OLAW Online Seminar

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

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

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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.

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

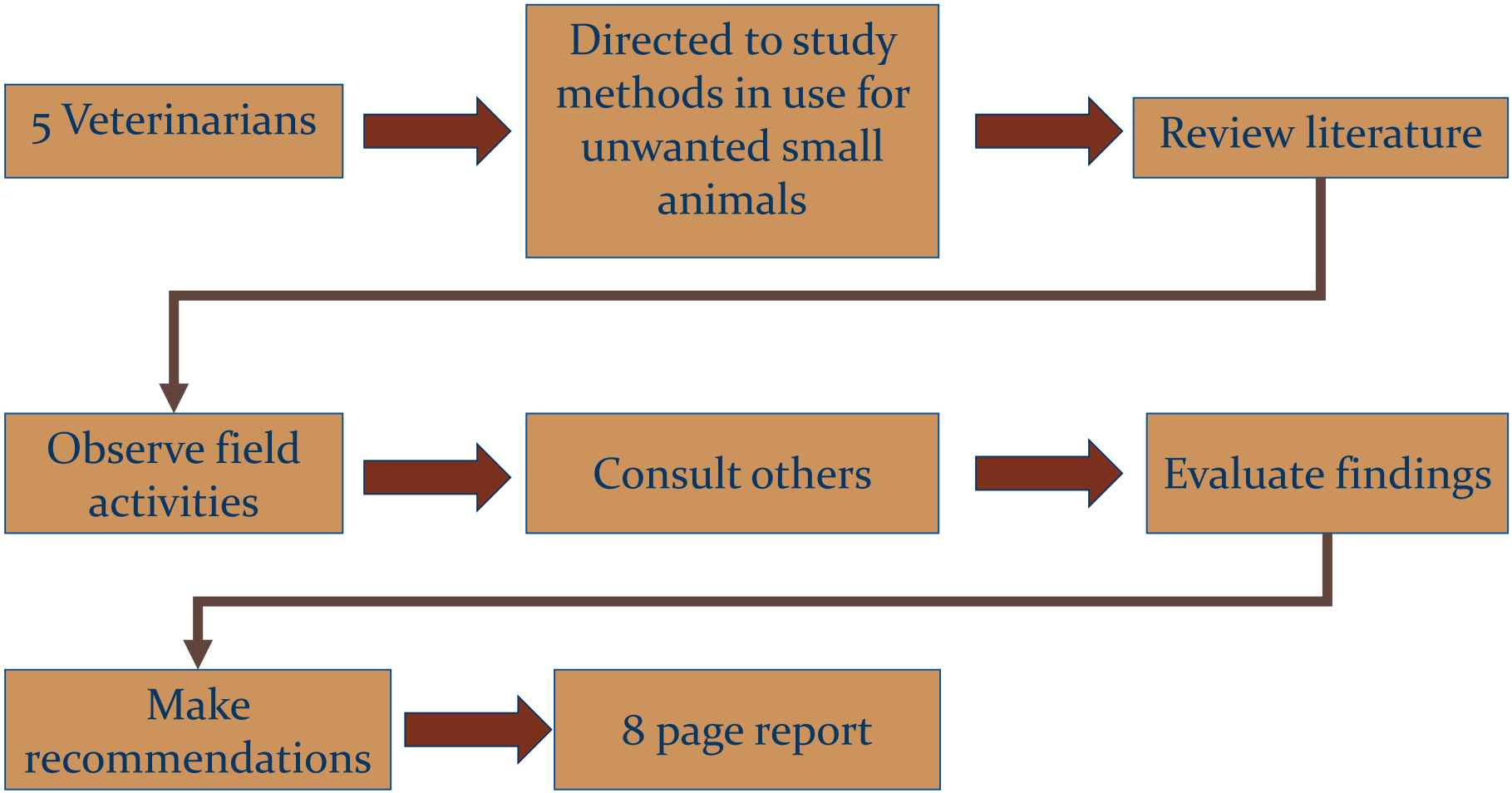
Sam Cartner, DVM, PhD, DACLAM

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



History

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

Members of the Panel on Euthanasia

Steven Leary, DVM, DACLAM (Chair); Washington University, St. Louis, Missouri
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The following individuals contributed substantively through their participation in the Panel's Working Groups and their assistance is sincerely appreciated.

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Laboratory Animals—James Artwohl, MS, DVM, DACLAM, Larry Carbone, DVM, PhD, DACLAM, Paul Flecknell, VetMB, MRCVS, PhD, DECVA, DECLAM, DACLAM, FRCVS, David P. Friedman, PhD, Kathleen Pritchett-Corning, DVM, DACLAM, MRCVS
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AVMA Guidelines on Euthanasia

1963	1993
1972	2000
1978	2007
1986	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

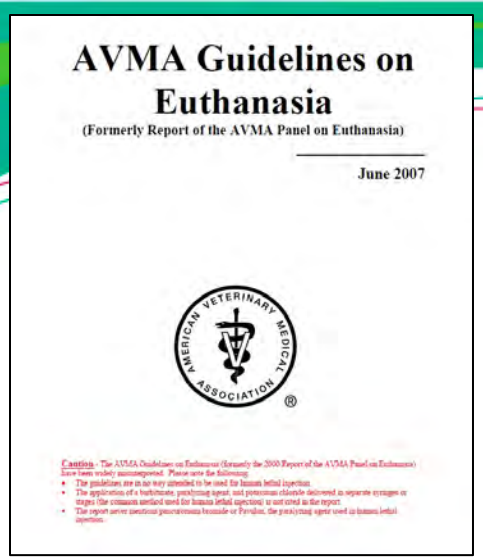
- IACUC was formally introduced in amendments to AWA (1985) and PHS Policy (1986)
- 1993
  - CO_2 - no change
  - Cervical dislocation - scientifically justified and approved by the IACUC
  - Decapitation - scientifically justified and approved by the IACUC
  - Added special considerations - equine, 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


AVMA Guidelines on Euthanasia

(Formerly Report of the AVMA Panel on Euthanasia)

June 2007

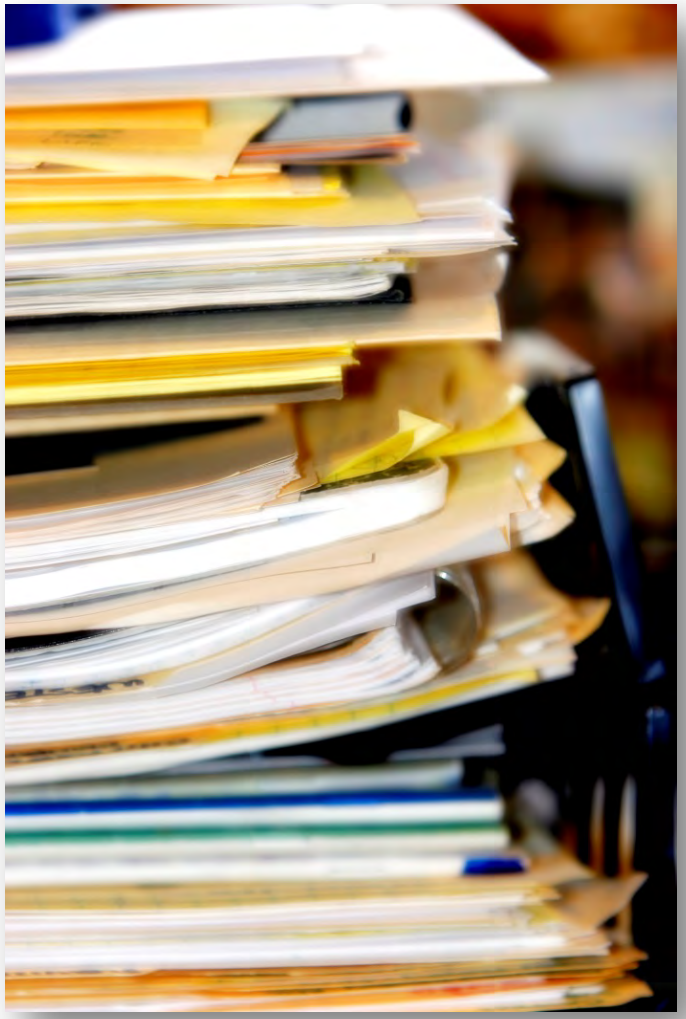
Caution Statement

"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

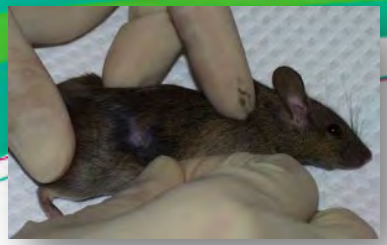
Acceptable with conditions methods are considered to be equivalent to acceptable methods when specific criteria for application of a method can be met.

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), CO_2, cervical dislocation, decapitation, microwave irradiation
    - CO_2 - 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

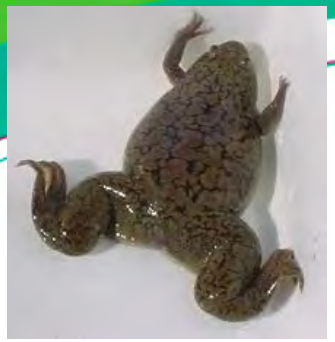
- **Acceptable**
  - Tricaine methanesulfonate (MS222) followed by physical adjunctive method or immersion in 5% sodium/calcium hypochlorite
- **Acceptable**
  - Rapid chilling (2 - 4°C) until loss of orientation and operculum movements followed by appropriate holding times (10 mins adults, 20 mins fry) 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)

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

- 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 CO_2 euthanasia?
  - Low flow CO_2 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

- CO_2 anesthesia due to ↓pHi
  - Reduces both basal and evoked neural activities
  - Produces unconsciousness and death over wide range of concentrations
- Does <u>not</u> rely on induction of hypoxia

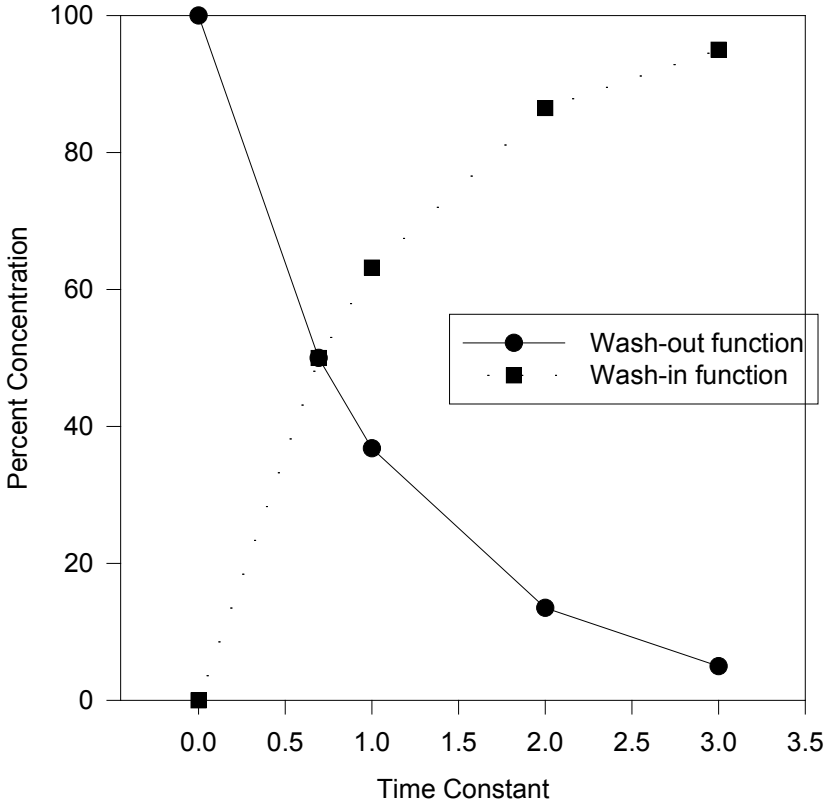
% CO2	% O2 remaining
0	20.98
10	18.882
20	16.784
30	14.686
40	12.588
50	10.49
60	8.392
70	6.294
80	4.196
90	2.098
100	0

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)

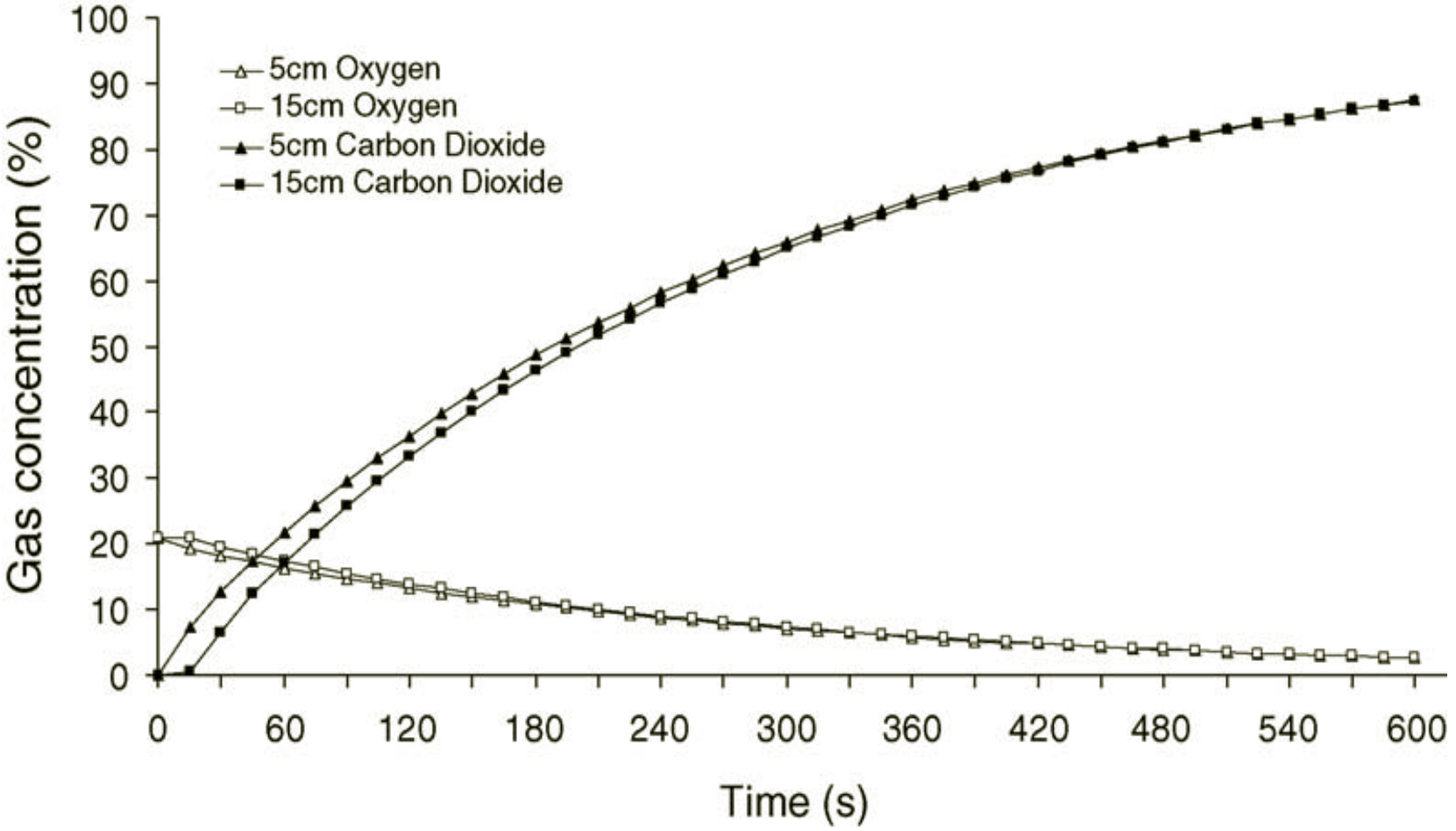
AVMA Recommends CO_2 Inflow Rate 10-30% of Chamber vol/min

- 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



Meyer and Morrow. J SHAP 2005; 13:210-217

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

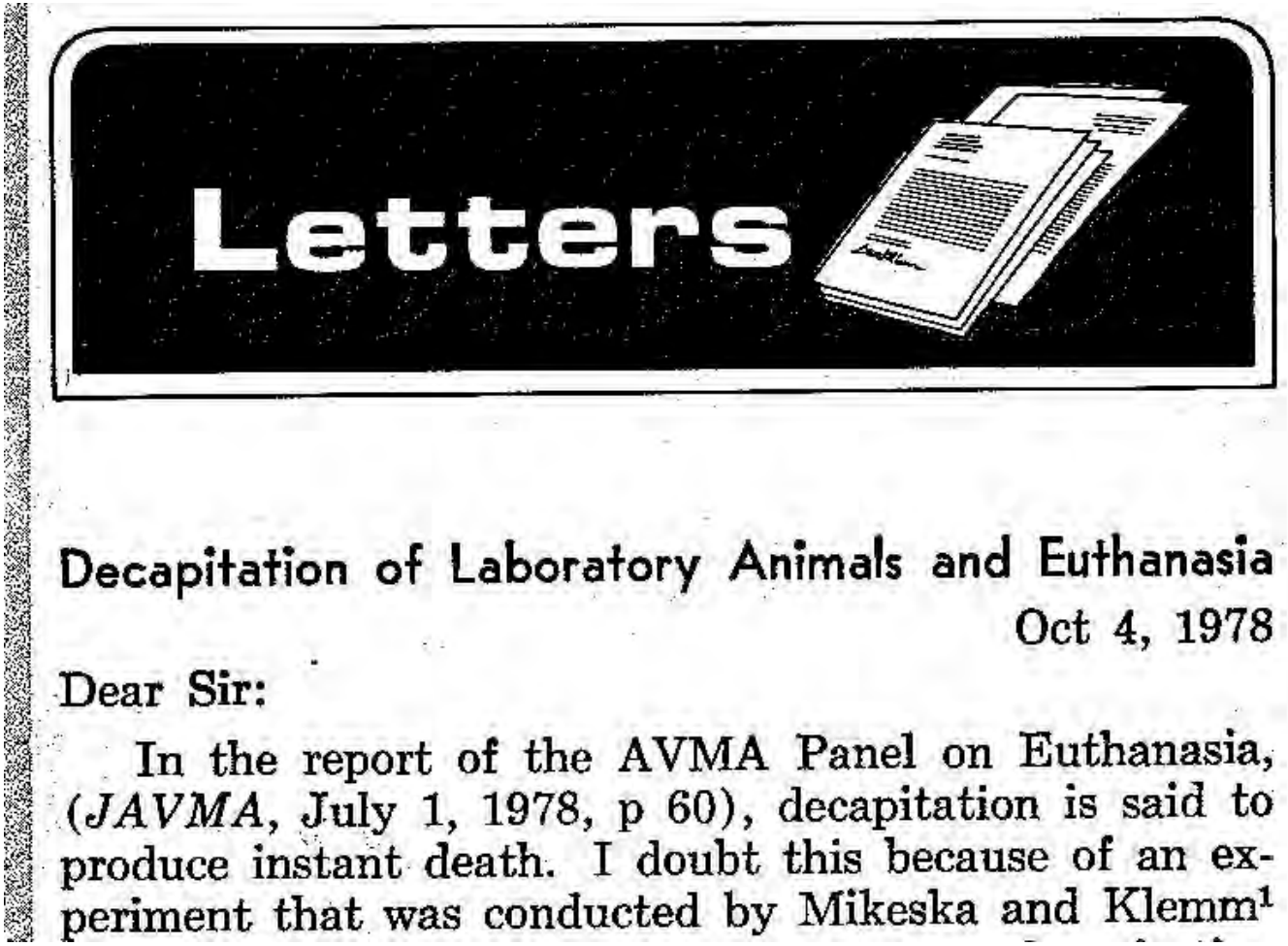
- Acceptable with conditions - personnel should be trained… demonstrate proficiency
- No requirement for scientific justification

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

1978 Warren Letter to Editor



Letters

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 Klemm[1]

EEG EVALUATION OF HUMANENESS OF ASPHYXIA AND DECAPITATION EUTHANASIA OF THE LABORATORY RAT[1,2,3]

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 euthansia. 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.*

Mikeska and Klemm, Laboratory Animal Science, Vol 23, No 2 1975.

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 CO_2 Inhalation

Samuel C Cartner,[1,*] Shayne C Barlow,[1,†] and Timothy J Ness[2]

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-CO_2 (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-CO_2. Similarly, these euthanasia methods also reduced VEP amplitude, although 100% PPV-CO_2 treatment affected VEP amplitude more than it did EEG activity. Thus, 100% PPV-CO_2 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-CO_2 administration. The data demonstrate that 100% PPV-CO_2, decapitation, and cervical dislocation lead to rapid disruption of cortical function as measured by 2 different methods. In comparison, 70% PPV-CO_2 and cardiac arrest due to intracardiac KCl injection had less rapid effects on cortical function.

Cartner, Barlow, and Ness, Comparative Medicine, Vol 57, No 6, 2007.

Question 6

- Would you review the Panel's reason for revising the recommendation concerning acceptability of rapid chilling of tropical fish, e.g., zebrafish?

Evaluation of Rapid Cooling and Tricaine Methanesulfonate (MS222) as Methods of Euthanasia in Zebrafish (*Danio rerio*)

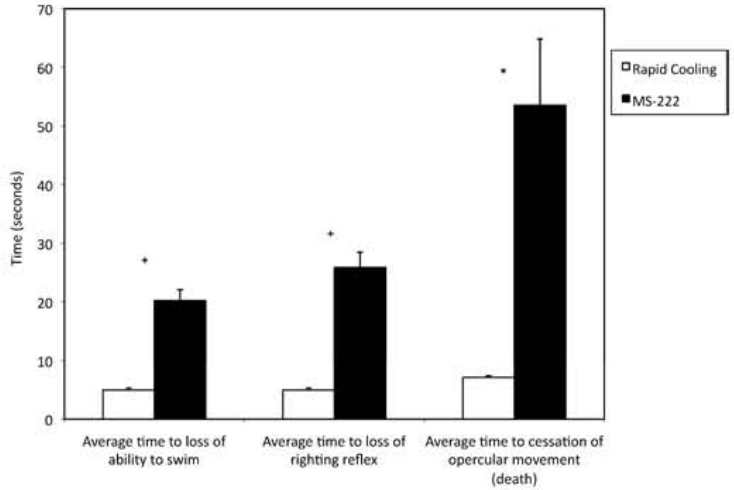


Figure 2. Euthanasia by rapid cooling compared with exposure to MS222. The time (s; mean ± SEM) from exposure until the fish lost the ability to swim was 4.90 ± 0.33 s for rapid cooling and 20.20 ±1.86 s for MS222. The time (s; mean ± SEM) from exposure until loss of the righting reflex was 4.90 ± 0.33 s for fish euthanized by rapid cooling and 25.90 ± 2.61 s for those exposed to MS222. The time (s; mean ± SEM) from exposure until death was 7.13 ± 0.27 s for rapid cooling compared with 53.52 ± 11.32 for MS222. For all observations, the difference between rapid cooling and MS222 was statistically significant (+, $P < 0.0001$; *, $P \leq 0.0005$).

"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