s after euthanasia by various methods. Tracheal cannulae (for positive-pressure ventilation, PPV) and cortical surface electrodes were placed in mice anesthetized with inhaled halothane. Succinylcholine was used to block spontaneous breathing in the mice, which then underwent continuous EEG recording. Photic stimuli (1 Hz) were presented to produce VEPs superimposed on the EEG. Anesthesia was discontinued immediately before euthanasia. Compared with that obtained before euthanasia, EEG activity during the 30-s study period immediately after euthanasia was significantly decreased after cervical dislocation (at 5 to 10 s), 100% PPV-CO₂ (at 10 to 15 s), decapitation (at 15 to 20 s), and cardiac arrest due to KCl injection (at 20 to 25 s) but not after administration of 70% PPV-CO₂. Similarly, these euthanasia methods also reduced VEP amplitude, although 100% PPV-CO₂ treatment affected VEP amplitude more than it did EEG activity. Thus, 100% PPV-CO₂ treatment significantly decreased VEP beginning 5 to 10 s after administration, with near abolition of VEP by 30 s. VEP amplitude was significantly reduced at 5 to 10 s after cervical dislocation and at 10 to 15 s after decapitation but not after either KCl or 70% PPV-CO₂ administration. The data demonstrate that 100% PPV-CO₂, decapitation, and cervical dislocation lead to rapid disruption of cortical function as measured by 2 different methods. In comparison, 70% PPV-CO₂ and cardiac arrest due to intracardiac KCl injection had less rapid effects on cortical function.
Question 6

- Would you review the Panel’s reason for revising the recommendation concerning acceptability of rapid chilling of tropical fish, e.g., zebrafish?
“Although we interpreted rapid opercular movements and erratic swimming as signs of distress after exposure to MS222, some of this activity may be normal behavioral changes as a fish passes through various anesthetic stages. However, neither of these behaviors occurred in animals placed in an ice–water bath.”

Wilson, Bunte, and Carty, J AALAS, Vol 48, No 6, 2009
Questions 7 and 8

- Do you need to use low flow CO$_2$ euthanasia in poultry?
- Can you use pre-filled chambers?
- How long in the chamber is required for euthanasia of chicks?
- Do you have a chart of appropriate size of poultry for cervical dislocation?
Upcoming OLAW Online Seminar

• December 12, 2013 – Topic: TBD