Update on the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

OLAW Online Seminar
September 10, 2020

Axel Wolff, MS, DVM, NIH, Office of Laboratory Animal Welfare
Samuel Cartner, DVM, PhD, DACLAM, University of Alabama at Birmingham
The PHS Policy References the AVMA Euthanasia Guidelines

OLAW is Committed to Using Sound Professional Guidance
The AVMA Guidelines are Based on Peer-Reviewed Scientific Literature
Co₂ Flow Rate Recommendations Revised Based on New Studies

10% Flow resulted in signs of prolonged distress

Scientific Debate Leads to Improvement
Decisions Should Not be Based on:

- Cost
- Convenience
- Tradition
- Unsupported Anecdotes
Public Comments


Notice Number: NOT-OD-20-069

Key Dates
Release Date: February 24, 2020
Response Date: April 29, 2020

Related Announcements
NOT-OD-20-143 - Grant and Contract Submission Requirements Regarding the Updated AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

Issued by
National Institutes of Health (NIH)

Purpose
This Request for Information (RFI) is to solicit input from the public on any concerns they may have with the updated American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2020 Edition ("Guidelines") and provide guidance to Public Health Service (PHS) and National Science Foundation funded institutions on implementation of the updated Guidelines.
Manual Blunt Force as a Method of Euthanasia
Cardiac Compression in Birds


Scientific Justifications Allow Flexibility

Dr. Sam Cartner
University of Alabama at Birmingham
Chair Laboratory Animals Working Group
September 10, 2020
2020

- 14 panel members, 11 working groups (3 techniques groups, 8 species groups), ethicist. Most participated on the 2013 Edition
- Opportunity for member comment
- Exist as a virtual entity to respond to inquiries
- Deliberate separation of euthanasia, humane slaughter, and depopulation → “Humane Endings”
- Commitment to data-based improvements that meet societal needs
History of the AVMA’s Panel on Euthanasia

2013

• Introduction emphasizes process prior to and after euthanasia
  ➢ “end of life decisions” and “life worth living”

• Diagrams and specific guidance on some techniques

• Glossary
  ➢ Unconsciousness=loss of righting reflex

• Changed “Conditionally Acceptable” to “Acceptable with Conditions”

• No reference/requirement for scientific justification
History of the AVMA’s Panel on Euthanasia

2013

• Cervical dislocation of poultry (turkeys)
  ➢ “Appropriate size”
• Thoracic compression
  ➢ Unacceptable
• Captive invertebrates
  ➢ Spiders, insects
• Emphasized 10-30% gradual displacement rate for laboratory rodents
Guidelines for the Euthanasia of Animals
Update for Laboratory Animals

Laboratory Animal Working Group

• Sam Cartner, DVM, PhD, DACLAM - Chair
• Larry Carbone, DVM, PhD, DACLAM
• Paul Flecknell, VetMB, MRCVS, PhD, DECVA, DECLAM, DACLAM
• David P. Friedman, PhD
• Debra Hickman, DVM, DACLAM, DACAW
• Kathleen Pritchett-Corning, DVM, DACLAM, MRCVS
Introduction

Distinguished between states of awareness

• **Unconscious** - loss of awareness and is associated with loss of righting reflex

• **Sedated** or **tranquilized** - can be aroused with sufficient stimulation
Laboratory Animals

Inhalants - Carbon Dioxide for Rodents

• CO₂ flow rate recommendation for rodents to 30-70%
• Previously 10-30%
• Based on much research looking at different flow rates
• Potential for distress at lower flow rates
• Potential for mucous membrane pain at higher flow rates
Physiological, Behavioral, and Histological Response of Male C57BL/6N Mice to Different CO₂ Chamber Replacement Rates

Gregory P Boivin, 1,2* Michael A Bottomly,3 Emily S Dudley,1 Patricia A Schiml,4 Christopher N Wyatt5, Dadja Grube6

“...activity levels, behavioral responses, plasma adrenocorticotrophic hormone and corticosterone levels, and lung pathology were not different between groups. We found no physiological, behavioral, or histologic evidence that 15% or 30% CO₂ CRR is less painful or distressful than is 50% or 100% CO₂ CRR. We conclude that 50% to 100% CO₂ CRR is acceptable for euthanizing adult male C57BL/6N mice.”
Figure 1. CO₂ concentration in the home cage with different CO₂ chamber replacement rates (CRR). The red arrow is the level at which pain occurs. Each of the yellow arrows represents the point of full recumbency (nose down) of the mice at the respective CO₂ CRR.
Evaluation of Low versus High Volume per Minute Displacement CO$_2$ Methods of Euthanasia in the Induction and Duration of Panic-Associated Behavior and Physiology

Debra L. Hickman $^{1,2,3,*}$, Stephanie D. Fitz $^{4}$, Cristian S. Bernabe $^{3,5}$, Izabela F. Caliman $^{5}$, Melissa M. Haulcomb $^{4}$, Lauren M. Federici $^{3,5}$, Anantha Shekhar $^{4,6}$ and Philip L. Johnson $^{3,5}$

Animals 2016, 6, 45; doi:10.3390/ani6080045

- Compared 10%, 30% and 100% displacement rates of chamber using rats
- 10% prolonged the panicogenic responses
- Higher flow volume increased agitation
Hickman - Prolonged Gasping

![Graph showing mean duration of gasping and comparison with different conditions over time.](image-url)
Hickman - Running Behavior

b. Mean Duration Running+/SEM

- Atm. Air VD
- 10% CO2 VD
- 30% CO2 VD
- 100% CO2 VD

* * vs. 100% CO2 VD
* + vs. baseline

onset VD

Time (sec)

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Laboratory Animals
Rabbits – Acceptable with Conditions

- Non-penetrating captive bolt
- CO₂ (50-60% volume change/min)
- Cervical dislocation
- Blunt force trauma – only for emergencies and when lacking resources
Efficacy of Blunt Force Trauma, a Novel Mechanical Cervical Dislocation Device, and a Non-Penetrating Captive Bolt Device for On-Farm Euthanasia of Pre-Weaned Kits, Growers, and Adult Commercial Meat Rabbits

Jessica L. Walsh, Aaron Percival and Patricia V. Turner
Animals 2017, 7, 100; doi:10.3390/ani7120100

- Historically blunt force trauma (BFT) most common on-farm method
- Discontinued in some abattoirs due to concerns about efficacy and operator fatigue
- Study evaluated 3 methods in 3 sizes of rabbits
- Insensibility: absence of brainstem and spinal reflexes, rhythmic breathing, and vocalizations
- Pathological evaluation of the degree of induced brain damage
Use non-dominant hand to restrain rabbit

Middle of forehead
Behind the eyes
Right in front of ears
A

B

Rabbit's head facing away from operator

Hands on either side of rabbit's hips, grasping skin and legs

45°
Efficacy of Blunt Force Trauma, a Novel Mechanical Cervical Dislocation Device, and a Non-Penetrating Captive Bolt Device for On-Farm Euthanasia of Pre-Weaned Kits, Growers, and Adult Commercial Meat Rabbits

Jessica L. Walsh, Aaron Percival, and Patricia V. Turner

<table>
<thead>
<tr>
<th>Method</th>
<th>Number</th>
<th>Successfully Euthanized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blunt Force Trauma (BFT)</td>
<td>58</td>
<td>78%</td>
</tr>
<tr>
<td>Non-Penetrating Captive Bolt (NPCB)</td>
<td>63</td>
<td>100%</td>
</tr>
<tr>
<td>Mechanical Cervical Dislocation (MCD)</td>
<td>49</td>
<td>94%</td>
</tr>
</tbody>
</table>
Euthanasia of meat rabbits with carbon dioxide: Behavioral and physiologic responses to gas chamber gradual- and fast-fill rates

Jessica L. Walsh, John Van de Vegte, Brianne Mercer, Patricia V. Turner

A=Increased Respiration
B=Loss of Righting Reflex
C=Loss of Corneal Reflex
D=Last Breath
F=Gas Off

28% volume displacement per minute
58% volume displacement per minute

Canadian Veterinary Journal 2019 60(7):770-778
On Farm CO₂ Euthanasia Chamber
Other Changes

2020 Edition

Livestock

• CO$_2$ Acceptable with Conditions for neonatal kid goats (< 3wk)

• Updated images for bovids to match the Guidelines for Humane Slaughter of Animals

• Updated images for small ruminants to match the Guidelines for Humane Slaughter of Animals

• New images for proper positioning for captive bolt use (llama, alpaca, deer, water buffalo, bison, chicken, turkey)

• Bovine poll shot not to be used as a primary method, as necessary by trained personnel as required for reasons of accessibility or safety

• Puntilla of camelids - Unacceptable
Selected Changes

2020 Edition

Avian Eggs
• Destruction by cooling or freezing must occur prior to 80% of incubation

Poultry
• Clarify CO₂ flow rate standards for laboratory rodents and rabbits do not apply to poultry or swine

Equine
• Anesthetized horses can be euthanized with
  ➢ 2% lidocaine 60 ml injected intrathecal
  ➢ KCl - IV/IC
  ➢ MgSO4 - IV
Selected Draft Changes

2018 Interim Update

Aquatics

• Addition of exsanguination as a secondary step
• Categorization of MS222 unchanged/acceptable
  ➢ Immersion time extended from 10 to 30 mins
• Metomidate would be considered acceptable if index status changes
• Information added about aversion and potential need for secondary step in some cases
Selected Draft Changes

2018 Interim Update

Unchanged at this time - Unacceptable
• Thoracic compression
• Rapid freezing of reptiles
• Drowning

Not added at this time
• Cranial injection of ethanol for ducks or other poultry
• Use of lidocaine with IP barbiturate
Comparison of intraosseous pentobarbital administration and thoracic compression for euthanasia of anesthetized sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*)

Paul-Murphy JR; et. al. American Journal of Veterinary Research, 2017;78(8)887-889

Gross pathological changes:

“Among birds euthanized by TC [thoracic compression], 9 of 10 sparrows and 5 of 7 starlings had grossly visible coelomic, pericardial, or perihepatic hemorrhage.”
AVMA Panel at Work

Next full update due ~2023
THANK YOU

Special Thanks to Drs. Emily Patterson-Kane, Dr. Cia Johnson, and the AVMA staff
Question 1:

The Guidelines address how to conduct the most humane methods of euthanasia for animals, but what about the people who perform such procedures?

Compassion fatigue is an important issue. Is there any guidance available on mitigating the impact of end-of-life decisions and euthanasia on care staff?
The AVMA recognizes this need and has initiated a Working Group under the oversight of the Steering Committee on Human Animal Interactions. They welcome a representative from OLAW.
Question 2:

Why were data from other mammalian species used to conclude that rodent fetuses are unconscious in utero? Rodents differ from humans and other vertebrates in some very significant ways. What data show that results from research in other species are applicable?
The Guidelines reference Dr. Mellor’s work from the Massey University in New Zealand. He has published many papers on mammalian development in many species. He describes that the general pattern of neurological development appears to be similar across most mammals, irrespective of when the capacities for sentience and conscious perception first appear in relation to the timing of birth.

The Panel on Euthanasia agreed with Mellor and, based on work in other mammalian species, concluded that rodent fetuses are likely to be unconscious in utero and that hypoxia, thereby, would not evoke a response.
Question 3:

On page 76, section 3.3.3 of the 2020 Guidelines, the correct application of manually applied blunt force trauma is categorized as an acceptable means of euthanasia for suckling pigs. If this is the case, why does the AVMA recommend actively searching for alternatives to this method?
The panel made this recommendation because they recognize that individuals performing blunt force trauma must be well trained and must not become physically fatigued such that performance of the technique is negatively impacted.

Further, manually applied blunt force trauma is aesthetically displeasing and potentially distressing to individuals asked to perform the technique. Psychological impacts on individuals performing the euthanasia technique and general societal acceptance are a few of the many variables that must be weighed when deliberating the choice of a euthanasia method.
Some literature strongly suggests that rapid freezing would meet the definition of euthanasia for amphibians and reptiles. In section 7.3.7, why does the AVMA limit rapid freezing to amphibians and reptiles < 4 g (0.1 oz) and require a secondary method?
Question 4: Answer

The AVMA continues to support the designation of hypothermia or freezing of amphibians and reptiles as unacceptable in animals > 4 g in weight. Rapid freezing should only be used for amphibians and reptiles < 4 g in weight and a secondary method should be used to ensure death has occurred and is irreversible.

This method is based on rodent models and likely will work for ectothermic vertebrates that fall within in this weight range. However, the use of hypothermia/freezing as a euthanasia method for these species lacks the appropriate scientific literature support to document that it meets the criteria set forth in the Guidelines for the Euthanasia of Animals: 2020 Edition.
The 2020 Guidelines specify a temperature range for rapid chilling of zebrafish from 2-4°C. However, a 2018 study by Wallace et al. suggests a range of 0-4°C may be more appropriate. Has the Panel considered the information in this publication when revising the Guidelines?

The Panel on Euthanasia thanks the commenter for bringing this additional publication to the Panel’s attention and will consider its content during the next update of the Guidelines.
Some research projects require specific methods of euthanasia that are not acceptable under the AVMA Guidelines but are necessary to produce valid scientific results. How can investigators and IACUCs address this issue while maintaining compliance?
Methods of euthanasia that do not follow the most current version of the AVMA Guidelines for the Euthanasia of Animals may be acceptable if there is:

- Scientific justification
- IACUC review and approval
Thank you!
Next OLAW Online Seminar: Topic to be Determined

OLAW Online Seminar
December 10, 2020