Responses Received to NIH Guide Notice NOT-OD-20-069

[Note: As noted in the Request for Information instructions, personally identifiable information have been removed by NIH prior to making comments publicly available.]

Responses received from organizations

American Physiological Society: (see attached letter)

American Society of Mammalogists: (see attached letter)

Federation of American Societies for Experimental Biology: (see attached letter)

Ornithological Council: (see attached letter)

Responses received from individuals

Response 1 (also see attached letter)

1. **Part I – Introduction and General Comments**
   (Submitter left answer blank)
2. **Part II – Methods of Euthanasia: M1. Inhaled Agents**
   (Submitter left answer blank)
3. **Part II – Methods of Euthanasia: M2. Noninhaled Agents**
   (Submitter left answer blank)
4. **Part II – Methods of Euthanasia: M3. Physical Methods:** Please see the attached letter concerning thoracic compression, specifically as it relates to euthanasia of small birds. The following is my response to OLAW's request for information concerning the implementation of the updated (2020) AVMA Guidelines for the Euthanasia of Animals. My comments are directed specifically at the issue of rapid cardiac compression (thoracic compression) for euthanasia of avian passerine species.
5. **Part III – Methods of Euthanasia by Species and Environment: S2. Laboratory Animals**
   (Submitter left answer blank)
6. **Part III – Methods of Euthanasia by Species and Environment: S5. Avians**
   (Submitter left answer blank)
7. **Part III – Methods of Euthanasia by Species and Environment: S6. Fish and Aquatic Invertebrates**
   (Submitter left answer blank)
8. **General Comments – All Other Parts**
   (Submitter left answer blank)

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Response 2

1. **Part I – Introduction and General Comments**
   (Submitter left answer blank)
2. **Part II – Methods of Euthanasia: M1. Inhaled Agents**

(Submitter left answer blank)

3. **Part II – Methods of Euthanasia: M2. Noninhaled Agents**
   (Submitter left answer blank)
4. **Part II – Methods of Euthanasia: M3. Physical Methods**
   (Submitter left answer blank)
5. **Part III – Methods of Euthanasia by Species and Environment: S2. Laboratory Animals:** On page 64, S2.5 Laboratory Fish, Amphibians, and Reptiles, the AVMA states the following: "As described in the aquatics section it is acceptable for zebrafish (Danio rerio) to be euthanized by rapid chilling (2° to 4°C) until loss of orientation and cessation of operculur movements. Subsequent additional exposure of the fish to chilled water for times specific to fish size and age." In reality, it is very difficult to maintain an ice water bath with a temperature not falling below 2 degrees centigrade. To do so, would require a heat source for the ice bath such as a costly circulator. The method described in this paper is more reasonable and sensible. Wallace CK, Bright LA, Marx JO, Andersen RP, Mullins MC, Carty AJ. Effectiveness of Rapid Cooling as a Method of Euthanasia for Young Zebrafish (Danio rerio). J Am Assoc Lab Anim Sci. 2018;57(1):58–63. Here the paper states 0° to 4°C with mentioning the need for a barrier between the fish and the ice such as a tea strainer. Please change the AVMA Guidelines to mention 0° to 4°C.
6. **Part III – Methods of Euthanasia by Species and Environment: S5. Avians**
   (Submitter left answer blank)
7. **Part III – Methods of Euthanasia by Species and Environment: S6. Fish and Aquatic Invertebrates**
   (Submitter left answer blank)
8. **General Comments – All Other Parts**
   (Submitter left answer blank)

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Response 3

1. **Part I – Introduction and General Comments**
   I think this updated guidance is very helpful and will improve animal welfare. thanks.
2. **Part II – Methods of Euthanasia: M1. Inhaled Agents**
   Thanks for the improved guidance in this section
3. **Part II – Methods of Euthanasia: M2. Noninhaled Agents**
   (Submitter left answer blank)
4. **Part II – Methods of Euthanasia: M3. Physical Methods**
   (Submitter left answer blank)
5. **Part III – Methods of Euthanasia by Species and Environment: S2. Laboratory Animals**
   Thank you for the improved guidance in this section.
6. **Part III – Methods of Euthanasia by Species and Environment: S5. Avians**
   (Submitter left answer blank)
7. **Part III – Methods of Euthanasia by Species and Environment: S6. Fish and Aquatic Invertebrates**
   (Submitter left answer blank)
8. **General Comments – All Other Parts** (Submitter left answer blank)

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Response 4

1. **Part I – Introduction and General Comments**
   I think this updated guidance is very helpful and will improve animal welfare. thanks.
2. **Part II – Methods of Euthanasia: M1. Inhaled Agents**
   Thanks for the improved guidance in this section
3. **Part II – Methods of Euthanasia: M2. Noninhaled Agents**
   (Submitter left answer blank)
4. **Part II – Methods of Euthanasia: M3. Physical Methods:** This document ignores comparative scientific literature and sound arguments based in physiology with respect to cooling and freezing, which is more humane than some of the drug usages proposed. Please see the following articles, especially the first-listed. LILLYWHITE, HARVEY B., RICHARD SHINE, ELLIOTT JACOBSON, DALE DENARDO, MALCOLM S. GORDON, CARLOS A. NAVAS, TOBIAS WANG, ROGER S. SEYMOUR, KENNETH B. STOREY, HAROLD HEATWOLE, DARRYL HEARD, BAYARD BRATTSTROM, and GORDON M. BURGHARDT. 2017. Anesthesia and euthanasia of amphibians and reptiles used in scientific research: Should hypothermia and freezing be prohibited? BioScience 67:53–61. SHINE, R., J.A. LESKU, & H.B. LILLYWHITE. 2019. Available evidence shows that cooling-then-freezing is a humane method of euthanasia for ectothermic animals. Journal of the American Veterinary Medical Association 255:48–50. SHINE, R, AMIEL J, MUNN AJ, STEWART M, VYSTOTTSKI AL, LESKU JA. 2015. Is “cooling then freezing” a humane way to kill amphibians and reptiles? Biology Open 00, 1–4 doi:10.1242/bio.012179.
5. **Part III – Methods of Euthanasia by Species and Environment: S2. Laboratory Animals**
   Thank you for the improved guidance in this section.
6. **Part III – Methods of Euthanasia by Species and Environment: S5. Avians**
   (Submitter left answer blank)
7. **Part III – Methods of Euthanasia by Species and Environment: S6. Fish and Aquatic Invertebrates**
   (Submitter left answer blank)
8. **General Comments – All Other Parts**
   (Submitter left answer blank)

Patricia A. Brown, V.M.D., M.S. April 29, 2020
Director
Office of Laboratory Animal Welfare
National Institutes of Health
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6705 Rockledge Dr.
Bethesda, MD 20892-7982

Dear Dr. Brown,

The following is my response to OLAW's request for information concerning the implementation of the updated (2020) AVMA Guidelines for the Euthanasia of Animals. My comments are directed specifically at the issue of rapid cardiac compression (thoracic compression) for euthanasia of avian passerine species.

In 2001, in response to the 2000 Report of the AVMA Panel on Euthanasia, I wrote a letter (ref #1 below) to the Journal of the American Veterinary Medical Association concerning the use of thoracic compression for euthanizing birds. The essential message of that letter, based on my understanding of the technique at that time, was that it produced death in birds by suffocation, a method of killing that is neither humane nor euthanasia. However, based on two reports published in 2017 & 2018 by Professor Joanne Paul-Murphy and colleagues at the University of California-Davis (2 & 3), studies in which I was not involved, I am personally compelled to retract my 2001 statement.

Professor Paul-Murphy et al's study describes euthanasia of sparrows and starlings either by thoracic compression or intraosseous pentobarbital treatment (IPT); the results clearly and convincingly show that correctly performed thoracic compression results in humane euthanasia of passerine birds. The authors also clearly demonstrate that a more accurate term for this technique is rapid cardiac compression (RCC). An important element for humane euthanasia is time: the time it takes to achieve key end-points, such as cessation of pulse, loss of consciousness, or isoelectric EEG. The Paul-Murphy et al data show that key end-points are quickly achieved when birds are euthanized by RCC. Importantly, these key time end-points are similar to those for birds euthanized by IPT.

As a veterinarian who has euthanized animals, including birds, I have considered an overdose of pentobarbital to be a gold standard for humane euthanasia; when used correctly it is fast, painless and effective. A technique that produces euthanasia within the same time frame as does pentobarbital and seemingly without stress to birds, should be considered a humane technique for euthanizing birds

The recent AVMA Panel on Euthanasia does not recognize rapid cardiac compression as acceptable for euthanizing birds. In my opinion the panel members failed in their task to develop science-based guidelines concerning euthanasia of birds. In section M3.12 Thoracic (Cardiopulmonary, Cardiac) Compression, page 43 of the 2020 Guidelines, the panel members indicate that cardiac compression has been used in mammals and birds and in support cite both of Dr. Murphy's articles (2 & 3). In neither article do the authors state that cardiac compression has been used in mammals; their articles focus only on birds! The panel members also state that "data supporting this method are limited," when in fact they were aware of the articles by Drs. Paul-Murphy and Engilis (2 & 3). The reason such data are limited is that there are few of these types of studies; they are extremely difficult to perform. The size of the birds, their unique anatomy, the difficulty of instrumenting them so as to acquire meaningful data can be daunting challenges. Having read the articles by Dr. Paul-Murphy and her colleagues, it is obvious to me that they successfully completed this very challenging study. In my mind they answered and resolved the question, is correctly performed rapid cardiac compression a humane techniques for euthanizing passerine birds in field setting? The answer is clearly 'yes'!

I urge the Office of Laboratory Animal Welfare to consider rapid cardiac compression as a humane technique for euthanizing birds, especially as an acceptable technique for euthanasia of birds in field research settings.

[Name redacted]

References

1. Ludders JW. Another reader opposing thoracic compression for avian euthanasia (lett). Journal of the American Veterinary Medical Association 2001; 218:1721.
2. Paul-Murphy JR et al. Comparison of intraosseous pentobarbital administration and thoracic compression for euthanasia of anesthetized sparrows (Passer domesticus) and starlings (Sturnus vulgaris). American Journal of Veterinary Research 2017; 78:887–899.
3. Engilis A, Paul-Murphy J. Rapid cardiac compression: An effective method of avian euthanasia. The Condor 2018; 120: 617.

April 29, 2020

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Re: NOT-OD-20-069: NIH Request for Information: Implementation of the Updated AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

Submitted electronically via portal and e-mail

Dear Dr. Brown,

The American Physiological Society (APS) appreciates the opportunity to provide comments to OLAW on implementation of the 2020 Edition of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals ("Guidelines").

Comments on the specific topics in the web form are provided below.

Comment Box 1 – Introduction and General Comments

Since 1963, the AVMA has developed Guidelines to "evaluate methods and potential methods of euthanasia for veterinarians who carry out or oversee the euthanasia of animals" (I2.1, p. 4). The first edition addressed only dogs, cats, and other small mammals, but subsequent editions were expanded to include other species and to address specialized settings, e.g., research animals, wildlife, food animals, etc. The Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) requires IACUCs to ensure that for covered activities, the "[m]ethods of euthanasia used will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia, unless a deviation is justified for scientific reasons in writing by the investigator." The PHS Policy's exclusive reliance upon these Guidelines reflects the belief that they are the definitive information source on euthanasia methods.

In the Preface to the new edition, the POE wrote that it "made every effort to identify and apply the best research and empirical information available" (I1, p. 4). It went on to say that "[a]s new research is conducted and more practical experience gained, recommended methods of euthanasia may change" (I1, p. 4). That is to be expected over time, but it means that the requirement for investigators to provide scientific justification for the use of euthanasia methods inconsistent with the Guidelines will become increasingly burdensome when there are long gaps between updates.

Our review of the Guidelines raised two broad concerns:

1. Are the AVMA Guidelines based upon sufficient information about common lab animal species?
2. Do the Guidelines provide adequate guidance for the broad range of situations that may arise in the diverse kinds of research that are subject to the PHS Policy?

With respect to common species of lab animals, even a cursory review allowed APS to identify an instance where the literature cited for a recommendation did not actually involve the species that was addressed. Specifically, the paper supporting the conclusion that rodent fetuses are "unconscious in utero and hypoxia does not evoke a response" was based upon recordings made in fetal sheep (S2.2.4.1, p. 62). [Mellor, D et al. (2005). The importance of 'awareness' for understanding fetal pain. Brain Research Reviews 2005; Volume 49, Issue 3, Pages 455-471] Such recommendations would be greatly strengthened if studies involving animals of the species in question were also cited.

With respect to providing information about diverse kinds of research, The *Guide for the Care and Use of Laboratory Animals* has devised a simple approach to grapple with this. Concerning field investigations, the *Guide* states that it "does not purport to be a compendium of all information regarding field biology and methods used in wildlife investigations." [National Research Council. 2011. Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/12910, p. 32] For that reason, the *Guide* encourages investigators to "consult with relevant professional societies and available guidelines." [*Guide,* p. 32]. Examples of such references include the *Guidelines for the Euthanasia for Rodent Fetuses and Neonates* developed by NIH's intramural program and recommendations by groups such as the American Society of Mammologists, American Ornithological Society, and American Society of Ichthyologists and Herpetologists. APS recommends that OLAW permit investigators to reference these and other relevant euthanasia guidelines without requiring them to provide further scientific justification.

APS also notes that the Guidelines provide little information about situations that may arise in the extraordinarily diverse kinds of research that are subject to the PHS policy, e.g. work funded by NSF, VA, and/or NASA. Since OLAW is currently looking for ways to reduce regulatory burden for scientists and IACUCs per the 21st Century Cures Act, APS encourages OLAW to collaborate with NSF, NASA, AVMA, USDA, and others to compile a list of recognized guidelines published by scientific societies such as those previously cited or developed by NIH's intramural program. This list would help scientists identify reputable references and greatly reduce the burden on IACUCs reviewing their protocols.

Comment Box 2: Part II – Methods of Euthanasia M2. Inhaled Agents

APS supports the recommendation to increase the displacement rate for carbon dioxide (CO_2) euthanasia for rodents from 10%-30% to 30%-70%. This recommendation comports with recent research findings showing a reduction in the potential pain and distress in rodents euthanized with higher flow rate, as noted below.

Hickman, D (2019). Wellbeing of Alcohol-preferring Rats Euthanized with Carbon Dioxide at Very Low and Low Volume Displacement Rates. J Am Assoc Lab Anim Sci. 2019; 58(1): 78-82
Moody CM, Chua B, and Weary DM (2014). The effect of carbon dioxide flow rate on the euthanasia of laboratory mice. Laboratory Animals. 2014; 48(4): 298 – 304
Boivin GP et al. (2017). Review of CO_2 as a Euthanasia Agent for Laboratory Rats and Mice. J Am Assoc Lab Anim Sci. 2017;56(5):491–499

Comment 4: Part II – Methods of Euthanasia M3. Physical Methods

Thoracic (cardiopulmonary, cardiac) compression is used extensively in field research. It is sometimes also needed in the laboratory settings when small birds and mammals urgently require euthanasia and the equipment or supplies needed for another method are not immediately available. The updated Guidelines classify thoracic compression as unacceptable for a primary euthanasia method: "Although it has been used extensively in the field, data supporting this method are limited, including the degree of distress induced and time to unconsciousness or death" [M3.12, p. 47]. According to the POE, "The consensus of veterinarians with field biology training and expertise is that portable equipment and alternate methods are currently available" and are "generally practical to use with minimal training and preparation" [M3.12, p. 47].

In its discussion, the POE mentioned a 2017 study by J.R. Paul-Murphy et al which concluded that thoracic compression effectively causes rapid cardiac arrest and "might be an efficient euthanasia method for small birds." Although the POE did not accept this conclusion, it is our understanding that many wildlife biologists view thoracic compression as the most humane and practical option in field settings. [Paul-Murphy JR et al. (2017). Comparison of intraosseous pentobarbital administration and thoracic compression for euthanasia of anesthetized sparrows (Passer domesticus) and starlings (Sturnus vulgaris). Am J Vet Res. 2017; 78(8): 887-899. https://www.ncbi.nlm.nih.gov/pubmed/28738007]

APS has no direct expertise in this area, but we encourage OLAW to heed input from stakeholders such as the American Society of Mammologists and the Ornithological Society with respect to thoracic compression in field research.

Comment box 5: Part III – Methods of Euthanasia by Species and Environment: S2. Laboratory Animals

The Society appreciates the Panel's clarification concerning euthanasia for laboratory rodents with altricial young (e.g., mice and rats) versus rodents with precocial young (e.g., guinea pigs) (S2.4.1, p. 62). At the same time, we are concerned about contradictory language regarding suckling pigs (S3.3.3.2.2, p. 76). This section first asserts that when manually-applied blunt force trauma is performed correctly, it "meets the definition of euthanasia." However, it also encourages those who rely on this method "to actively seek alternatives to ensure that criteria for euthanasia can be consistently met." It is unclear why it is necessary to "actively seek alternatives" if those who will perform the euthanasia have been properly trained. This contradictory language will impose additional burdens on researchers and the IACUCs that review their protocols. To avoid this confusion and hopefully also reduce regulatory burden, the Society calls upon OLAW to provide clear instruction on how to interpret and implement this section.

As noted above, we are also concerned that a study with fetal sheep was cited to justify a recommendation for the euthanasia of rodent fetuses. This is described in greater detail in Comment Box 1.

Comment Box 7: Part III – Methods of Euthanasia by Species and Environment: S5. Avians

Please see our comments about the acceptability of thoracic compression in field studies involving and birds in Box 4, above.

Comment Box 8: General Comments – All Other Parts

The Society appreciates the distinction the Guidelines made between sedation and anesthesia (I5.6, p. 15).

APS is grateful for the opportunity to provide feedback on this RFI. We thank OLAW and the AVMA Panel on Euthanasia for their engagement with the scientific community on this important issue. We encourage timely communication with stakeholders once decisions are made on the Guidelines to clarify expectations.

Sincerely,

[Name redacted]

American Society of Mammalogists

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27 April 2020

National Institutes of Health
Office of Extramural Research
RE: Comments on the updated (2020) AVMA Guidelines for the Euthanasia of Animals.

To Whom It May Concern,

The American Society of Mammalogists (ASM) appreciates the opportunity to comment on the NIH Office of Laboratory Animal Welfare's (OLAW's) planned implementation of the 2020 version of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals. Although these guidelines are useful when applied to animals in captivity, the ASM believes that they are of limited use when applied to free-ranging wildlife and, thus, for wildlife research in general. We argue that for such investigations, both investigators and oversight bodies should rely on the various peer-reviewed, taxon-specific guidelines produced by professional societies that were developed specifically at the recommendation of the National Science Foundation to fill the gap between biomedical and wildlife settings (Orlans 1988). Specific issues regarding application of the AVMA guidelines to free-ranging wildlife in field settings include:

PART I – Introduction and General Comments

- The AVMA guidelines state that "*[the Guidelines were designed for use by members of the veterinary profession who carry out or oversee the euthanasia of animals.*"[P.5:12.3] Veterinarians seldom accompany investigators into the field, and wildlife researchers are rarely overseen by a veterinarian. Instead, activities by field researchers typically occur under the oversight of an Institutional Animal Care and Use Committee (IACUC) or similar oversight body of which the veterinarian is a member.

- The AVMA guidelines acknowledge that: "*... the quickest and most humane means of terminating the life of free-ranging wildlife in a given situation may not always meet all criteria established for euthanasia (ie, distinguishes between euthanasia and methods that are more accurately characterized as humane killing).”*[P.97:S7.6.1] However, the AVMA guidelines expressly *“are not intended to address slaughter, depopulation, or other killing methods.”*[P.82:S6.1.1] Hence, the AVMA guidelines simply do not apply to most situations involving free-ranging wildlife, while noting that *“the best methods possibly under the circumstances must be applied.”*[P. 97, S7.6.1]

- The model Veterinary Practice Act endorsed by the AVMA as of August 2019 (https://www.avma.org/policies/model-veterinary-practice-act), with regard to establishment of a VCPR states:
  Section 5 – Veterinarian-Client-Patient Relationship Requirement 1. No person may practice veterinary medicine in the State except within the context of a Veterinarian-Client-Patient Relationship (VCPR). A Veterinarian-Client-Patient Relationship (VCPR) cannot be established solely by telephonic or other electronic means. Without a VCPR, any advice provided through electronic means shall be general and not specific to a patient, diagnosis or treatment. Veterinary telemedicine shall only be conducted within an existing VCPR, with the exception for advice given in an emergency until that patient can be seen by a licensed veterinarian.

  The Veterinary Practice Act in effect for most states does not recognize establishment of a VCPR without examination of an animal by the veterinarian, even for wildlife. As a consequence, no VCPR can be established. This reality further removes the veterinarian from being able to provide oversight regarding euthanasia of wildlife in field settings.

- Although the AVMA guidelines recognize many controlled substances as approved for euthanasia, use of these compounds requires DEA licensing. The Veterinary Mobility Act of 2014 permits veterinarians, but not other registrants, to use controlled substances away from the location specified in the license. Thus, use by investigators in field settings who are not also veterinarians or who do not have a veterinarian present is technically illegal.

S7.6.3.2.2 Physical methods

- With regard to euthanasia by gunshot, the AVMA guidelines state:
  “Gunshot is acceptable with conditions for euthanasia of free-ranging, captured, or confined wildlife, provided that bullet placement is to the head (targeted to destroy the brain).” The guidelines recognize gunshot as acceptable for a variety of species including

free-ranging birds (S 7.6, also Appendix I) and wild mammals; however, the AVMA guidelines also specify that the firearm and ammunition selected *"must achieve a muzzle energy of at least 300 feet-lb (407 J) for animals weighing up to 400 lb (180 kg). For animals larger than 400 lb, 1,000 feet-lb (1,356 J) is required.* (M 3.5.2, pg 43).
As noted elsewhere (Sikes et al. 2016), this energy level would require a cartridge on the order of a .357 magnum to euthanize any animal up to 400 lbs (180 kg). This power is grossly inappropriate for smaller mammals, as is targeting of the brain because skulls of wild mammals are commonly saved as voucher specimens and for both teaching and research collections. Equally important, it is not possible to meet the AVMA requirements for euthanasia using any cartridge that fires multiple projectiles (i.e., shotguns), a concern that applies to any flying or running bird or mammal, because the multiple projectiles could never be aimed to ensure passage through the brain nor could they ever deliver the energy specified.

Although most of the foregoing points regarding lack of applicability to free-ranging wildlife are addressed in the *2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education* (Sikes et al. 2016), the 2020 revision of the AVMA guidelines for euthanasia continue to reference the older (Sikes et al. 2011) version of the ASM guidelines for euthanasia of wild mammals.

For the reasons detailed here, the American Society of Mammalogists feels that application of the AVMA Guidelines for the Euthanasia of Animals to free-ranging animals is frequently inappropriate and even contrary to humane dispatch of individual animals. As a result, we argue that decisions as to whether and under what conditions these guidelines are applied to wildlife should be left to the discretion of the IACUC or similar oversight body. Our intent is not to question the logic or rationale of the AVMA guidelines as applied to traditional veterinary practice and to research animals (including captive wildlife) at institutions, but to emphasize the lack of relevance and therefore utility when applied to free-ranging wildlife. Under such conditions, more relevant guidance can be found in the taxon-specific guidelines that were developed at the urging of the National Science Foundation.

Sincerely,

[Name redacted]

References

Orlans, F. B. (ed.). 1988. Field research guidelines: impact on Animal Care and Use Committees. Scientists Center for Animal Welfare, Bethesda, Maryland.

Sikes, R. S., W. L. Gannon, and the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy **92**:235-253.

Sikes, R. S. and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy **97**:663–688.

FASEB
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Representing Over 130,000 Researchers

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April 7, 2020

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RE: NIH Request for Information: Implementation of the Updated AVMA Guidelines for the Euthanasia of Animals: 2020 Edition [FR Doc. 2020-03607 and NOT-OD-20-069]

Submitted electronically via portal and via e-mail: olaw@od.nih.gov

Dear Dr. Brown,

The Federation of American Societies for Experimental Biology (FASEB) appreciates the opportunity to provide comments on the Request for Information (RFI) (NOT-OD-20-069) seeking input on the implementation of the updated American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2020 Edition ("Guidelines"). As one of the primary issues leading to the establishment of FASEB, humane care and use of animals in biological and biomedical research remains a fundamental priority of the Federation and its 28 member societies.

FASEB commends AVMA's efforts to engage with the research community in drafting the updates to the Guidelines. As implementation proceeds, we encourage both OLAW and AVMA to preserve strong stakeholder participation to ensure the process is both transparent and inclusive of multiple research perspectives. Sustained engagement with the community is vital for the promotion and protection of laboratory animal care and welfare.

Comments on specific sections of the Guidelines are noted below.

Part I – Introduction and General Comments

In reviewing the Introduction and General Comments section, we particularly appreciated the discussion on the human-animal relationship *(Section 15.5 – Human Behavior)*. While the Guidelines recognize that owners, veterinarians, and animal care staff can be psychologically affected when performing or observing euthanasia, the topic of compassion fatigue was only mentioned once, with minimal guidance for ways institutions and veterinary clinics can strengthen support for those working with laboratory animals. Given AVMA's exceptional leadership on this issue, FASEB encourages OLAW to coordinate with AVMA and other laboratory animal organizations to include language in the Guidelines that will facilitate institutions' ability to identify and mitigate the risks of compassion fatigue and euthanasia stress for personnel. For example, among the numerous resources AVMA offers for personal and professional wellbeing are lists of individual and organizational symptoms of compassion fatigue. Broader distribution of such educational resources will increase awareness and encourage institutions to be proactive within their respective animal programs.

Part II – Methods of Euthanasia: M1. Inhaled Agents

FASEB applauds the change in displacement rate for carbon dioxide (CO_2) euthanasia for rodents from [10% - 30%] to [30% to 70%], consistent with recent research findings that have shown higher flow rates to reduce potential pain and distress for rodents[1,2,3]. The purpose of euthanasia is to minimize potential pain and discomfort for laboratory animals, and we appreciate the Panel's commitment to this aim by integrating evidence-based guidelines.

Part II – Methods of Euthanasia M3. Physical Methods

Thoracic (cardiopulmonary, cardiac) compression is extensively used in field research as well as university laboratories that study wild small mammals and birds. Frequently, this method is the most humane alternative available in certain research scenarios. While we recognize the Guidelines seek to maintain a precautionary approach towards euthanasia methods, we disagree with the language that states, *"…data supporting this method are limited, including degree of distress induced and time to unconsciousness or death" (Section M3.12, pg. 47)* as this is inconsistent with recent literature. For example, a study published in 2017 suggested that thoracic compression is an efficient euthanasia method for small birds, as it effectively obstructs venous return, subsequently causing rapid circulatory arrest[4].

The Guidelines are also in direct conflict with previous AVMA policy statements on this issue. A 2011 factsheet referenced on OLAW's website, "Welfare Implications of Thoracic Compression," concludes that while not an acceptable method of euthanasia, thoracic compression *"…should not be prohibited where its use is necessary to minimize animal suffering or is scientifically justified (such as under the oversight of an Institutional Animal Care and Use Committee (IACUC))."* Contradictory language between the Guidelines and previous AVMA statements obscures the role of IACUC in seeking compliance with both the Guide and AVMA Guidelines.

Therefore, FASEB recommends revising this language in the Guidelines to reflect recent publications currently informing wildlife protocols at numerous institutions, as well as AVMA and OLAW policy. Additionally, OLAW may want to reach out to stakeholders with specific expertise in wildlife and avian research, such as the Ornithological Society and American Society of Mammologists, to provide input on this language.

[1] Hickman, D (2019). Wellbeing of Alcohol-preferring Rats Euthanized with Carbon Dioxide at Very Low and Low Volume Displacement Rates. *J Am Assoc Lab Anim Sci*. 2019; 58(1): 78-82.
[2] Moody CM, Chua B, and Weary DM (2014). The effect of carbon dioxide flow rate on the euthanasia of laboratory mice. *Laboratory Animals*. 2014; 48(4): 298 – 304.
[3] Boivin GP et al. (2017). Review of CO_2 as a Euthanasia Agent for Laboratory Rats and Mice. *J Am Assoc Lab Anim Sci*. 2017;56(5):491–499.
[4] Paul-Murphy JR et al. (2017). Comparison of intraosseous pentobarbital administration and thoracic compression for euthanasia of anesthetized sparrows (Passer domesticus) and starlings (Sturnus vulgaris). *Am J Vet Res*. 2017; 78(8): 887-899.

Part III – Methods of Euthanasia by Species and Environment: S2. Laboratory animals

FASEB appreciates the clarification in euthanasia procedures for laboratory rodent with altricial young (e.g., mice and rats) versus rodents bearing precocial young (e.g., guinea pigs) in *Section 2.2.4.1, Fetuses and Neonates – Acceptable Methods (pg. 62)*. While we acknowledge AVMA's utilization of scientific literature to inform these updates, in many instances, the evidence cited does not align with the laboratory animal being discussed. For example, the conclusion that rodent fetuses are *"…unconscious in utero and hypoxia does not evoke a response"* is based on recordings from fetal sheep (Mellor et al., 2005).To ensure researchers and animal care personnel employ guidelines established on comprehensive evidence, we recommend incorporating citations beyond farm animal studies. In particular, we urge the integration of NIH's *Guidelines for the Euthanasia for Rodent Fetuses and Neonates*, a resource that provides detailed, evidence-based guidance for euthanasia procedures with this commonly used species.

Finally, we wish to express concern regarding the contradictory language located in section *S3.3.3 Suckling Pigs – Manually applied blunt force trauma (pg. 76*). The beginning of this section asserts that this method fulfills the definition of euthanasia when performed correctly, yet the paragraph concludes by encouraging researchers and staff to actively seek alternatives *"…to ensure that criteria for euthanasia can be consistently met."* While we recognize and support the search for alternatives as a key component of the 3Rs, inconsistent messaging within the Guidelines may prove counterintuitive to this objective and risks confusion for researchers utilizing this species. Therefore, FASEB recommends clarifying the meaning of "active search for alternatives" or striking this language altogether.

FASEB values the synergistic relationship between animal welfare and biomedical research progress and appreciates the opportunity to provide feedback on this RFI. We thank OLAW and the AVMA Panel on Euthanasia for their engagement with the scientific community on this important issue and encourage timely communication with stakeholders once the policy is finalized to clarify agency expectations and ensure seamless implementation.

Sincerely,

[Name redacted]

The Ornithological Council

PROVIDING SCIENTIFIC INFORMATION ABOUT BIRDS

American Ornithological Society

Association of Field Ornithologists

Birds Caribbean

CIPAMEX (Sociedad para el Estudio y Conservación de las Aves en México)

North American Crane Working Group

Neotropical Ornithological Society

Pacific Seabird Group

Raptor Research Foundation

Society of Canadian Ornithologists/ Société de Ornithologistes du Canada

The Waterbird Society

Wilson Ornithological Society

22 April 2020

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Submitted via grants.nih.gov

Dear Dr. Brown,

Please accept these comments in response to OLAW's announcement (24 February 2020) requesting information on implementation of the updated AVMA Guidelines for the Euthanasia of Animals: 2020 Edition (NOT-OD-20-069). These comments are submitted by the Ornithological Council, a consortium of 11 scientific societies of ornithologists; these societies span the Western Hemisphere and the research conducted by their members spans the globe. Their cumulative expertise comprises the knowledge that is fundamental and essential to science-based bird conservation and management, a critical need given that North American wild bird populations have declined by nearly 30% (Rosenberg et al. 2019). It is essential – now more than ever – that any restrictions imposed on this research be science-based. More germane to these comments is the fact that these scientists have considerably more experience in field biology and methods of euthanasia suitable to their research than does the AVMA, most of the members of the AVMA Panel on Euthanasia, or the consultants who provided input to that panel.

As always, we applaud and thank OLAW for requesting and considering comments on actions that are of critical importance to ornithologists. We know it is a time-consuming process and a burden on the already over-burdened staff. That being said, we hope that the effort is productive, resulting in "departures" from the blanket acceptance of the AVMA Guidelines, where warranted by the staff's independent review of the scientific evidence.

We reiterate what we suggested to OLAW upon the release of the 2013 edition of the AVMA Guidelines for the Euthanasia of Animals.

It is time for OLAW to formally recognize alternate, biologically appropriate standards for wildlife research that is conducted in the field. There is no reason why OLAW must adopt only a single standard, and, in fact, requiring a single standard that is not factually or scientifically suitable for a particular type of research may very well result in less humane treatment of study animals, by limiting the availability of humane methods.

OLAW should recognize that scientific merit and integrity are the touchstones of the validity of the standards it imposes upon its grantees and the grantees of the other agencies that have contracted with OLAW to administer animal welfare programs.

There are basic, objective standards that the OLAW should employ when evaluating the adoption of any standard, be it a standard developed internally or one developed by an outside organization. These include an evaluation of the credentials of the authors, assessment of potential or actual bias of the authors, and independent peer review. The evaluation of any particular method should be based on the best available science. Before OLAW imposes the AVMA Guidelines on researchers, it should undertake at least this basic assessment. We realize that it would be difficult, if not impossible, for the OLAW staff to assess the substantive merits of the AVMA Guidelines, but these basic processes should be reviewed. It is also difficult to understand how OLAW can accept these standards without a review of the scientific accuracy, completeness, and merits. For that reason, an independent peer review is essential.

There is also no reason why OLAW must adopt outside standards in their entirety. Nothing prevents OLAW from "departing" (i.e., making exceptions) when scientifically warranted. OLAW can and should use its own scientific judgment to evaluate scientific information and determine if a different conclusion is warranted.

Applying these precepts to the proposed adoption of the AVMA Guidelines on Euthanasia leads to the following conclusions, discussed in depth, below:

1. By its very terms, the AVMA Guidelines do not apply to most forms of research, including most wildlife biology conducted in the field setting.
2. There are other guidelines pertaining to euthanasia that OLAW should consider and allow grantees to use if basic, objective standards are met and if they are as appropriate, or more appropriate, factually and scientifically, to specific situations. This is the case with regard to ornithological research.
3. The AVMA Guidelines fail to meet the objective, well-recognized principles elucidated above. There is no indication of independent peer review, the credentials of the authors and consultants are not provided, and there is evidence of discipline bias that OLAW should investigate before adopting the AVMA Guidelines. For instance, one method needed by ornithologists — rapid cardiac compression — was determined to be unacceptable and the best available science and views of prominent wildlife veterinarians and highly experienced ornithologists was disregarded, whereas methods used in other disciplines – particularly the biomedical fields - that entail far more pain and distress and are of much longer duration were considered acceptable or acceptable with conditions. There is also evidence of discipline and personal bias.

4. Whether or not the AVMA Guidelines are adopted, OLAW should recognize an exception for the use of rapid cardiac compression (Part III, Section 5. Avians) in wildlife research in the field.

Discussion

By its very terms, the AVMA Guidelines do not apply to some forms of research, including most wildlife biology conducted in the field setting. It is therefore scientifically inappropriate for the Public Health Service (PHS) to recognize these standards as appropriate for compliance with PHS Policy with regard to those types of research.

Specific text in the document makes this clear:

> - The Guidelines set criteria for euthanasia, specify appropriate euthanasia methods and agents, and are intended to assist veterinarians in their exercise of professional judgment. (p.4).
> - The Guidelines are designed for use by members of the veterinary profession who carry out or oversee the euthanasia of animals. (p.5).
> - The POE's objective in creating the Guidelines is to provide guidance for veterinarians about how to prevent and/or relieve the pain and suffering of animals that are to be euthanized. (p.6)

In comparison to biologists, veterinarians relatively rarely conduct wildlife research (field or lab) though they sometimes participate as a cooperating specialist. When they participate, these guidelines would apply to them. These guidelines were not developed for wildlife field researchers who are not veterinarians. Wildlife researchers rarely work in conditions similar to veterinary clinical practice in an office or in agricultural settings and rarely have access to most of the injectable and inhalant drugs used by veterinarians, both for legal reasons (e.g., restricted substances) and practical reasons (e.g., inability to transport materials and equipment to remote field sites or obtain materials and supplies in remote areas).

The AVMA continues to equate euthanasia with the single, limited purpose of bringing about death to end suffering, i.e., that the termination of life is for the benefit of the animal.

> Humane disposition reflects the veterinarian's desire to do what is best for the animal and serves to bring about the best possible outcome for the animal… Euthanasia as a matter of humane disposition occurs when death is a welcome event and continued existence is not an attractive option for the animal as perceived by the owner and veterinarian. (p. 6).

The issue of humane technique arises only after it has been determined that the decision to euthanize (again, which is co-extensive with the good of the animal) has already been made:

> When the decision has been made to euthanize and the goal is to minimize pain, distress, and negative effect to the animal, the humaneness of the technique (i.e, how we bring about the death of animals) is also an important ethical issue. As veterinarians and human beings [*sic*] it is our responsibility to ensure that if an animal's life is to be taken, it is done with the highest degree of respect, and with an emphasis on making the death as painless and distress free as possible. (p.7).

Thus, the AVMA has defined euthanasia in a way that expressly excludes a significant part of both biomedical and wildlife animal research, as well as a number of wildlife management practices. In biomedical research, healthy animals are often killed because they cannot be used in subsequent experiments. In wildlife management, healthy animals are often culled to reduce populations to prevent harm to livestock and crops or to reduce other conflicts with human interests. By the very terms used in these guidelines, this would not constitute euthanasia and would thus violate PHS Policy, no matter how humane the technique. Wildlife research likewise often entails the deliberate take of the life of the animal for research purposes, which is obviously not "what is best for" or the "best possible outcome for" the individual animal.

The AVMA definition would apply to wildlife research only in situations where a study animal was accidentally injured and it would be more humane to end its life than to leave it to suffer and die over a longer time —though the Migratory Bird Treaty Act and the Endangered Species Act prohibit the taking of life for all but three species of birds found in the United States.

As the AVMA Guidelines are, by definition, inapplicable to most taking of animal life in the context of wildlife research, it would be impossible for a wildlife researcher to comply with PHS Policy.

No matter how humane the technique, when a healthy animal is sacrificed for scientific research, it is not, as the AVMA defines euthanasia, an

> … act for the sake of the animal or its interests, because the animal will not be harmed by the loss of life. Instead, there is consensus that the animal will be relieved of an unbearable burden. (p.6)

Although the AVMA Guidelines delve into numerous methods of killing non-domestic or non-pet animals, the entire document is based on a premise that entails the ending of life to relieve suffering.

> "Death, in this case, may be a welcome event and euthanasia helps to bring this about, because the animal's life is not worth living but, rather, is worth avoiding." (p.6)

This is a fine statement with regard to the traditional use of euthanasia by most veterinarians – the ending of a life of a pet or other domestic animal suffering the effects of old age, injury, or illness. It could be extended to the ending of life of animals studied in research if the research resulted in injury or other conditions that cause suffering, but otherwise is not relevant to the purpose of ending the lives of animals studied in field biology, which more often entails the termination of the life of a healthy animal.

It is so essential a point that it bears repeating: the AVMA Guidelines, insofar as they rest on a central concept that limits euthanasia to a taking of life for a limited reason that is almost always irrelevant to wildlife research (or indeed, much biomedical research), are inapplicable and to wildlife biology. No matter how humane the technique, wildlife biologists would not be in compliance with PHS Policy if compliance is based on the AVMA Guidelines.

The termination of the life of animals in most wildlife research would, under the AVMA terms, constitute humane killing. The AVMA distinguishes between euthanasia and humane killing, basing the distinction on the purpose for putting an animal to death. This is a biologically irrelevant distinction. A method is either humane or not, or more or less humane than another method, regardless of the purpose of the killing. It matters not to the animal why it is being put to death. The extent of the fear, pain, or suffering, if any, does not vary with the intent of the human. The measure of whether a method is humane should be the rapidity to loss of consciousness and the extent of pain and suffering prior to that point.

That being said, in the case of wildlife research, the need to minimize the extent and duration of holding time and handling is also important. The AVMA Guidelines are primarily designed for ending the lives of domestic and agricultural animals and pets, most often under circumstances where they can be gently restrained and are not under extreme stress. These animals are accustomed to human presence, touch, and handling; they are not inherently stressed or fearful simply because a human is present or because they are being restrained. These are not the circumstances that prevail when field biologists capture birds. These animals struggle, may painfully injure themselves, and exhibit signs of stress and fear during the several minutes it takes to safely draw up infectible euthanasia drugs. They struggle during penetration of the needle and such struggling may result in more pain and poor injection placement. Similarly, the time required to safely set up a gas anesthesia chamber and to manipulate a bird into it can be measured in minutes and the birds fight and flutter in fear before gas takes effect.

Simply stated, the amount of time to remove a small passerine bird from a mist net and kill it by cardiac compression can be measured in seconds, whereas killing it by injection or inhalants takes a number of minutes, all the while the bird is fearful, stressed and often struggling. If time from capture to death, rather than time from application of the method to cessation of consciousness is considered, methods that kill rapidly are obviously more 'humane' to an objective observer, and certainly for the animal involved.

The AVMA expressly states that its guidelines do not apply to humane killing. "The methods described in the Guidelines serve as guidance for veterinarians and others who may need to perform euthanasia. The Guidelines are not intended to specifically address slaughter or humane killing….Neither slaughter nor humane killing is addressed by this document." (at S.6.1.1 on p.68). Thus, according to the AVMA, the most humane method of killing is not considered to be euthanasia if it is not done to end suffering. In other words, the taking of life for scientific research could never constitute euthanasia, no matter how humane the technique, unless the animal also happened to be suffering.

Despite the statement that the AVMA Guidelines are not intended to address humane killing, those Guidelines discuss a number of methods that the AVMA then deems "humane killing." The examples given of humane killing do not include or exclude scientific research.

We urge OLAW to consider revising its policies to include humane killing. Doing so would encompass the ending of an animal's life outside the AVMA rubric of "doing what is best for the animal" and would thus encompass scientific research. Aligning the terminology used in the PHS Policy with that used by the AVMA Guidelines would at least resolve the difference between *the AVMA's definition of euthanasia and the reality of scientific research involving live animals, which would, in turn, accommodate IACUC approval of humane methods that do not qualify as euthanasia as defined by AVMA.*

That being said, we ask OLAW to note that the 16 veterinarians who signed the letter to AVMA (August 2019) opposing AVMA's decision to continue to classify rapid cardiac compression as unacceptable stated that:

> It was suggested by a member of the POE [AVMA Panel on Euthanasia]that cardiac compression might be acceptable to the AVMA as a method of HUMANE KILLING, however it is our opinion that words matter. When some techniques are distinguished as NOT euthanasia, we are labeling them as NOT as good, possibly even less humane. It is our opinion that cardiac compression meets all the criteria put forth by POE in the AVMA Guidelines for the Euthanasia of Animals when evaluating methods of euthanasia. We are requesting that the AVMA change the current listing of thoracic compression to an acceptable method of euthanasia for wild birds, with conditions such as being limited to small birds and as for any method, training personnel to perform cardiac compression correctly is vital.

The Ornithological Council concurs with this argument. As we stated earlier – a method is humane or not, regardless of the intent or purpose of the human in ending the life of the animal. However, if OLAW should accept the AVMA 2019 revision in its entirety, without exceptions, then the recognition of humane killing – however biologically incoherent that distinction might be – would at least address the fact that OLAW would be accepting a standard that by its terms, actually excludes most research.

OLAW should expressly allow IACUCs to use other guidelines pertaining to euthanasia if basic, objective standards are met and if they are as appropriate, or more appropriate, factually and scientifically, to specific situations, particularly if the method at variance with AVMA Guidelines provides a more rapid and less stressful death. This is certainly the case with regard to ornithological research.

Use of other standards that safeguard animal welfare, generally

OLAW should consider conforming its policy to that used by the APHIS Animal Care Program, which recognized, several years ago, that the Animal Welfare Act regulations (9 CFR 1.1) define euthanasia as:

- The humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, **OR**
- A method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death. (Animal Welfare Inspection Guide, March 2020 rev.; last accessed 1 April 2020 at https://www.aphis.usda.gov/animal_welfare/downloads/Animal-Care-Inspection-Guide.pdf)

Conforming PHS Policy to that of the APHIS Animal Care Program is not only biologically appropriate but it also furthers the stated intent of OLAW to comply with the mandate of Section 2034 (d) of the 21st Century Cures Act (Public Law No: 114-255:

(1) identify ways to ensure such regulations and policies are not inconsistent, overlapping, or unnecessarily duplicative, including with respect to inspection and review requirements by Federal agencies and accrediting associations;
(2) take steps to eliminate or reduce identified inconsistencies, overlap, or duplication among such regulations and policies; and
(3) take other actions, as appropriate, to improve the coordination of regulations and policies with respect to research with laboratory animals.

By imposing a separate and more restrictive standard, OLAW is, in essence, re-writing the AWA regulations as to any PHS-funded research, along with research funded by the National Science Foundation, NASA, the Food and Drug Administration, and the Veteran's Health Administration. An institution funded by any of these agencies would have to meet the PHS policy (and thus, the AVMA Guidelines) rather than the regulatory standards imposed under the Animal Welfare Act, which allow for a substantive review to be made as to the particular methods under particular circumstances.

Alternate guidelines that are more appropriate for the study of wild birds in field conditions

We call on the PHS to issue guidance that states clearly that in the case of wildlife, it is appropriate for the IACUC to approve research protocols that will be consistent with the Ornithological Council *Guidelines to the Use of Wild Birds in Research* (Fair *et al*. 2010) and the separate guidance on rapid cardiac compression published by the Ornithological Council. These two peer-reviewed documents are based peer-reviewed literature, on the extensive experience of thousands of ornithologists, and on consultation with wildlife veterinarians.

With regard to rapid cardiac compression, the AVMA states without support of any kind that "The consensus of veterinarians with field biology training and expertise is that portable equipment and alternate methods are currently available to field biologists for euthanasia of wildlife under field conditions, in accordance with current standards for good animal welfare." (p. 47). If OLAW is going to accept anecdotal, anonymous evidence, then OLAW should also accept this evidence: many veterinarians with whom the Ornithological Council has consulted agree that in many, if not most, field conditions, portable equipment and alternate methods are not available or are highly problematic at best, and can actually result in less humane death.

If OLAW is willing to accept experiential evidence from the AVMA, then it should also accept such evidence from highly experienced ornithologists, both as to the rapidity of loss of consciousness resulting from rapid cardiac compression and as to the unavailability or extreme impracticality of alternatives in many, if not most, field studies in which euthanasia is an essential component of the research. Again, we call on OLAW to make an independent scientific determination rather than accepting a conclusion that ignores the scientific evidence and that is based on unsubstantiated and selective information.

Further, if experiential evidence and knowledge are in fact a sufficient basis for decision-making, it is puzzling that the AVMA continues to ignore the evidence submitted by the Ornithological Council in 2013:

> The consensus* was that birds weighing less than 100 g should be unconscious within 5 seconds and dead within 15-20 seconds. Birds between 100-250g become unconscious within 10-20 seconds and could take 20-60 seconds to be verifiably dead. More confidence was universally attributed to estimates of smaller birds, and less confidence in estimates and greater variation in bird response were described for larger birds.
>
> *Consensus of five of the most active and experienced field ornithologists in attendance. Each has used thoracic compression on ~500 >1000 birds over a career of field collecting and each was interviewed privately, without knowledge of the statements made by the others.

Further, veterinarians who take part in or conduct ornithological research in the field have stated their support for a change in the classification of rapid cardiac compression, both at AVMA conferences on the euthanasia guidelines and in writing. A letter submitted by 16 veterinarians to the AVMA (30 August 2019) has apparently been ignored by the AVMA. That letter stated, in part:

> However, several veterinarians and field scientists are not in agreement with the POE's determination to retain the designation of the field technique of THORACIC (CARDIOPULMONARY, CARDIAC) COMPRESSION as an unacceptable means of euthanizing animals that are not deeply anesthetized or insentient. The individuals signing this letter support the use of cardiac compression as an acceptable means of field euthanasia of small birds that may not be deeply anesthetized or insentient at the initiation of the technique. Our concerns are specifically aimed at field conditions in which no veterinarian is physically present to administer anesthetics prior to application of cardiac compression.

It is evident that the AVMA Guidelines are, in fact, actually insufficient as to euthanasia methods for wild birds in field settings. For instance, the AVMA touts alternate methods but fails to specify what those methods might entail. Perhaps this is because they are alluding to isoflurane, enflurane, sevoflurane, and desflurane, which can be obtained without Drug Enforcement Agency licenses. There are, however, state restrictions and other concerns (see Appendix).

Moreover, the AVMA Guidelines make clear that these substances can be problematic for euthanasia. For instance:

> Isoflurane is less soluble than halothane, and it induces anesthesia more rapidly. However, it has a pungent odor and onset of unconsciousness may be delayed due to breath holding." And "Although sevoflurane is reported to possess less of an objectionable odor than isoflurane, some species may struggle violently and experience apnea when sevoflurane is administered by face mask or induction chamber.[19] Like enflurane, sevoflurane induces epileptiform electrocortical activity.[20] Desflurane is currently the least soluble potent inhaled anesthetic, but the vapor is quite pungent, which may slow induction. This drug is so volatile that it could displace O_2 and induce hypoxemia during induction if supplemental O_2 is not provided. Both diethyl ether and methoxyflurane are highly soluble, and may be accompanied by agitation because anesthetic induction is quite slow. Diethyl ether is irritating to the eyes, nose, and respiratory airways; poses serious risks due to flammability and explosiveness; and has been used to create a model for stress." (p.24). Although inhaled anesthetics are routinely used to produce general anesthesia in humans and animals, these agents may be aversive and

distressful under certain conditions. Flecknell et al[19] reported violent struggling accompanied by apnea and bradycardia in rabbits administered isoflurane, halothane, and sevoflurane by mask or induction chamber, and concluded these agents were aversive and should be avoided whenever possible. Leach et al. [25–27] found inhaled anesthetic vapors to be associated with some degree of aversion in laboratory rodents, with increasing aversion noted as concentration increased; halothane was least aversive for rats, while halothane and enflurane were least aversive for mice. (p. 25)

Note that with regard to these agents, the AVMA does not discuss the duration of the time the animal remains conscious. The aversive impacts described for each of these agents could last far longer than any pain or distress experienced in the seconds to loss of consciousness following rapid cardiac compression. And, in fact, the veterinarians' August 2019 comments to AVMA made exactly that point:

> The Proposed 2019 Updates to the AVMA Guidelines for the Euthanasia of Animals does not provide recommendations how to measure the degree of distress induced by any euthanasia process. Studies that report signs of distress associated with euthanasia are limited and primarily report behavioral indexes in mammals. There is currently no data measuring the degree of distress induced by any method of avian (non-poultry) euthanasia, including current AVMA approved methods. Therefore, having limited or no data supporting degree of distress induced by cardiac compression of small birds, is not a valid reason to disallow it, since it has not yet been a substantiated criterion applied to any other method of avian euthanasia.

OLAW should recognize an exception for the use of rapid cardiac compression in wildlife research in the field, for the reasons discussed below (Part III, Section 5. Avians).

The AVMA ignores the best available scientific evidence that demonstrates conclusively that rapid cardiac compression is at least as rapid, if not more so, than other methods deemed by the AVMA to be acceptable. The AVMA offers no evidence to the contrary.

The AVMA Guidelines state that:

> Thoracic compression (also known as cardiopulmonary or cardiac compression) is a method that has been used by biologists to terminate the lives of wild small mammals and birds, mainly under field conditions.[272] Although this method has been used extensively in the field, data supporting its use, such as degree of distress induced and time to unconsciousness or death, are limited.[273] Given current knowledge of the physiology of small mammals and birds, it cannot be assumed that thoracic compression does not result in pain or distress before animals become unconscious. Consequently, thoracic compression is an unacceptable method of euthanizing animals that are not deeply anesthetized or insentient due to other reasons, but is appropriate as a secondary method for animals that are insentient. (p.47 and pp. 80-81).

This text is virtually unchanged from the 2013 edition. That it completely ignores the two peer-reviewed studies published since then – one in an AVMA journal – both confirming that the method as used with birds does NOT entail compression of the thorax is inexcusable and evidences bias, discussed at more length, below. The detailed descriptions offered by Paul-Murphy et al. (2017) and Engilis et al. (2018) along with the photographs included in the latter paper make this abundantly clear and indisputable. The

2019 revision has deleted the references upon which this erroneous nomenclature was based. The 2013 revision cited letters (Bennett 2001; Ludders 2001**) that were based on an inaccurate understanding of the method that has now been disproved by the Paul-Murphy and Engilis papers. Although the AVMA deleted those citations following receipt of the August 2019 veterinarians' letter, the text remains unchanged. There may be some explanation other than bias for the refusal to consider the best, peer-reviewed scientific evidence and for continuing to rely on disproved assertions in letters to journals, but given the totality of the circumstances, bias is certainly a plausible explanation.

> **Note that Dr. Ludders, by letter to the editor of the Journal of the American Veterinary Medical Association, retracted his 2001 letter. The retraction occurred prior to the revision of the AVMA Guidelines. The editor acknowledged receipt and noted that the Panel on Euthanasia was in the process of updating the AVMA Guidelines. It is therefore curious that the AVMA would continue to cite it in support of its decision. Dr. Ludders, who also signed onto the 19 August 2019 letter, gave the Ornithological Council permission to share this information and excerpts from his letter:
>
> "Professor Paul-Murphy et al's study describes euthanasia of sparrows and starlings either by thoracic compression or intraosseous pentobarbital treatment (IPT); the results clearly and convincingly show that correctly performed thoracic compression results in humane euthanasia of passerine birds. The authors also clearly demonstrate that a more accurate term for this technique is rapid cardiac compression (RCC). An important consideration for humane euthanasia is the time it takes to achieve key end points, such as cessation of pulse, loss of consciousness, or isoelectric EEG. The Paul-Murphy et al data show that key end points are very quickly achieved when birds are euthanized by RCC. Furthermore, the RCC key time end points are similar to those for birds euthanized by IPT.
>
> As a veterinarian who has euthanized animals, I have considered an overdose of pentobarbital to be a gold standard for humane euthanasia; when used correctly it is fast, painless and effective. A technique that produces euthanasia within the same time frame as does pentobarbital and seemingly without stress to the bird, should be considered a humane technique for euthanizing birds, and I encourage the AVMA Panel on Euthanasia to consider it as such."

The AVMA stated in 2013 that "data supporting this method are not available, including degree of distress induced and time to unconsciousness or death." The work of Paul-Murphy and Engilis subsequently provided that data. Now, the AVMA states that the data are limited. True, the data are limited, but all available data show clearly that the method is, in fact, very rapid, with a cessation of pulse ranging from 0 (zero) seconds to 18.5 seconds (Paul-Murphy et al. 2017). The lack of a pulse indicates a cessation of blood (and therefore oxygen) to the brain, resulting in a rapid loss of consciousness. The first two criteria cited by AVMA Guidelines are (1) ability to induce loss of consciousness and death with a minimum of pain and distress; (2) time required to induce loss of consciousness (p.9)

These rates reported by Paul-Murphy et al. (2017) compare favorably to those reported for loss of consciousness resulting from the use of carbon dioxide. The AVMA classifies CO_2 as "acceptable with conditions," Time to unconsciousness with CO_2 is dependent on the displacement rate, container volume, and concentration used. In rats, unconsciousness is induced in approximately 12 to 33 seconds with 80 to 100% CO_2 and 40 to 50 seconds with 70% CO_2 (citation omitted). Similarly, a rapidly increasing concentration (flow rate > 50% of the chamber volume per minute) induces unconsciousness in only 26 to 48 seconds (citations omitted). Leake and Waters (citation omitted) found that dogs exposed to 30% to 40% CO_2 were anesthetized in 1 to 2 minutes. For cats, inhalation of 60% CO_2 results in loss of consciousness within 45 seconds, and respiratory arrest within 5 minutes (citation omitted). For pigs, exposure to 60 to 90% CO_2 causes unconsciousness in 14 to 30 seconds (citations omitted) with unconsciousness occurring prior to onset of signs of excitation (p.30)."

Prior to loss of consciousness, animals subjected to the use of CO_2 may experience pain and distress as described by the AVMA Guidelines:

> Carbon dioxide has the potential to cause distress in animals via 3 different mechanisms: (1) pain due to formation of carbonic acid on respiratory and ocular membranes, (2) production of so-called air hunger and a feeling of breathlessness, and (3) direct stimulation of ion channels within the amygdala associated with the fear response. Substantial species and strain differences are reported. (p.28).

OLAW should take note of this gross inconsistency. For the use of carbon dioxide in a variety of species, pain and distress for 12 to 60 seconds prior to loss of consciousness is deemed acceptable to the AVMA but for rapid cardiac compression in birds, pain and distress prior to loss of consciousness in 0 to 18.5 seconds is not.

We expressly request that OLAW make its own, independent determination by reviewing the Paul-Murphy and Engilis papers and the discussion presented in this letter and issue a statement in conjunction with its decision as to the AVMA 2020 revision that as to birds, rapid cardiac compression is an acceptable method of euthanasia. Note that when AAALAC International adopted the 2013 revision of the AVMA euthanasia guidelines, it expressly stated that:

> The Council on Accreditation recognizes the need for the use of thoracic compression in conscious wild small birds and mammals in situations where alternate techniques are not feasible or objectives of the protocol are such that the Institutional Animal Care and Use Committee (IACUC), and/or competent authority, grants approval for this method, training for the technique is provided, and its continued approval is re-evaluated as more scientifically-based data regarding its use becomes available. (Most recently accessed from https://www.aaalac.org/pub/E9014167-DD8B-4261-7D62-B028BC9D677C on 1 April 2020).

AAALAC made this exception before the publication of the Paul-Murphy and Engilis papers and based its decision on a practicality issue. The Ornithological Council will now ask AAALAC to revise this exception based on the biological evidence rather than the practicality and we ask that OLAW do the same.

Although there is ample scientific basis for OLAW to issue its own exception to the AVMA Guidelines, if OLAW for some reason is unwilling to do so, the Ornithological Council asks OLAW to issue a statement that as to the use of rapid cardiac compression for birds, there is adequate scientific justification for an IACUC to approve a departure from the ILAR Guide for the Care and Use of Laboratory Animals.

The AVMA Guidelines show evidence of bias

The AVMA in 2000 and 2007 classified what was then called thoracic compression and is now accurately described — as to birds — as rapid cardiac compression as conditionally acceptable. There was no new or additional information to justify the 2013 change in the classification. Now, with two peer-reviewed papers, one published in an AVMA journal, reporting a study by a highly renowned research veterinarian who received the 2019 AVMA Animal Welfare Award and who served on the avian working group for the 2013 AVMA Guidelines, the AVMA continues to (a) refuse to accept the scientific evidence that the appropriate name is rapid cardiac compression and (b) refuse to change the classification to conditionally acceptable or acceptable. Together with the fact that the verbiage is virtually unchanged from the 2013 edition, leaves little conclusion but that the decision was based not on science but on some other basis. And, in fact, it has been reported to the Ornithological Council that at the AVMA Humane Endings conference in 2014, at least one member of the AVMA Euthanasia Panel continued to assert that the manner of death was hemorrhage, i.e., that the birds bleed to death, despite the presentation by Dr. Paul-Murphy of her findings, indicating that this was not the case. In addition, one member of the panel apparently conducted her own study (unpublished to the best of our knowledge) of rapid cardiac compression. The study was apparently presented it at the 2014 AVMA conference on euthanasia. For reasons known only to this veterinarian, the study methods did not resemble the actual method known as rapid cardiac compression in any way. At that point, the original version of the Ornithological Council fact sheet describing the methods was available and had the veterinarian asked, the Ornithological Council would gladly have helped the veterinarian in finding an ornithologist experienced in the method to assist in the study. Apparently, this veterinarian placed anesthetized birds in a blood pressure cuff, or a device similar to a blood pressure cuff, and inflated the device until the birds died. Of course, this is not how the method is performed. No pressure is placed on the thorax and the bird is not squeezed around the thorax. In fact, as demonstrated in the Paul-Murphy paper and a subsequent methods paper by Engilis et al., the compression is applied directly to the heart. Hence, the name change to rapid cardiac compression. It is impossible to delve into the minds of this panelist or the other committee members as to how this study influenced their thinking but it is certainly possible that this presentation in fact influenced this outcome. Finally, we were made aware in 2013 that a member of the panel – who also served on the 2020 panel – made a number of false, derogatory statements about the Ornithological Council staff, some to colleagues who alerted the Ornithological Council – suggesting that personal animus might be a factor in this decision. And, in fact, that panel tacitly admitted making these comments in an e-mail to the Ornithological Council staff member in question. This individual also reportedly stated, in the context of the 2020 revision, that classification of the rapid cardiac compression will never be changed, implying that scientific information, regardless of quality or quantity, would be the basis upon which the classification would be made.

The apparent selective acceptance (and selective rejection) of evidence, without explanation, also evidences bias. As noted above, the AVMA panel ignored comments submitted by the 16 highly qualified veterinarians who signed a letter to the AVMA in 2019 that noted (as above) that the AVMA has no data on the level of distress associated with *any* method of avian euthanasia and yet overlooked the absence of such data when evaluating other methods – as demonstrated by the examples discussed above (CO_2) and below (inhalants and kill traps).

Worse than inconsistent application of unsubstantiated standards, the veterinarians pointed out that the AVMA's assumptions about the pain and distress associated with rapid cardiac compression were simply wrong. The letter stated that:

> Field biologists, experienced with application of the cardiac compression method, attest to observing minimal behavioral responses to the technique when applied in conscious wild birds (Engilis Jr et al., 2018). One of the veterinary authors of the cardiac compression study (Paul-Murphy et al., 2016) has observed the technique applied to conscious small wild birds and did not observe any of these distress-like behaviors.

The AVMA cites absolutely no evidence in support of its assumptions; there is no indication that any members of the panel have even seen the method performed.

Bias might also explain the inconsistency between the classification of carbon dioxide and that of rapid cardiac compression. For instance, abundant proof of pain, distress, and prolonged duration results in an "acceptable with conditions" rating for CO_2, even where alternatives such as injectable substances are readily available, though undoubtedly these are less convenient for euthanasia of the numbers of animals killed in biomedical research; by contrast, documented evidence that loss of consciousness following rapid cardiac compression is so fast that pain is experienced for only a few seconds is dismissed by the AVMA.

Similarly, bias could underlie the inconsistency seen in the AVMA's discussion of kill traps, which, it says, were "found to meet standards for certain species" (p.46). The traps that apparently met the AVMA standards included one trap for which mean (±SE) estimated times to loss of consciousness and heartbeat were ≤ or = 55 sec and 305 (±8) sec, respectively after firing the trap; this study confirmed that the trap can be expected to render ≥ or = 70% of captured fishers irreversibly unconscious in ≤ or = 3 min ($P < 0.05$). (Proulx and Barrett 1993). The trap described in another paper cited in this context had similarly long duration: "The C120 Magnum trap, equipped with a 66 x 69 mm pan trigger, which favored double strikes in the neck and thorax regions, successfully killed nine of nine wild mink (*Mustela vison*) in simulated natural conditions. Average times to loss of consciousness and heartbeat were estimated at less than 72 (±24) sec and 158 (±48) sec, respectively, after firing of the trap. This study confirmed that the C120 Magnum trap can be expected to render greater than 79% of all captured mink unconscious in less than or equal to 3 min (P less than 0.05)." (Proulx et al. 1990). These times – along with the reported times in other papers cited by the AVMA as reaching "the required level of efficacy" – far exceed the times reported for rapid cardiac compression (Paul-Murphy et al. 2017).

The AVMA concludes that:

> Kill traps do not consistently meet the POE's criteria for euthanasia, and may be best characterized as humane killing under some circumstances. At the same time, it is recognized they can be practical and effective for scientific animal collection or pest control when used in a manner that ensures selectivity, a swift kill, and no damage to body parts needed for field research. (p.46)

It is difficult to understand, given the data reported by the papers cited by the AMVA, how kill traps could *ever* meet the AVMA's criteria for humane killing, much less euthanasia, given that these traps are not used to "bring about a welcome death" or end suffering, which are the hallmark characteristics of euthanasia, according to the AVMA. It is equally implausible that kills traps could be characterized as humane killing, given that it could take a minute before the unanesthetized animal is unconscious and, presumably, is experiencing pain and distress during that time – the very reasons that the AVMA gave for classifying rapid cardiac compression as unacceptable, though rapid cardiac compression is far faster. This extreme inconsistency could be the product of bias.

Conclusion

We hope that NIH will take seriously the need to evaluate independently the standards of an outside organization before adopting those standards and imposing them on grantees and others. Standards lacking scientific merit should not become the policy of the NIH. The AVMA guidelines pertaining to field research methods generally and specifically to thoracic compression lack scientific merit.

At the very least, OLAW should convene its own panel of experts, adhering to the requirements of the Federal Advisory Committee Act, to determine appropriate compliance standards. It is inappropriate for OLAW to simply accept the standards of an outside organization, without a robust and independent peer review, and then impose those standards on others without regard to the process by which those standards were developed. This is particularly true if OLAW intends to accept only one set of standards, even if those standards are not, by their own terms, suited to the research funded by NIH and other federal research agencies and if they were developed by individuals without the requisite expertise.

We thank OLAW for considering our concerns and hope that these comments have proved useful. The Ornithological Council and the researchers who comprise our member societies take animal welfare very seriously. We support OLAW's efforts to assure and improve the care and treatment of animals used in research, and we know that the NIH will take such measures as are necessary to assure that the standards by which research protocols are judged and animal care programs are assessed are of the highest possible quality and are biologically sound.

Sincerely,

[Name redacted]

Literature cited

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Engilis, Jr., Andrew Jr., Irene E. Engilis, and Joanne Paul-Murphy. 2018. Rapid cardiac compression: An effective method of avian euthanasia. The Condor: Ornithological Applications 120: 617:621.

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Rosenberg, Kenneth V., Adriaan M. Dokter, Peter J. Blancher, John R. Sauer, Adam C. Smith, Paul A. Smith, Jessica C. Stanton, Arvind Panjabi, Laura Helft, Michael Parr, and Peter P. Marra. Decline of the North American avifauna *Science.* 04 Oct 2019: 120-124

Appendix: Availability of alternatives

Introductory note

The best scientific evidence supports the assertion that rapid cardiac compression is an acceptable means of euthanasia for birds. We discuss alternatives because we are mindful of the PHS and AWA requirements to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animal, although of course, the terms "slight," "pain," and "momentary," and "distress" are not quantified. It is not clear that any adverse effects felt by birds as a result of rapid cardiac compression rise to this level, as to either duration or severity.

We also discuss alternatives to assist OLAW in understanding that in fact these unspecified alternatives to which the AVMA alludes are, in fact, not available in most circumstances and, in fact, do not reduce pain and suffering. To the contrary, they may lead to more pain and suffering than would result from the use of rapid cardiac compression alone.

Finally, we note that this emphasis on alternatives seems to be the primary basis for the AVMA conclusion and that the same issue seemingly plays little or no role in the decisions as to other methods. For instance, the lengthy discussion about CO_2 expounds at length about the disadvantages and adverse effects, yet the AVMA did not make the same type of statement with regard to CO_2. Having determined that is "acceptable with conditions, notwithstanding the distress associated with suffocation and the pain resulting from the formation of carbonic acid on the respiratory and ocular membranes, and the prolonged duration (depending on initial concentrations and fill rates), the AVMA chose not to list CO_2 as unacceptable notwithstanding the ready availability of alternatives in the laboratory environment, including injectables. We can only speculate that the failure to insist that the most humane method that is readily available in that setting be used in lieu of one that is far less humane; it is likely that practicality – the need to euthanize large numbers of animals, often at one time – was a consideration. However, if practicality is a valid consideration as to the use of CO_2 particularly in terms of alternatives, then it is surely a valid consideration as to the use of alternatives as to the use of rapid cardiac compression.

What veterinarians have actually said about alternatives

The AVMA states, without substantiation, that:

> "The consensus of veterinarians with field biology training and expertise is that portable equipment and alternate methods are currently available to field biologists for euthanasia of wildlife under field conditions, in accordance with current standards for good animal welfare." (p.47)

Without providing evidence, such as letters or statements from veterinarians with field biology training, it is possible that this consensus – if it exists at all – exists only among the members of the panels who wrote the 2019 revision of the AVMA Guidelines. The veterinarians who allegedly concur with this statement are not identified and their credentials - particularly as to ornithological field experience – are not provided. Even if there are some veterinarians who have appropriate experience who, in fact, support this statement, it is clearly not a consensus. In August 2019, 16 veterinarians signed a letter to AVMA objecting to the AVMA's refusal to reclassify rapid cardiac compression. They expressed concern both about the availability of alternatives and about the adverse impacts of said alternatives.

*Veterinarians expressed concerns that alternatives may result in **more** pain and stress:*

In their August 2019 letter to the AVMA, the veterinarians expressed concern about the adverse impacts of the alternatives:

> The statement regarding "other methods" is assumed to be referring to the later sentence about open-drop and injectable agents, however it may also be referring to other inhaled methods. The open-drop method uses an inhalation anesthetic. Because the distress component of cardiac compression has been called into consideration, there are similar concerns about inhalation anesthesia and inhaled agents for euthanasia, some of which have been documented for several vertebrate species including poultry and finches (Scott, et al. 2017). Scott, et al. provides data to conclude "When conscious zebra finches were held and injected intracoelomically with sodium pentobarbital, respiratory arrest was achieved much faster than when sodium pentobarbital was used after sedation with isoflurane. In addition to isoflurane followed by sodium pentobarbital taking longer (334 s) to achieve euthanasia than sodium pentobarbital alone (122 s), birds placed in the isoflurane chamber for sedation hit the walls as they tried to escape; wing flapping and total wing movement were recorded during this time."
>
> It is very insightful that the AVMA Guidelines include the following:
>
> *…all inhaled methods have the potential to adversely affect animal welfare because onset of unconsciousness is not immediate. Distress may be created by properties of the agent (eg, pungency, hypoxia, hypercarbia) or by the conditions under which the agent is administered (eg, home cage or dedicated chamber, gradual displacement or prefilling of the container), and may manifest itself behaviorally (eg, overt escape behaviors, approach- avoidance preferences [aversion]) or physiologically (eg, changes in heart rate, sympathetic nervous system [SNS] activity, hypothalamic-pituitary axis [HPA] activity). (Lines 1122-1127)*
>
> *Furthermore, use of SNS and HPA activation to assess distress during inhalation of euthanasia agents is complicated by continued exposure to the agents during the period between loss of consciousness and death. (Lines 1132-1133)*
>
> *Through preference and approach-avoidance testing, all inhaled agents currently used for euthanasia have been identified as being aversive to varying degrees. (Lines 1137-1138)*
>
> *For virtually all animals, being placed in a novel environment is stressful (Line 772, 80–83)*
>
> Therefore, under these constraints of remote field work by non-DVM individuals, physical methods must often be used to secure an avian specimen or euthanize an injured wild bird. Some physical methods, such as cervical dislocation and decapitation, can cause irreparable damage to the cadaver and are not suitable

> when collecting specimens for research and archiving in museums. Thoracic (cardiac) compression is the preferred method used by field biologists because it yields a quick death and high-quality specimens (Fair JM et al., 2010, Engilis Jr et al., 2018).

Further, IACUC veterinarians reviewing protocols for more than a hundred museums, universities, and research institutions have approved the use of rapid cardiac compression in field conditions. That they do so is strong evidence that there is no consensus that portable methods and (unspecified) alternates are available for use in field conditions.

Availability of unidentified alternatives

The 2019 revision repeats the assertion made in the 2013 revision:

> Anesthetics can be administered prior to application of thoracic compression. Depending on taxa, open-drop methods that do not require DEA registration can be used. (p.47)

As the AVMA did not think it necessary to identify the agents that might be used by open-drop method, we can only surmise that they allude to inhalation anesthetics such as isoflurane and sevoflurane. It is true that such substances are not regulated at the federal level, i.e., by the DEA. However, as the AVMA certainly knows, these substances are regulated at the state level and therefore available only to state-licensed physicians. Thus, a veterinarian must be willing to obtain it and provide it to the ornithologist for use in field research. As of 2019, all but four states (Arkansas, Connecticut, Delaware, and the District of Columbia) restrict the use of substances by licensees to situations where a Veterinary-Client-Patient Relationship exists. According to the AVMA, this relationship is established only when “the veterinarian has sufficient knowledge of the animal(s) to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s). This means that the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of an examination of the animal(s), or by medically appropriate and timely visits to the premises where the animal(s) are kept. The veterinarian is readily available, or has arranged for emergency coverage, for follow-up evaluation in the event of adverse reactions or the failure of the treatment regimen.”

Only two states – California and Oregon - exempt work involving wild or feral animals from the VCPR requirement.

Of course, the VCPR conditions are essentially inapplicable to most field research or to the methods of euthanasia used in the context of field research. The veterinarian has no knowledge of the study animals at all unless the veterinarian is in the field every day with the biologists and is present with each of the field technicians who may be working independently at some distance from one another. Even if present, there would be no reason for the veterinarian to diagnose medical conditions nor is that even relevant unless it happens to be a study question. There would be no need for follow-up or emergency coverage. However, as the VCPR is a legal restriction in most states, veterinarians in those states may be unwilling to provide it to field researchers. In some states, the license restricts the use of the substance to a particular location, making it impossible to use the substance legally at a field site.

In some countries, such inhalants are not available to anyone but licensed physicians and veterinarians, who are not permitted to supply it to others. We have attempted to investigate the availability of isoflurane in other countries and have received responses ranging from “available without a prescription” (Guatemala) to “very hard to get, requiring orders from a licensed veterinarian” (Mexico, South Africa) and “sold only to veterinarians and even then, difficult to obtain because it is fairly expensive and not widely used here” (Japan).

Apart from the issue of legal access, such resources are not available in all field situations. Such is certainly the case where investigators are presented with opportunities to capture small birds or mammals that represent important specimens in the course of conducting other research. For instance, while looking around old buildings or moving woody debris investigators can uncover shrews or native mice, or they might retrieve a bat from a well shaft. In these instances, the investigators are almost always without euthanasia equipment or supplies of any kind.

Even on planned collecting expeditions researchers frequently work in very remote areas reached on foot or are otherwise without access to fresh supplies, or where circumstances do not permit the substance to be stored under the conditions recommended by the manufacturer (20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature]. On collecting expeditions researchers frequently work in very remote areas without access to fresh supplies. A 36-day expedition based out of the University of New Mexico returned to the United States having collected nearly 700 bird specimens. Although one liter of isoflurane would probably have been sufficient (depending on the size of the birds collected), carrying a liter container, cotton, and numerous containers or devices for administering the isoflurane for the 10 k daily hikes from the field camp would have been highly impractical. Had they inadvertently spilled or lost the isoflurane, they would have been unable to obtain more. They were at least 24 hours from an urban area where they might have been able to replenish their supply. An expedition of this sort will ordinarily result in the collection of about 600 bird specimens.

It is unsafe to carry such inhalants on small aircraft for obvious reasons– a spill could incapacitate the pilot. Researchers who reach remote sites by small aircraft would not be able to carry such substances. Carrying isoflurane on commercial aircraft poses additional challenges. The IATA Dangerous Goods Manual treats the liquid form as "Aviation regulation liquid, not otherwise specified" and places it in hazard class 9. Though the amount that can be carried on a passenger aircraft is not problematic, it requires IATA training and certification and there are, of course, specific packaging requirements. Unfortunately, it is often the case that even when substances are properly packaged and labeled, airlines will reject them. We have had countless reports of experiences where airlines rejected various items that are expressly allowed under the IATA and DOT regulations: dry shippers (essentially, large thermos bottles that are flushed with liquid nitrogen that is then discarded before the shipper is filled and sealed - these are unregulated and yet airlines have refused to accept them); whole-animal specimens, even when completely dry; blood samples, feathers, and other tissues; dry ice; and more. We have no confidence that an airline will agree to carry inhalant anesthetics even when properly packaged and labeled. The IATA manual is voluminous and very complex. Airlines' staffers are not trained in the IATA regulations and will readily reject an unfamiliar item simply because they don’t know how to determine if it is acceptable.

Circumstances such as those described above afford few alternatives for euthanasia and isoflurane and pharmaceuticals are not among them. Veterinarians frequently will refuse to give controlled substances to researchers, particularly for off-label use, due to AMDUCA restrictions and out of concern for potential abuse. Some IACUCs and universities will refuse to allow their use unless a veterinarian is present. These substances frequently cannot be carried legally into other countries. Researchers working in these circumstances are essentially limited to firearms and rapid cardiac compression. Some institutions resist allowing the use of firearms for safety reasons and, it should be noted, firearms are often problematic because their use is often restricted by state or local law.

The veterinarians who wrote the August 2019 letter to AVMA confirm the challenge in obtaining inhalants:

> It is not stated what injectable agents, that do not require DEA registration, are being recommended. IACUC protocols for field scientists at research institutions are expected to comply with The AVMA Euthanasia Guidelines. For remote collecting or fieldwork being done by scientists and those without a license to administer anesthetics and controlled drugs, a veterinarian is needed to provide access to non-controlled drugs if being used. In an email discussion with a member of the California Veterinary Medical Board, the comments were "the veterinarian would be responsible for the use of said drugs under his/her license. Not sure I would want that accountability".

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1931 North Meacham Road, Suite 100
Schaumburg, IL 60173-4360

To Whom it May Concern,

We, the individuals with signatures below, would like to thank the AVMA Panel on Euthanasia (POE) for the thoughtful revisions being considered in the Proposed 2019 Updates to the AVMA Guidelines for the Euthanasia of Animals. It is greatly appreciated that the revisions have considered new information to address several issues regarding euthanasia and animal welfare.

However, several veterinarians and field scientists are not in agreement with the POE's determination to retain the designation of the field technique of THORACIC (CARDIOPULMONARY, CARDIAC) COMPRESSION as an unacceptable means of euthanizing animals that are not deeply anesthetized or insentient. The individuals signing this letter support the use of cardiac compression as an acceptable means of field euthanasia of small birds that may not be deeply anesthetized or insentient at the initiation of the technique. Our concerns are specifically aimed at field conditions in which no veterinarian is physically present to administer anesthetics prior to application of cardiac compression. The following statement in Proposed 2019 Updates to the AVMA Guidelines for the Euthanasia of Animals captures some of this sentiment:

> *The POE recognized there will be less-than-perfect situations in which a method of euthanasia that is listed as acceptable or acceptable with conditions may not be possible, and a method or agent that is the best under the circumstances will need to be applied. (Line 471- 473)*

These concerns for the Proposed 2019 Updates to the AVMA Guidelines for the Euthanasia of Animals will not fit into the provided Euthanasia-Guidelines-Comment-Form. Please read the points of discussion below and take these comments under serious consideration. Note that when the term "avian" or "bird" is used, this is referring to non-poultry species.

The current language used for thoracic compression in two sections of the interim 2019 marked changes for comment of the AVMA Euthanasia guidelines is pasted and italicized below. The areas that require further discussion for change are highlighted and will be addressed.

M3.12 THORACIC (CARDIOPULMONARY, CARDIAC) COMPRESSION

Thoracic (cardiopulmonary, cardiac) compression is a method that has been used by biologists to terminate the lives of wild small mammals and birds, mainly under field conditions. Although it has been used extensively in the field, data supporting this method are limited, including degree of distress induced and time to unconsciousness or death. (Paul-Murphy et al., 2016). Based on current knowledge of the physiology of both small mammals and birds, thoracic compression can result in substantial pain and distress before animals become unconscious, thus lacking key humane considerations that can be addressed by other methods. Various veterinary and allied groups do not support thoracic compression as a method of euthanasia. Consequently, thoracic compression is an unacceptable means of euthanizing animals that are not deeply anesthetized or insentient due to other reasons, but is appropriate as a secondary method for animals that are insentient. The consensus of veterinarians with field biology training and expertise is that portable equipment and alternate methods are currently available to field biologists for euthanasia of wildlife under field conditions, in accordance with current standards for good animal welfare. Anesthetics can be administered prior to application of thoracic compression. Depending on taxa, open-drop methods or injectable agents that do not require DEA registration can be used. These alternate methods are generally practical to use with minimal training and preparation as standard procedures prior to embarking upon fieldwork. (Lines 3018-3036)

Thoracic (cardiopulmonary, cardiac) compression is a method that has been used by biologists to terminate the lives of wild, small mammals and birds mainly under field conditions when other methods are not available. Although thoracic compression has been used extensively in the field, data supporting this method, including level of distress and times to unconsciousness or death, are not available. Based on current knowledge of avian physiology and euthanasia, thoracic compression can result in significant levels of pain and distress before animals become unconscious, thus lacking key humane considerations that can be addressed by other methods. Various veterinary and allied groups do not support thoracic compression as a method of euthanasia. Consequently, thoracic compression is generally an unacceptable means of euthanizing animals that are not deeply anesthetized or insentient due to other reasons but is appropriate as a secondary method for animals that are insentient. (Lines 5279-5289)

1) Response to statements: *"data supporting this method are limited, including ……. time to unconsciousness or death. (Paul-Murphy et al., 2016)"* and "*data supporting this method, including … times to unconsciousness or death, are not available."*

The data supporting most methods of euthanasia in birds other than poultry are very limited, including IV pentobarbital, the acceptable method of euthanasia for birds in the AVMA Guidelines for the Euthanasia of Animals. There are only two publications with data on euthanasia methods for birds other than poultry (Paul-Murphy et al., 2016 and Scott, et al. 2017). The Paul-Murphy et al., 2016 study was designed to compare time to circulatory arrest, time to isoelectric EEG, and other end points of interest between intraosseous pentobarbital (an accepted method of euthanasia) and cardiac compression (an *unacceptable means of euthanizing animals that are not deeply anesthetized or insentient*) in 2 small passerine species. In this study it was determined that the median time to isoelectric EEG (it is well accepted that an isoelectric EEG is compatible with an unconscious state), did not differ significantly between treatment groups for sparrows or starlings. In addition, median times to cessation of pulse were significantly shorter for cardiac compression than for intraosseous pentobarbital in sparrows and starlings. Cessation of pulse is an essential component for determination of death. In both sparrows and starlings, thoracic compression resulted in a more rapid cessation of pulse than did pentobarbital. Digital compression directly over the heart in the cardiac compression technique, causing obstruction of venous return and stopping cardiac output, led to rapid, and in some cases immediate, pulse cessation. (Paul-Murphy et al., 2016).

2) Response to statement: *"data supporting this method are limited, including degree of distress induced ….. (Paul-Murphy et al., 2016)"* This statement is contradicted later in the same paragraph by *"Based on current knowledge of the physiology of both small mammals and birds,* *thoracic compression can result in substantial pain and distress before animals become unconscious"*

The Proposed 2019 Updates to the AVMA Guidelines for the Euthanasia of Animals does not provide recommendations how to measure the degree of distress induced by any euthanasia process. Studies that report signs of distress associated with euthanasia are limited and primarily report behavioral indexes in mammals. There is currently no data measuring the degree of distress induced by any method of avian (non-poultry) euthanasia, including current AVMA approved methods. Therefore, having limited or no data supporting degree of distress induced by cardiac compression of small birds, is not a valid reason to disallow it, since it has not yet been a substantiated criterion applied to any other method of avian euthanasia.

There is no evidence that cardiac compression causes substantial pain and distress, substantial being a subjective term and not driven by data. This statement: "*the conclusion being based on current knowledge of physiology of both small mammals and birds*" was present in the original 2013 AVMA Guidelines for the Euthanasia of Animals. In 2010, when the AVMA POE Bird Working Group met to renew the guidelines for the 2013 edition, veterinarians in that group did not understand the thoracic compression technique because it was poorly described and no one making that decision had any experience with the technique. Two letters in the 2001 Journal of the American Veterinary Association inaccurately describe the technique as "What thoracic compression does do is prevent movement of the keel and thoracic wall and, thus, prevents the movement of air through the pulmonary system. The end result is that birds die by suffocation, a method of killing that is not humane." (Bennett RA, 2001, Ludders JW, 2001). These letters in the AVMA journal were strongly influential to the decision for avian guidelines, and are still referenced in the Proposed 2019 Updates to the AVMA Guidelines for the Euthanasia of Animals, despite the current availability of an accurate description of the technique with drawings and images demonstrating that the placement of fingers is in the cranial-lateral space dorsal to the sternum, directly over the region of the heart (Paul-Murphy et al., 2016, Engilis Jr et al., 2018).

The AVMA Guidelines for the Euthanasia of Animals includes a description of associated distress behaviors as the following; "*responses to noxious stimuli in conscious animals include distress vocalization, struggling, attempts to escape, and defensive or redirected aggression" (Line 801-802)*. Field biologists, experienced with application of the cardiac compression method, attest to observing minimal behavioral responses to the technique when applied in conscious wild birds (Engilis Jr et al., 2018). One of the veterinary authors of the cardiac compression study (Paul-Murphy et al., 2016) has observed the technique applied to conscious small wild birds and did not observe any of these distress-like behaviors. We ask the AVMA to consider that cardiac compression be considered with support from statements in the following AVMA Guidelines:

- *"euthanasia techniques that result in "rapid loss of consciousness" and "minimize pain and distress" should be strived for, even where it is difficult to determine that these criteria have been met." (Line 690-692)*

- *"As a general rule, a gentle death that takes longer is preferable to a rapid, but more distressing death; however, in some species and under some circumstances, the most humane and pragmatic option may be exposure to an aversive agent or condition that results in rapid unconsciousness with few or no outward signs of distress."(Line 1173-1175)*

Cardiac compression meets the criteria of rapid loss of consciousness and the technique itself is rapid (substantiated with a peer-reviewed study), thereby minimizing pain and distress. The killing of many healthy birds would be necessary if additional studies were required to provide data to establish the degree of pain and distress in cardiac compression compared to the standard of care, IV pentobarbital. In an email discussion that I have permission to share, Lisa Tell DVM, Dipl. ACZM commented " …I do know that for hummingbirds, I believe if it is done the correct way, cardiac compression is the fastest and probably least stressful way to euthanize a hummingbird. We can tell when the birds are stressed because they drop feathers like crazy. In my experience CO2 is not a pleasant way to kill a bird and having to use injectables on such a small bird is also painful and stressful. …. Our need to euthanize birds is very rare but having the option to do it in the most humane manner would be monumental.

It can be argued that the current AVMA approved method of euthanasia of birds, IV pentobarbital, *"can result in substantial pain and distress before animals become unconscious"*. Most veterinarians experienced in using barbiturates for euthanasia of birds agree that IV barbiturate administration can be a challenging procedure and recommend sedation prior to restraint for IV or IO injection of euthanasia solutions. There are several references addressing concerns about the distress associated with the handling, restraint and administration of euthanasia solution to animals, including small birds:

- Obtaining venous access in a small bird requires training and skill and often increases handling time (Greenacre CB, 2016)

- …intravenous injection of pentobarbital is considered a humane ending, minimizing fear and distress associated with handling is a major concern. (Hess L. 2005)

- *Intravenous injection of an injectable euthanasia agent is the quickest and most reliable means of euthanizing birds when it can be performed without causing fear or distress. Wild, fearful, or excited birds may require a sedative or anesthesia before IV injection can be performed. (Lines 5153-5156)*

- *Handling animals that are not accustomed to humans or that are severely injured or otherwise compromised may not be possible without inducing stress, so some latitude in the means of euthanasia is needed in some situations. (Lines 482-482)*

- *When the restraint necessary for giving an animal an IV injection is likely to impart added distress to the animal or pose undue risk to the operator, sedation, anesthesia, or an acceptable alternate route or method of administration should be used. Aggressive or fearful animals should be sedated prior to restraint for IV administration of the euthanasia agent. (Lines 1926-1929)*

3) Response to "*key humane considerations that can be addressed by other methods"*

   and

   "Anesthetics can be administered prior to application of thoracic compression. Depending on taxa, open-drop methods or injectable agents that do not require DEA registration can be used."

The statement regarding "other methods" is assumed to be referring to the later sentence about open-drop and injectable agents, however it may also be referring to other inhaled methods. The open-drop method uses an inhalation anesthetic. Because the distress component of cardiac compression has been called into consideration, there are similar concerns about inhalation anesthesia and inhaled agents for euthanasia, some of which have been documented for several vertebrate species including poultry and finches (Scott, et al. 2017). Scott, et al. provides data to conclude "When conscious zebra finches were held and injected intracoelomically with sodium pentobarbital, respiratory arrest was achieved much faster than when sodium pentobarbital was used after sedation with isoflurane. In addition to isoflurane followed by sodium pentobarbital taking longer (334 s) to achieve euthanasia than sodium pentobarbital alone (122 s), birds placed in the isoflurane chamber for sedation hit the walls as they tried to escape; wing flapping and total wing movement were recorded during this time."

It is very insightful that the AVMA Guidelines include the following:

…all inhaled methods have the potential to adversely affect animal welfare because onset of unconsciousness is not immediate. Distress may be created by properties of the agent (eg, pungency, hypoxia, hypercarbia) or by the conditions under which the agent is administered (eg, home cage or dedicated chamber, gradual displacement or prefilling of the container), and may manifest itself behaviorally (eg, overt escape behaviors, approach- avoidance preferences [aversion]) or physiologically (eg, changes in heart rate, sympathetic nervous system [SNS] activity, hypothalamic-pituitary axis [HPA] activity). (Lines 1122-1127)

Furthermore, use of SNS and HPA activation to assess distress during inhalation of euthanasia agents is complicated by continued exposure to the agents during the period between loss of consciousness and death. (Lines 1132-1133)

Through preference and approach-avoidance testing, all inhaled agents currently used for euthanasia have been identified as being aversive to varying degrees. (Lines 1137-1138)

For virtually all animals, being placed in a novel environment is stressful (Line 772, 80–83)

It is not stated what injectable agents, that do not require DEA registration, are being recommended. IACUC protocols for field scientists at research institutions are expected to comply with The AVMA Euthanasia Guidelines. For remote collecting or fieldwork being done by scientists and those without a license to administer anesthetics and controlled drugs, a veterinarian is needed to provide access to non-controlled drugs if being used. In an email discussion with a member of the California Veterinary Medical Board, the comments were "the veterinarian would be responsible for the use of said drugs under his/her license. Not sure I would want that accountability".

Therefore, under these constraints of remote field work by non-DVM individuals, physical methods must often be used to secure an avian specimen or euthanize an injured wild bird. Some physical methods, such as cervical dislocation and decapitation, can cause irreparable damage to the cadaver and are not suitable when collecting specimens for research and archiving in museums. Thoracic (cardiac) compression is the preferred method used by field biologists because it yields a quick death and high-quality specimens (Fair JM et al., 2010, Engilis Jr et al., 2018).

4) Response to *"Various veterinary and allied groups do not support thoracic compression as a method of euthanasia."*

This statement: *"Various veterinary and allied groups do not support thoracic compression as a method of euthanasia."* was present in the original 2013 AVMA Guidelines for the Euthanasia of Animals. In 2010 when the AVMA POE Bird Working Group met to renew the guidelines eventually developed for the 2013 edition, veterinarians in that group were associated with veterinary and allied groups, including American Association of Avian Veterinarians, American Association of Zoo Veterinarians, and Association of Wildlife Veterinarians. However, the individuals did not have experience with thoracic compression and did not understand how the technique was applied. The working groups relied heavily on two opinion letters in the 2001 Journal of the American Veterinary Association, both with inaccurate descriptions of the technique (Bennett RA, 2001, Ludders JW, 2001). These letters are still referenced in the Proposed 2019 Updates to the AVMA Guidelines for the Euthanasia of Animals, despite the current availability of accurate descriptions of the technique with drawings and images (Paul-Murphy et al., 2016, Engilis Jr et al., 2018). In 2014, at the AVMA Humane Endings Conference, a member of the AVMA POE Bird Working Group and current member of the AVMA POE, presented results of a pilot study in which a blood pressure cuff was secured and inflated around the thorax of birds to simulate euthanasia by thoracic compression. This example is used only to illustrate how easily misunderstood the technique of thoracic compression has been.

It was suggested by a member of the POE that cardiac compression might be acceptable to the AVMA as a method of HUMANE KILLING, however it is our opinion that words matter. When some techniques are distinguished as NOT euthanasia, we are labeling them as NOT as good, possibly even less humane. It is our opinion that cardiac compression meets all the criteria put forth by POE in the AVMA Guidelines for the Euthanasia of Animals when evaluating methods of euthanasia. We are requesting that the AVMA change the current listing of thoracic compression to an acceptable method of euthanasia for wild birds, with conditions such as being limited to small birds and as for any method, training personnel to perform cardiac compression correctly is vital.

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Sincerely,

[Names redacted]

Comparison of intraosseous pentobarbital administration and thoracic compression for euthanasia of anesthetized sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*)

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OBJECTIVE
To compare intraosseous pentobarbital treatment (IPT) and thoracic compression (TC) on time to circulatory arrest and an isoelectric electroencephalogram (EEG) in anesthetized passerine birds.

ANIMALS
30 wild-caught adult birds (17 house sparrows [*Passer domesticus*] and 13 European starlings [*Sturnus vulgaris*]).

PROCEDURES
Birds were assigned to receive IPT or TC (n = 6/species/group). Birds were anesthetized, and carotid arterial pulses were monitored by Doppler methodology. Five subdermal braided-wire electrodes were used for EEG. Anesthetic depth was adjusted until a continuous EEG pattern was maintained, then euthanasia was performed. Times from initiation of euthanasia to cessation of carotid pulse and irreversible isoelectric EEG (indicators of death) were measured. Data (medians and first to third quartiles) were summarized and compared between groups within species. Necropsies were performed for all birds included in experiments and for another 6 birds euthanized under anesthesia by TC (4 sparrows and 1 starling) or IPT (1 sparrow).

RESULTS
Median time to isoelectric EEG did not differ significantly between treatment groups for sparrows (19.0 and 6.0 seconds for TC and IPT, respectively) or starlings (88.5 and 77.5 seconds for TC and IPT, respectively). Median times to cessation of pulse were significantly shorter for TC than for IPT in sparrows (0.0 vs 18.5 seconds) and starlings (9.5 vs 151.0 seconds). On necropsy, most (14/17) birds that underwent TC had grossly visible coelomic, pericardial, or perihepatic hemorrhage.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that TC might be an efficient euthanasia method for small birds. Digital pressure directly over the heart during TC obstructed venous return, causing rapid circulatory arrest, with rupture of the atria or vena cava in several birds. The authors propose that cardiac compression is a more accurate description than TC for this procedure. (*Am J Vet Res* 2017;78:887–899)

Euthanasia is the humane termination of an animal's life. According to the AVMA Guidelines for the Euthanasia of Animals, methods of euthanasia must cause rapid loss of consciousness followed by cardiac or respiratory arrest and death and must minimize pain, distress, and anxiety prior to loss of consciousness.[1] Evaluation of euthanasia methods for birds is limited, and the available peer-reviewed reports primarily address euthanasia and slaughter methods for commercially raised poultry.[2] In ornithological research, birds are collected in the field for specific purposes such as procurement of tissue or for use as specimens in museums and teaching collections.[3] Euthanasia of wild birds may also be necessary to relieve pain or address welfare concerns when a bird is injured or debilitated during collection such that it has a low probability of survival. The AVMA recognizes that recommended modes of euthanasia for captive animals are not always feasible in field situations.[1] However, the challenges presented by field conditions do not release investigators from the responsibility of minimizing the pain and distress of animals to be euthanized.[3]

ABBREVIATIONS

EEG	Electroencephalogram
P_{ETCO_2}	End-tidal partial pressure of carbon dioxide
F_{IO_2}	Fraction of inspired oxygen
IPT	Intraosseous pentobarbital treatment
TC	Thoracic compression

The AVMA euthanasia guidelines include methods that are acceptable or acceptable with conditions for euthanasia of birds. The IV injection of a euthanasia agent, such as pentobarbital, is currently indicated as an acceptable method. The guidelines state that this is the quickest and most reliable method when it can be performed without causing fear or distress; however, wild, fearful, or excited birds might require a sedative or anesthetic before IV injection can be performed.[1] It is generally understood that the capture and handling of unsedated wild birds induce fear and distress. In addition, obtaining venous access in a small bird requires training and skill and often increases handling time.[2] Therefore, although IV injection of pentobarbital is considered a humane ending, minimizing fear and distress associated with handling is still a concern.

The most common AVMA-approved method preferred by veterinarians for euthanasia of companion birds is to anesthetize or sedate a bird prior to IV administration of an overdose of pentobarbital.[4,5] Pentobarbital sodium is labeled for use in the United States and Canada for euthanasia of all animal species by IV administration, although it is not FDA-approved for use in birds. As a controlled substance (US Department of Justice–DEA schedule II drug), it can be used only by or under the supervision of a licensed veterinarian. Furthermore, data supporting the effects of IV administration of pentobarbital to birds other than poultry, including times to unconsciousness or death, are not available.

Wildlife biologists, wildlife managers, and wildlife health professionals are often required to procure specimens from wild avian populations using capture methods such as mist nets or net guns. For remote collecting or international fieldwork by field biologists and those without a license to administer anesthetics and controlled drugs, the administration of injectable drugs as a means to perform or aid euthanasia of birds is often illegal, and the use of inhalation anesthetics in remote areas is often impractical; therefore, physical methods must be used to secure the specimen. Some physical methods, such as cervical dislocation and decapitation, can cause irreparable damage to the cadaver and are not suitable when collecting specimens for research and archiving in museums. Thoracic compression is the preferred method used by field biologists because it yields a quick death and high-quality specimens.[3] Currently, the AVMA considers TC to be an unacceptable method of euthanasia for sentient birds because data supporting this method, including levels of pain, distress, and times to unconsciousness or death, are not available.[1] To the authors' knowledge, no such data are available for any method of euthanasia in nonpoultry avian species.

The use of EEG activity to determine when consciousness is lost is considered by some investigators to be the most objective means of assessing unconsciousness,[6,7] while others believe that the EEG is not a direct measure of consciousness and does not determine the exact moment when unconsciousness occurs because changes can be gradual and subtle.[8] However, there is sufficient agreement that an isoelectric EEG pattern (also known as a flatline pattern or electrocerebral inactivity) is not compatible with consciousness and is an indicator of cessation of normal cerebral function.[6] Electroencephalography results have been used in welfare evaluations of poultry slaughter and euthanasia techniques.[7,9,10] Additionally, an isoelectric EEG pattern combined with nonreversible states such as apnea or cessation of pulse has been used to define death.[7,9,10]

The objective of the study reported here was to compare an overdose of pentobarbital sodium solution (as IPT) to TC as methods of euthanasia for small passerine birds, with 2 common species used as subjects. The study was designed to compare time to circulatory arrest, time to isoelectric EEG, and other end points of interest between the 2 euthanasia methods among birds within each species, but it was not designed to compare outcomes between the 2 species. To our knowledge, no previous studies have evaluated changes in cerebral electrical activity and other physiologic effects of the 2 methods of euthanasia in anesthetized passerine birds. We hypothesized that there would be no difference in the time to cessation of arterial pulses or to isoelectric EEG between the 2 methods. Gross and histologic postmortem data were collected and reported to provide information necessary to understand the physical cause of death following TC.

Materials and Methods

Birds and treatment allocation

The study was conducted with wild-caught adult house sparrows (*Passer domesticus*) and European starlings (*Sturnus vulgaris*). These species were selected to represent 2 body sizes (and the typical size range involved in most avian field studies globally) and because both species are considered overpopulated and invasive in North America. The capture of wild sparrows and starlings was conducted by 2 experienced investigators (AE and IEE). Sparrows were captured by use of mist nets and were promptly removed from the nets and placed into small holding pens (30.8 X 30.8 X 61.6 cm; 2 birds/pen). Pens were covered with a blanket and shuttled to the study facility at 2-hour intervals until the desired number of birds was collected. Starlings were captured before sunrise by use of hand nets at a large roost (estimated as > 250,000 birds). Birds were quickly removed from the net and placed into a small holding pen (30.8 X 30.8 X 61.6 cm; 2 birds/pen), which was covered with a blanket for transfer to the study facility (arriving approx 30 minutes after capture). Methods for netting birds followed the Ornithological Council Guidelines to the Use of Wild Birds in Research,[3] and the protocols were approved by the University of California-Davis Institutional Animal Care and Use Committee.

Because mist netting of nontarget species could not be excluded during sparrow capture, a federal banding permit (No. 23383, issued to AE) was in place. All migratory birds caught in nets were released immediately at the point of capture. The study was approved by the University of California-Davis Institutional Animal Care and Use Committee.

In the present study, euthanasia end points were measured and anesthetized birds were used. These included 12 sparrows (body weight, 25.7 ± 2.0 g) and 12 starlings (body weight, 71.1 ± 3.4 g). The sample size was selected with the welfare of research animals in mind; a pilot investigation with 10 Japanese quail (involved in another study for which euthanasia was required) was performed to refine the procedures (data not shown), and the number of birds was kept small to reduce the number of animals used. Birds of each species were arbitrarily assigned to the TC and IPT groups (6 birds/species/group). Sexes were confirmed at necropsy. For the sparrows, the TC group included 5 males and 1 female, and the IPT group included 6 males. For starlings, the TC group included 3 males and 3 females, and the IPT group included 4 males and 1 female; sex of 1 bird was undetermined.

Another 6 birds that had been captured were anesthetized and euthanized by TC or IPT at the conclusion of the study to be examined by necropsy. These included 5 sparrows (3 females and a male that had TC and 1 female that had IPT) and 1 female starling that had TC. All birds that were brought into captivity were euthanized during or at the conclusion of the study.

Housing and husbandry

Sparrows and starlings were collected and housed at separate sequential periods to minimize time in captivity. Birds were housed in groups of 3 or 4 (sparrows) or 2 (starlings) in 27.5 X 20.0 X 15.0-in wire mesh cages at ambient temperature (approx 21° to 26°C) with a 12-hour light to dark cycle. Water was available ad libitum in a water bowl and by drip line in each cage. Sparrows were provided with a commercial seed mix,[a] and starlings were provided with a mixture of dry dog food and poultry mash daily.[11]

Anesthetic and monitoring procedures

Birds were anesthetized with isoflurane[b] in oxygen delivered via a small mask covering the head for induction. Isoflurane (2% to 4%) in oxygen (2 L/min) was delivered to the mask through a nonrebreathing circuit. The birds were endotracheally intubated, and isoflurane and oxygen flow were reduced to 2% and 1 L/min, respectively. Sparrows were intubated with a 16-gauge IV catheter with a silicone tip,[c] and starlings were intubated with a 2-mm uncuffed endotracheal tube.[d] A mechanical ventilator[e] was used during placement of monitoring equipment, but birds were allowed to breathe spontaneously when data collection began. A side port on the endotracheal tube was connected to a monitor[f] for measurement of P_{ETCO_2}, end-tidal isoflurane concentration, and F_{IO_2}. The F_{IO_2} began at 1.0 for all birds and was lowered during the course of anesthesia, with a goal of reaching 0.21 (equivalent to field applications without supplemental oxygen administration) prior to euthanasia. Ten starlings and 9 sparrows had the F_{IO_2} maintained at ≤ 0.40, including 6 birds at F_{IO_2} of 0.21. Two starlings and 3 sparrows had the F_{IO_2} maintained at ≥ 0.70.

A Doppler crystal[g] with gel applied was placed over the right carotid artery to monitor pulse rate. An intraesophageal temperature probe was inserted to the level of the thoracic esophagus. The equipment used for EEG and ECG was a digital electrophysiological monitoring system with integrated video monitoring.[h] Feathers were plucked from the skin over the region of the skull to facilitate electrode placement. Five 25-gauge braided subdermal wire electrodes[i] were placed SC in contact with the calvarium in the following positions: rostral to the opening of the left (A_1) and right (A_2) auditory canals, on the dorsal midline between A_1 and A_2 (C_Z), between C_Z and A_1 (C_3), and between C_Z and A_2 (C_4; **Figure 1**). A ground electrode was inserted rostral to the elec-

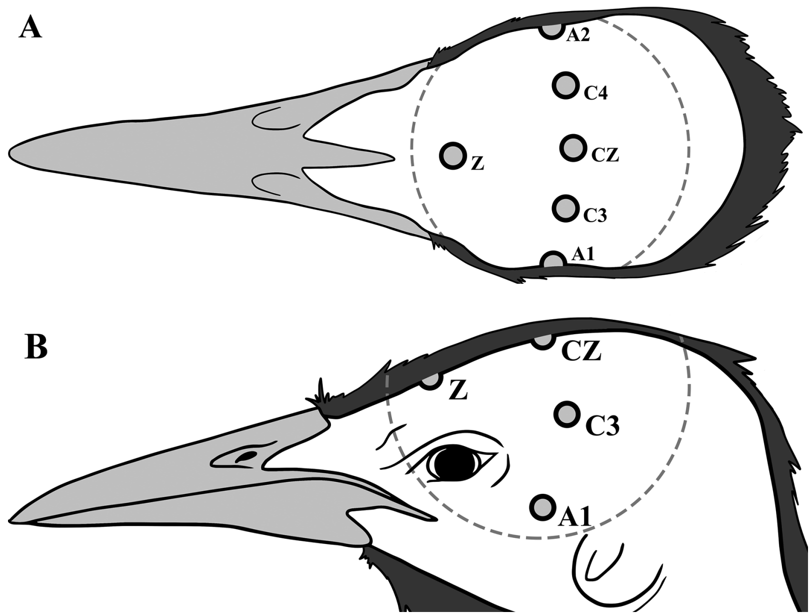

Figure 1—Schematic drawings of electrode placement on the dorsal aspect (A) and lateral aspect (B) of the head of a bird for EEG recording in a study to compare aspects of euthanasia by TC and IPT in wild-caught house sparrows (*Passer domesticus*) and European starlings (*Sturnus vulgaris*). Electrodes (represented by gray circles) were placed SC in contact with the calvarium of anesthetized birds in the following positions: rostral to the opening of the left (A_1) and right (A_2) auditory canals, on the dorsal midline between A_1 and A_2 (C_Z), between C_Z and A_1 (C_3), and between C_Z and A_2 (C_4). A ground electrode was inserted rostral to C_Z (Z).

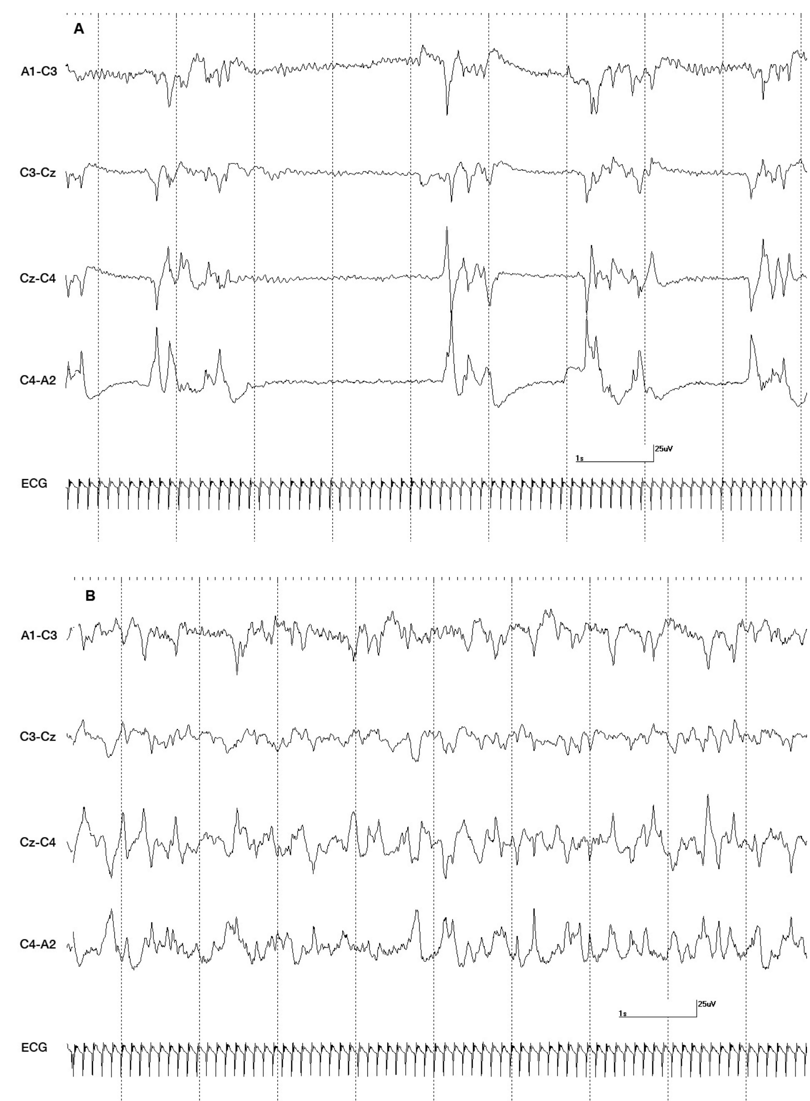

Figure 2—Representative EEG and ECG recordings from a starling anesthetized with isoflurane (A) and a continuous rate infusion of propofol (by intraosseous administration; B). Notice the burst suppression pattern (a mixture of high-voltage electrical activity with periods of no activity) associated with isoflurane anesthesia. The ECG tracings were obtained from electrodes placed on the proximal aspect of the left wing and proximal part of the left pelvic limb. The calibration voltage applies to EEG only. ***See*** Figure 1 for remainder of key.

trode designated as C_Z. Additional electrodes[j] were placed on the proximal aspect of the left wing and on the left thigh for ECG monitoring. Data were displayed with a transverse bipolar montage (pairs A_1-C_3, C_3-C_Z, C_Z-C_4, and C_4-A_2 and ECG). The sampling rate was 500 Hz, with sensitivity set at 5 µV/mm (100 µV/mm for ECG), sweep speed of 10 s/screen, a time constant of 0.1 seconds, and high-frequency filter of 70 Hz. To reduce electrical interference, a 60-Hz notch filter was applied.

In all birds, the medullary space of a tibiotarsal bone was catheterized with a 25-gauge, 5/8-in needle to administer propofol.[b] Intraosseous catheter placement was preferred over an IV catheter for its ease of placement, minimal blood loss, and repeatability in the small passerines. Following instrumentation, isoflurane administration was discontinued, and a continuous rate infusion of propofol (via the intraosseous needle) was used to maintain anesthesia. The mean ± SD last end-tidal isoflurane concentration prior to initiation of propofol administration was 0.54 ± 0.26% for sparrows and 0.43 ± 0.09% for starlings. The propofol dose to obtain and maintain a light plane of anesthesia ranged from 5.7 to 13.2 mg/kg/min for sparrows and from 1.9 to 5.6 mg/kg/min for starlings. A light plane of anesthesia in propofol-anesthetized birds was identified by physical signs and an EEG showing continuous activity devoid of burst suppression (an EEG pattern characterized by a mixture of high-voltage electrical activity with periods of no activity; **Figure 2**). The duration of time that birds were without inhalant anesthesia and titrated with propofol to eliminate burst suppression varied.

Euthanasia procedures

For pentobarbital sodium administration, propofol infusion was stopped, and pentobarbital solution (392 mg/mL) was slowly injected (duration < 60 seconds) through the intraosseous catheter. A fixed volume of 0.05 mL of pentobarbital was delivered to sparrows, resulting in a dose range of 693 to 754 mg/kg, twice the recommended dose of 0.2 to 1 mL/kg.[12] The first 2 starlings that underwent the procedure received 100 mg of pentobarbital/kg; however, the time to cessation of arterial pulse as measured by the Doppler method was prolonged (> 5 minutes) in 1 of these birds, so the remaining 4 starlings received a pentobarbital dose of 220 mg/kg; both doses were within the recommended range.[12] With the exception of the prolonged time to cessation of pulse in 1 bird, all of the monitoring parameters and time intervals for these 2 birds were within the range obtained for the starlings that received the 220-mg/kg dose. Therefore, data from these birds were included in the analysis.

For the TC technique, propofol administration was stopped and TC was performed by 2 of the authors who were trained and highly experienced in this method (AE and IE). Field application of TC involves holding the bird to ensure proper finger placement. In all cases, the bird should be handled with both hands, and the dominant hand should be used for the underwing positions and compression, with the nondominant hand used for keel positioning and

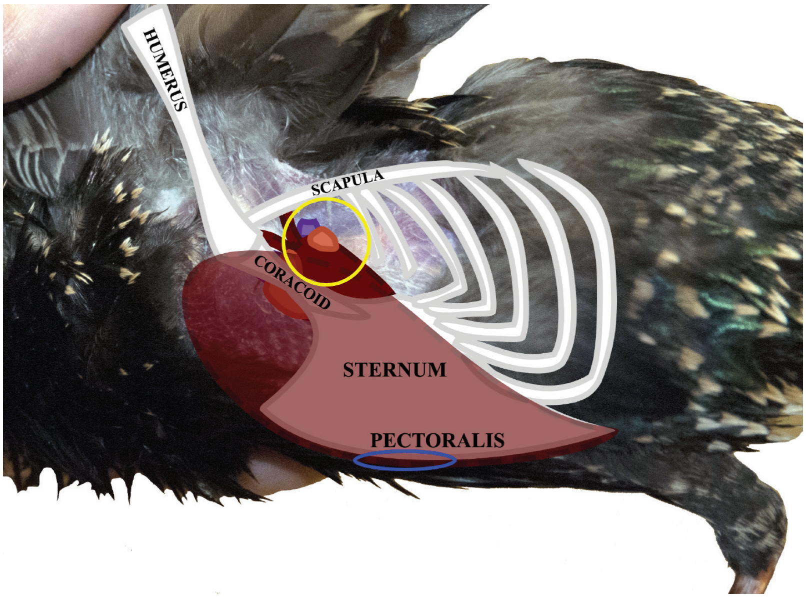

Figure 3—Schematic drawing of anatomic points and finger placement for TC overlaid on a digital photograph of the left body wall of a starling. The thumb and index or middle finger of the dominant hand are placed on opposite sides of the thorax in a space ventral to the scapula, dorsal to the coracoid, and caudal to the humerus when the humerus is extended above the body (yellow circle); the dorsum of the bird is against the palm of this hand during the procedure. With correct finger placement, cardiac contractions are easily palpable. In field applications, the fore and middle fingers of the opposite hand are placed along the ventral aspect of the sternum for additional support (blue circle); in the study, anesthetized and instrumented birds were placed in ventral recumbency on the flat surface of an examination table, and finger placement on the sternum was not used. Rapid, firm compression (maintained for ≥ 60 seconds) was performed with the thumb and forefingers over the heart.

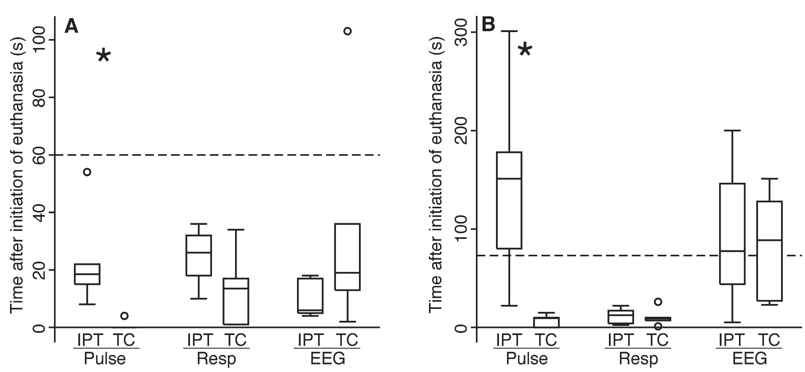

Figure 4—Box plots of times from initiation of euthanasia to end points of interest (time to cessation of arterial pulse as detected by Doppler method [Pulse], apnea as determined by capnography and observation of video recordings [Resp], and isoelectric EEG signal [EEG]) in anesthetized wild-caught house sparrows (n = 6; A) and European starlings (6; B) euthanized by TC or IPT. The horizontal line within each box represents the median, and boxes represent the range from the first to third quartiles. Whiskers represent the minimum and maximum values within 1.5 times the interquartile range of the median, and white circles represent values outside that range. The horizontal dashed line in each panel indicates the maximum time that the TC pinch was held. Asterisks indicate significant ($P < 0.05$) differences between IPT and TC groups. For the sparrows in the TC group (A), notice that cessation of pulses was immediate for all but 1 animal, so the median, box, and whiskers were all superimposed at the x-axis.

stability. With the bird's keel against the palm of the nondominant hand, the bird is held with the thumb and index or middle finger of the dominant hand (approaching from the dorsum) under the bird's wings. The thumb and finger are positioned on either side of the body cavity so that they are opposite each other in the triangular region formed by the pectoral muscle (ventrally), coracoid (cranially), and scapula (dorsally; **Figure 3**). The bird is readjusted in the nondominant hand so that the forefinger and middle finger are placed against the ventral edge of the keel, just below the furculum, to keep the bird in proper position when TC is initiated.[13] The thumb and index or middle finger are then pinched rapidly together to stop cardiac activity and held in place for ≥ 60 seconds. Because the study birds were anesthetized and attached to monitoring equipment, the described field method was modified as follows: the bird was placed in ventral recumbency with the sternum on the examination table. The researcher placed the dominant hand over the wings, gently lifting them to position the index finger and thumb in the correct placement. The table surface was used to keep the sternum stable, replacing the nondominant hand. The result was similar to the field method, with the table surface providing stability while TC was applied with the dominant hand. Monitoring was stabilized before TC was applied, and then the forefinger and thumb were rapidly and firmly pressed together to compress the heart. The bird was not lifted from the table during this time. The pinch was maintained for ≥ 60 seconds for each bird.

Necropsy procedures

Postmortem radiographs (ventrodorsal and right lateral projections) of sparrows (n = 6) and starlings (6) euthanized by TC were obtained. Digital radiography was performed with settings of 55 to 60 kVp and 5 mAs. The birds were placed directly against the detector panel without use of a radiographic grid.

Carcasses were stored at 4°C within 4 hours after death, and gross examination or tissue fixation was performed within 48 hours after death. Most starlings (n = 6 and 5 that were euthanized by TC and IPT, respectively) were examined as fresh carcasses. Skin was removed from the ventral aspect of the body, and the body wall, including the ribs and clavicles, was cut along the costal margins to remove the keel and the attached pectoral musculature. The heart and viscera were examined in situ, and any coelomic hemorrhage was noted. The trachea, esophagus, and associated soft tissues were cut away from the neck, and the viscera (excluding the lungs) were removed from the body. The heart and great vessels were dissected free from surrounding tissues, and any pericardial or perihepatic hemorrhage and gross tissue damage were noted. The lungs were removed and examined separately, and any gross tissue damage was noted.

Tissues from all sparrows (n = 10 and 7 euthanized by TC and IPT, respectively) and 2 starlings (1 euthanized by each method) were fixed in neutral-buffered 10% formalin solution prior to dissection. The keel was removed from the starlings and most of the sparrows (n = 12) as previously described, the presence of coelomic hemorrhage was recorded, and the head, limbs, and skin were removed before fixation of tissues for ≥ 3 days. After fixation, dissection proceeded as previously described.

Five sparrows (4 and 1 that underwent TC and IPT, respectively) were skinned and then fixed with the keel intact after removal of the head and limbs. After fixation, the keel was removed as described above and the viscera (including the lungs) were dissected free. Gross examination was performed by use of a dissecting microscope, and specimens were then prepared for histologic examination. The viscera were then trimmed en bloc and sectioned in the sagittal plane to include the heart, lungs, cranial aspect of the liver, and associated vessels. Tissue was embedded in paraffin, sectioned at a thickness of 4 µm, and stained with H&E stain. Step sections were made as necessary to evaluate structures of interest. Histologic sections were evaluated by a board-certified veterinary pathologist (MKK) who was blinded to the methods of euthanasia for these 5 birds.

Data analysis

Continuous recordings of monitoring data were captured during the procedure. End points were defined as apnea determined by capnography (to determine that ventilation ceased) and by observation of coelomic movement or excursions on video recordings; cessation of pulse as detected by the Doppler crystal; onset of any abnormal ECG pattern (ie, arrhythmia, fibrillation, loss of ventricular complexes, or asystole); and onset of isoelectric EEG as measured from recordings by use of the end of detectable electrical activity determined not to be artifactual. Time from the onset of the euthanasia technique (beginning of the pentobarbital injection [IPT] or application of the compressive pinch [TC]) to each of the end points, as well as the time between the different end points, was measured for each bird by means of a stopwatch and by marking the onset and end points on the EEG and ECG recordings. The esophageal temperature, heart rate, respiratory rate, P_{ETCO_2}, and total time of anesthesia were recorded immediately prior to initiation of the euthanasia technique. Subjective data were also recorded for occurrence of feather erection, gaping of the beak, or other body movements following initiation of TC or IPT.

Data distributions were assessed with the Shapiro-Wilk test. Several of the measured end point times were not consistent with a normal distribution, even after logarithmic transformation. Therefore, summary statistics were presented as median and first and third quartiles (calculated by inversion of the empiric distribution function), and nonparametric statistical tests were used. Values > 1.5 times the interquartile range above and below the median were indicated on box plots, but were not excluded from data analy-

Table 1—Summary statistics for end points of interest (time to cessation of arterial pulse as detected by Doppler method, apnea as determined by capnography or observation of video recordings, isoelectric EEG signal, and onset of abnormal ECG patterns) in anesthetized wild-caught house sparrows (*Passer domesticus*) and European starlings (*Sturnus vulgaris*) euthanized by TC (n = 6) or IPT (6).

	TC		IPT		
Species and variable	**Median**	**1st–3rd quartile**	**Median**	**1st–3rd quartile**	***P* value**
Sparrows					
Time to end point (s)					
Cessation of pulse	0.0	(0.0–0.0)	18.5	(15.0–22.0)	0.003
Apnea	13.5	(1.0–17.0)	26.0	(18.0–32.0)	0.109
Isoelectric EEG	19.0	(13.0–36.0)	6.0	(5.0–17.0)	0.199
Abnormal ECG	87.3	(59.2–175.7)	30.0	(14.9–223.5)	0.337
Starlings					
Time to end point (s)					
Cessation of pulse	9.5	(0.0–10.0)	151.0	(80.0–178.0)	0.004
Apnea	9.0	(7.0–10.0)	12.5	(4.1–17.0)	0.647
Isoelectric EEG	88.5	(27.0–128.0)	77.5	(44.0–146.0)	0.873
Abnormal ECG	103.9	(98.2–136.1)	360.0	(227.8–485.1)	0.025

Time to each end point was measured from the initiation of the indicated method (the moment when TC was started or the beginning of injection for IPT). The *P* values reflect results of comparison between treatment groups by Wilcoxon-Mann-Whitney rank sum test for each end point. Values of $P < 0.05$ were considered significant.

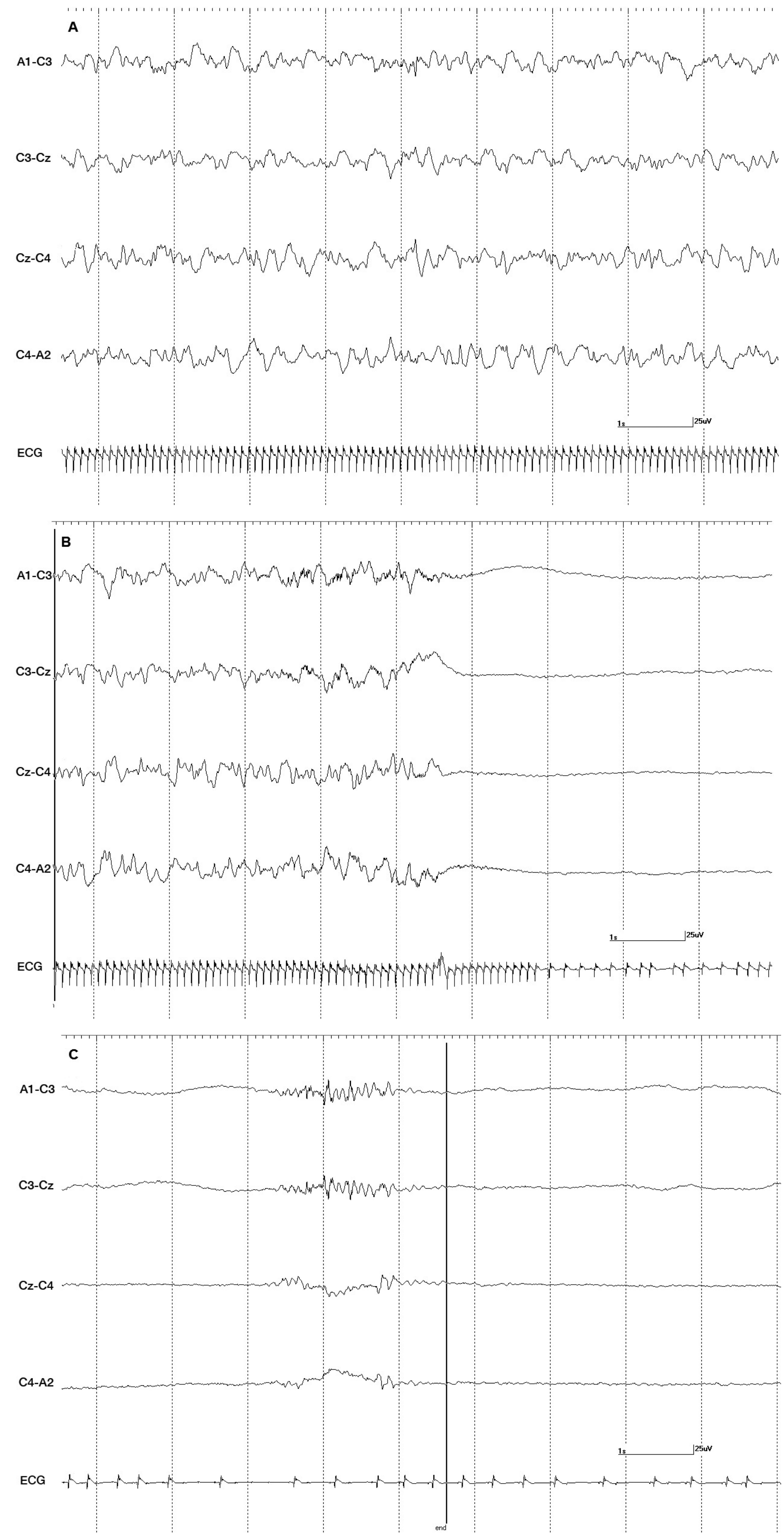

Figure 5—Representative EEG and ECG recordings from a sparrow during anesthesia maintained by propofol administration prior to euthanasia (A), during euthanasia by IPT with propofol discontinued (the solid vertical line far left indicates the onset of the euthanasia method; B), and at the cessation of all EEG activity (vertical line labeled end; C). Seventeen seconds elapsed between the times indicated by the vertical bars. Notice the transition from continuous EEG to burst suppression to isoelectric EEG. ***See*** Figures 1 and 2 for key.

sis except for comparison as otherwise noted. Data from starlings and sparrows were analyzed separately because species differences in drug metabolism and body size could be expected to affect time intervals for each euthanasia technique. Comparisons of time intervals were made between the TC and IPT groups for each species with the Wilcoxon-Mann-Whitney rank sum test, including calculation of confidence intervals for the difference in median values between groups. The Tukey method was used to define outliers for the time interval data. The proportion of birds with and without coelomic hemorrhage identified at necropsy was compared between the TC and IPT groups with the Fisher exact test. Values of $P < 0.05$ were considered significant. All analyses were performed with statistical software.[k]

Results

From the time of initiation of each euthanasia method, variables of interest were recorded for each bird, and summary statistics were calculated for each group **(Table 1)**. Both euthanasia techniques resulted in rapid conversion to an isoelectric EEG, and there was no significant difference in time from initiation of the euthanasia method to isoelectric EEG between TC and IPT groups for either sparrows ($P = 0.199$) or starlings ($P = 0.873$; **Figure 4**). One sparrow in the TC group had a prolonged time to isoelectric EEG (> 4 times the interquartile range for this group); there was still no significant ($P = 0.360$) difference in time to conversion to isoelectric EEG between methods for sparrows when this data point was excluded. Examples of EEG changes in each species were provided **(Figures 5 and 6)**. Breathing was detectable (by capnography and observation of excursions on recorded video) after the onset of application for the TC technique in 4 of 6 sparrows and 6 of 6 starlings. The exact time of apnea could not be determined for 1 starling euthanized with TC, despite careful review of the capnographic data and video images. Without this data point, there was no significant difference in time from initiation of euthanasia to apnea between the TC and IPT groups for starlings ($P = 0.647$), and the results were

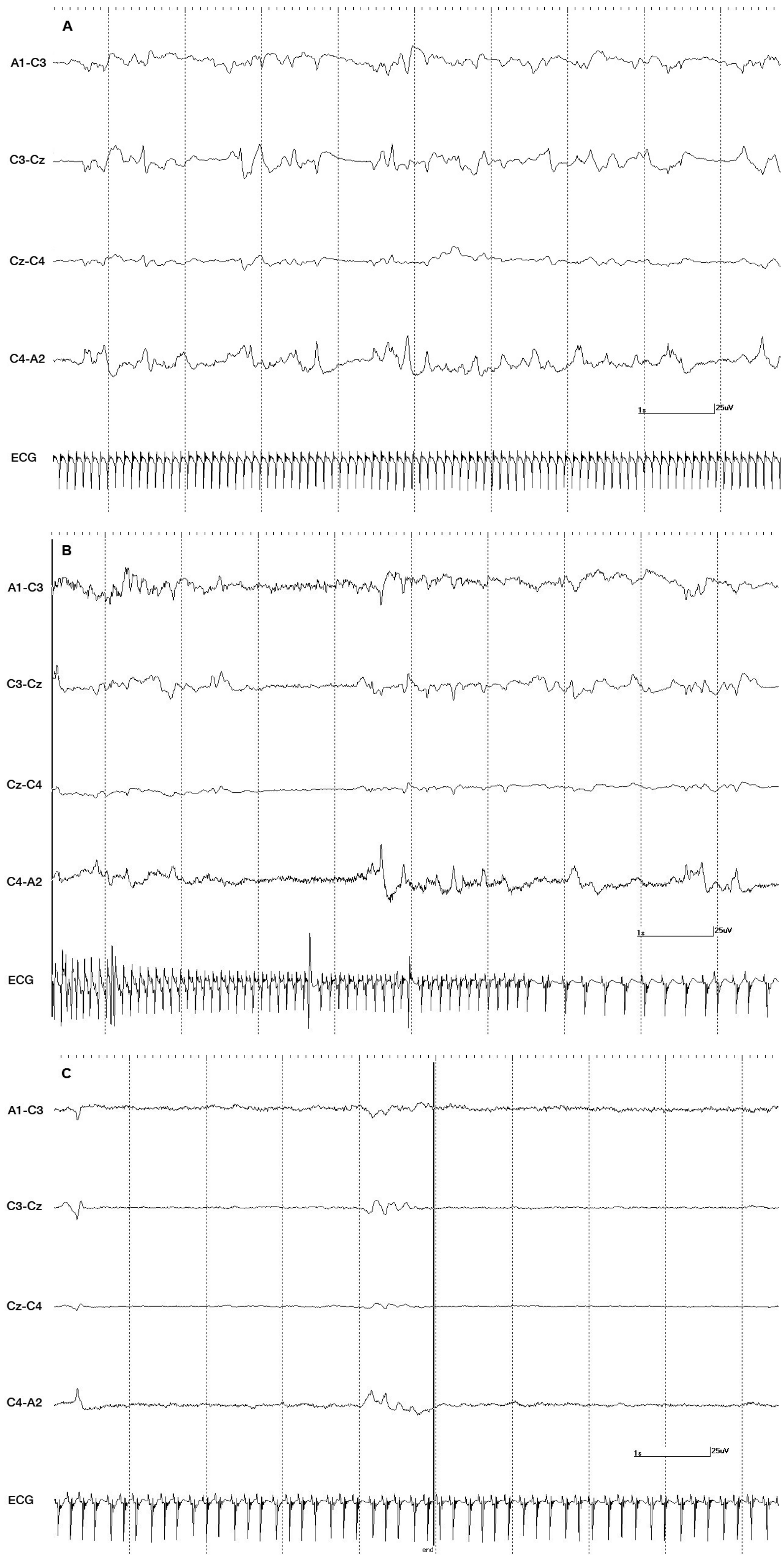

Figure 6—Representative EEG and ECG recorded from a starling during anesthesia maintained by propofol administration prior to euthanasia (A), during euthanasia by TC with propofol discontinued (B), and at the cessation of all EEG activity (C). Twenty-seven seconds elapsed between the times indicated by the vertical lines. Notice the transition from nearly continuous EEG (brief [< 1 second] voltage attenuations can be seen) to burst suppression to isoelectric EEG. ***See*** Figures 1, 2, and 4 for key.

similar for sparrows (P = 0.109; Figure 4). Apnea occurred prior to conversion to isoelectric EEG for all starlings, with no significant (P = 0.715) difference between groups (median intervals, –57.0 seconds for TC and –61.5 seconds for IPT). For sparrows, the timing of apnea relative to conversion to isoelectric EEG was not consistent in the TC group, whereas apnea occurred shortly after conversion to isoelectric EEG in the IPT group; there was no significant difference between groups when the 1 severe outlier was excluded (median intervals, –6.5 seconds for TC and 13.5 seconds for IPT; P = 0.068).

The time from initiation of euthanasia to cessation of arterial pulse detection by the Doppler method was significantly shorter for the TC group, compared with that for the IPT group, among sparrows (P = 0.003) and starlings (P = 0.004; Figure 4). One starling in the IPT group that received the 100-mg/kg dose had a prolonged time to cessation of pulse (> 5 minutes), although the value was not a statistical outlier. The difference in time to cessation of pulse between euthanasia techniques for this species remained significant (P = 0.006) when this bird was removed from the analysis. Cessation of pulse occurred simultaneously with or preceded apnea in all sparrows, with no significant (P = 0.197) difference between groups (median intervals, –11.5 seconds for TC and –1.0 second for IPT). For starlings, cessation of pulse also occurred prior to apnea in 4 of 5 birds euthanized by TC for which time to apnea could be determined, whereas apnea occurred prior to cessation of pulse in all starlings euthanized by IPT, resulting in a significant (P = 0.014) difference between groups (median intervals, –1.0 second for TC and 138.5 seconds for IPT). This difference remained significant (P = 0.021) when the 1 starling with prolonged time to cessation of pulse was excluded. For all birds euthanized by TC, pulses ceased prior to conversion to isoelectric EEG; the median interval was 17.0 seconds for sparrows and 76.0 seconds for starlings. For all sparrows euthanized by IPT, conversion to isoelectric EEG occurred prior to cessation of pulses, with a median interval of 10.5 seconds.

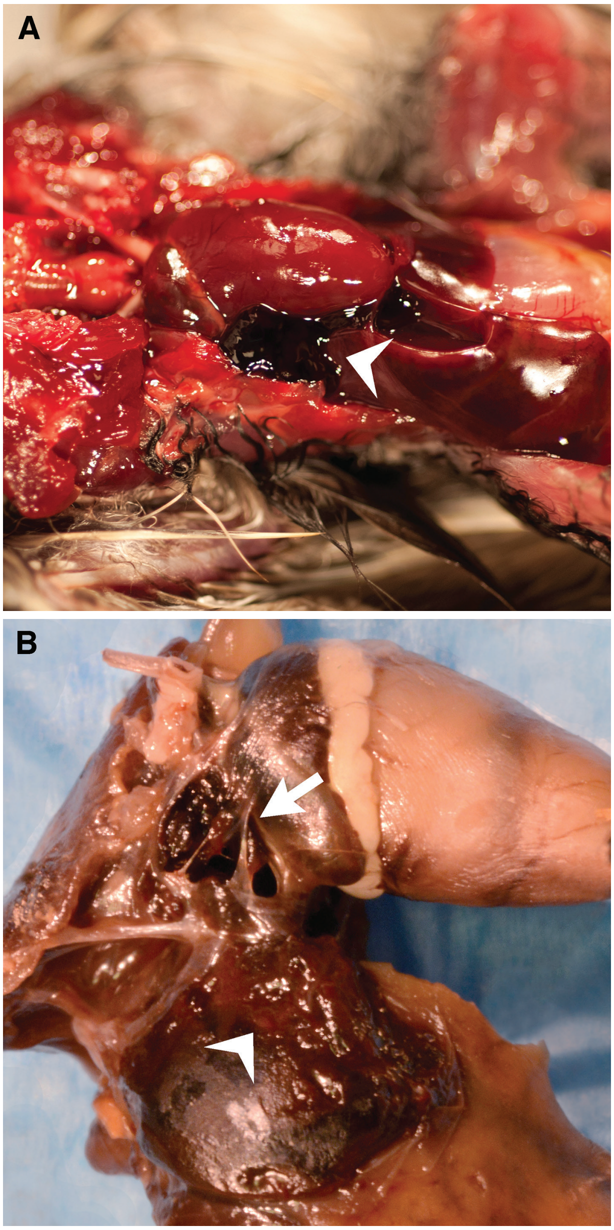

Figure 7—Digital photographs obtained during necropsies of a starling (A) and a sparrow (B) following euthanasia by TC. In panel A, the starling was dissected as a fresh carcass. The image is oriented with the head to the left and the ventral aspect toward the top. Notice hemorrhage between the heart and the liver (arrowhead). In panel B, the heart and partial liver of a sparrow dissected after fixation in neutral-buffered 10% formalin solution are shown. The organs are placed so that orientation approximates that in panel A. Notice the rupture of the vena cava at its junction with the right atrium (arrow) and associated intracoelomic hemorrhage (arrowhead).

Four of the 6 starlings euthanized by IPT converted to isoelectric EEG prior to cessation of pulses (median, 36.0 seconds). The remaining 2 starlings received pentobarbital doses at the high end of the range and had cessation of pulses prior to isoelectric EEG. Consequently, there were significant differences between the TC and IPT groups for the interval between cessation of pulses and isoelectric EEG among sparrows ($P = 0.010$) and starlings ($P = 0.004$). The difference remained significant ($P = 0.018$) for starlings when the 1 bird with prolonged time to cessation of pulses was removed from analysis.

Electrocardiographic activity, although frequently with an abnormal rate or rhythm, continued in all birds after conversion to isoelectric EEG and past the absence of a detectable Doppler pulse signal (Table 1). After administration of IPT and onset of ventricular fibrillation, a regular cardiac rhythm reoccurred in 1 sparrow for 2 minutes, followed by asystole. The remaining birds did not have recovery of normal ECG complexes after the onset of any ECG abnormality (arrhythmia, fibrillation, loss of ventricular complexes, or asystole). There was no significant ($P = 0.337$) difference in the time to onset of abnormal ECG between the TC and IPT groups for sparrows, but the onset of abnormal ECG was significantly ($P = 0.025$) more rapid for starlings euthanized by TC than for those euthanized by IPT.

For each species, 4 of 6 birds in the TC group had F_{IO_2} maintained at ≤ 0.40 prior to euthanasia, and 2 of 6 had F_{IO_2} maintained at ≥ 0.70. There were no significant differences between birds that had low and high F_{IO_2} for the time from initiation of euthanasia to the time of apnea (sparrows, $P = 0.814$; starlings, $P = 0.564$), cessation of Doppler pulse detection (sparrows, $P = 0.480$; starlings, $P = 0.634$), onset of isoelectric EEG (sparrows, $P = 0.355$; starlings, $P = 0.355$), or onset of abnormal ECG (sparrows, $P = 0.355$; starlings, $P = 0.643$).

In addition to time intervals between events, physiologic and anesthesia-related variables were compared between groups. There were no significant differences in esophageal temperature (sparrows, $P = 0.521$; starlings, $P = 0.109$), heart rate (sparrows, $P = 0.245$; starlings, $P = 0.868$), respiratory rate (sparrows, $P = 0.748$; starlings, $P = 0.810$), P_{ETCO_2} (sparrows, $P = 1.0$; starlings, $P = 0.262$), or total time of anesthesia prior to initiating euthanasia (sparrows, $P = 0.053$; starlings, $P = 0.688$) between the IPT and TC groups.

During the first 45 seconds of TC, feather erection followed by rapid relaxation of the feathers occurred in 3 of 6 sparrows and 2 of 6 starlings, and gaping of the beak occurred in 1 of 6 sparrows and 2 of 6 starlings. During infusion of pentobarbital, muscle movements occurred in 2 birds, including stretching of the wings in 1 sparrow and toe curling in 1 starling.

Gross pathological changes

Pathological findings were reviewed for the 6 birds/species/treatment group used for evaluation of euthanasia end points, as well as for the additional 6 birds captured and euthanized at the conclusion of the study. Among birds euthanized by TC, 9 of 10 sparrows and 5 of 7 starlings had grossly visible coelomic, pericardial, or perihepatic hemorrhage

(Figure 7). No hemorrhage was noted in 7 of 7 sparrows and 6 of 6 starlings euthanized by IPT. The difference in the proportion of birds with and without hemorrhage was significant between the 2 euthanasia techniques for both species (sparrows, $P < 0.001$; starlings, $P = 0.021$). The most common location for hemorrhage was between the heart and the cranial margin of the right liver lobe. Clotted blood in this location was typically bounded by membranes, but it was generally not possible to determine whether these were cranial thoracic air sacs, hepatopericardial or hepatoperitoneal membranes, or serosa of the liver. Three of 7 starlings and 7 of 10 sparrows had this pattern of hemorrhage observed. Clotted blood was in the pericardium of 1 of 10 sparrows and 2 of 7 starlings and was free in the coelom of 1 of 10 sparrows and 2 of 7 starlings.

In both species used in the study, the cranial portion of the liver tightly surrounded the apex and dorsal aspect of the heart, enveloping the caudal vena cava to its junction with the right atrium. Rupture of the caudal vena cava was suspected as a cause for the perihepatic hemorrhage noted on gross examination. However, dissection to separate the heart and vena cava from the liver resulted in artifactual damage in some of the starlings examined as fresh carcasses, complicating the interpretation of apparent ruptures in this vessel. Subsequently, 2 starlings (1 that had TC and 1 that had IPT) and all sparrows were fixed in formalin prior to dissection, and the dissection technique was modified to allow visual examination of this critical region with minimal disruption of the tissue. Among the birds examined after fixation, a rupture of the right atrium or vena cava was noted in the 1 starling and in 7 of 10 sparrows euthanized by TC (Figure 7). No ruptures were found in the 1 starling and 7 sparrows euthanized by IPT. The difference in proportions of birds with these findings could not be tested for starlings, but was significant for sparrows ($P = 0.002$). The most common location for rupture was at the junction of the caudal vena cava with the right atrium, noted in the starling and 4 of 7 sparrows. Two sparrows had a rupture of the cranial vena cava proximal to the junction with the right atrium, and 1 had a rupture of the caudal vena cava approximately 2 mm distal to the right atrium.

There was little evidence of trauma to other tissues on gross evaluation. Two starlings in the TC group had hemorrhage within the parenchyma of the cranial portions of the liver. None of the birds in either group had any grossly apparent damage to the lungs or any hemorrhage within the body wall suggestive of recent rib fractures. No fractures or other lesions suggestive of bone trauma were visible on postmortem radiographs of 6 sparrows and 6 starlings euthanized by TC.

Histopathologic changes

Histologic evaluation of 5 sparrows (4 and 1 from the TC and IPT groups, respectively) revealed that blood was present in various amounts around the heart base, between the caudal vena cava and the liver, and beneath the liver capsule in all birds of the TC group. Blood was also variably present surrounding the kidneys, gonads, spleen, proventriculus, lungs, or airways, but hemorrhage was not associated with parenchymal trauma in those organs and likely represented suffusion from the caval or atrial rupture sites. The liver was congested in 2 birds of the TC group, and 1 other bird in this group had congested pulmonary veins, consistent with obstruction of venous return to the heart at the time of death. In the bird euthanized by IPT, the heart and larger blood vessels were distended by coagulated blood, consistent with effects of pentobarbital injection, but there was no hemorrhage or vascular congestion present. There were also no rents or other penetrating defects in the wall of the heart or in the large blood vessels.

Ruptures of the vena cava had been identified grossly in 2 of the sparrows from the TC group that were subsequently prepared for histologic examination (Figure 7). The rupture sites were identified in the histologic sections and had attenuation of muscle fibers and hemorrhage dissecting through the vascular walls, atrial walls, or both, consistent with perimortem occurrence rather than artifactual damage to the fixed tissue. Full-thickness defects were not identified histologically in the 2 other sparrows of the TC group that did not have grossly apparent ruptures, even with stepwise sections performed to examine the area more extensively. However, in both birds, hemorrhages dissected partially through the walls of the caudal vena cava or right atrium. Both birds also had a pattern of suffusive hemorrhage similar to that seen in birds with identified ruptures. This pattern of hemorrhages suggested that cardiovascular ruptures occurred in these birds as well, although they were not represented in the histologic sections.

Discussion

In the present study, performed with passerine birds under a light plane of general anesthesia to compare TC and IPT for euthanasia, conversion to an isoelectric EEG was rapid in both sparrows and starlings, with similar time intervals measured from initiation of euthanasia for the 2 methods within each species. The isoelectric EEG pattern was continuously recorded for several minutes and appeared to be irreversible, with no recovery of EEG activity. This was used as an indicator of cessation of normal electrical activities of the brain and, in combination with irreversible apnea and cessation of arterial pulse detection by Doppler methods, was used to define death in this study. Determination of death after barbiturate infusion in other species often includes the absence of brainstem reflexes such as corneal and palpebral reflexes[1], but these variables could not be assessed in our study owing to the use of propofol anesthesia.

Cessation of pulse is an essential component for determination of death. In both sparrows and starlings, TC resulted in a more rapid cessation of pulse than did IPT. Digital compression directly over the heart for ≥ 60 seconds in the TC technique led to rapid, and in some cases immediate, pulse cessation, which occurred prior to conversion to isoelectric EEG in all birds. The cessation of pulse was likely due to the loss of effective cardiac contractions and, in most cases, rupture of the vena cava or atrium, leading to rapid loss of blood circulation. No return of pulses was detected after digital pressure was released from the thorax. Decreased blood flow to the brain creates tissue hypoxia, which rapidly leads to an isoelectric EEG, as reflected by these results. Conversely, the IPT in this study resulted in isoelectric EEG before the loss of cardiac function and cessation of pulse in 6 of 6 sparrows and 4 of 6 starlings. This finding was expected because of the known anesthetic effects of pentobarbital sodium and findings in horses euthanized by overdose of the same drug in another study.[14]

The time from initiation of euthanasia to an abnormal ECG or asystole was extremely variable with both methods used in the present study. For both methods in both species, the change in ECG occurred after conversion to isoelectric EEG and after cardiac output had ceased, as evidenced by the lack of Doppler detection of the arterial pulse. Similar findings in the aforementioned study[14] of horses supported the proposal that cardiac death occurs earlier and that ongoing ECG activity represents ineffective contraction with no cardiac output (electrical-mechanical dissociation) as the remaining cardiac muscle ATP is used. The presence of cardiac electrical activity does not imply effective cardiac pumping because the ECG is only a 2-D recording at the body surface of electrical fields generated by the heart, and it does not reflect the mechanical status of the heart.[8,15]

The time from initiation of euthanasia to apnea in birds was short (median time, ≤ 26 seconds for sparrows and ≤ 12.5 seconds for starlings) for both euthanasia methods in the present study, with no significant differences between methods for either species. In mammals, barbiturates depress the CNS beginning with the cerebral cortex, resulting in loss of consciousness that progresses to anesthesia. With an overdose, deep anesthesia progresses to apnea due to depression of the respiratory center, and this is followed by cardiac arrest.[1] Because birds were under anesthesia at the onset of both euthanasia techniques in the present study, it cannot be determined whether loss of consciousness would precede or follow apnea with either technique if used on an awake bird. Apnea occurred prior to conversion to isoelectric EEG in all starlings regardless of euthanasia method. The relative timing of apnea and conversion to isoelectric EEG was less consistent in sparrows, but there was no significant difference between groups for either species. Cessation of pulse was simultaneous with or preceded apnea in all birds euthanized by TC. Therefore, it is unlikely that apnea can be considered the cause of death with TC.

The AVMA-approved method to euthanize conscious birds is IV injection of pentobarbital, and TC has historically been used as a field technique to provide a rapid death of small birds. For the present study, we elected to use anesthetized birds under controlled experimental conditions to provide instrumentation and monitoring necessary to obtain accurate physiologic end point data for each technique and to reduce covariables in determination of similarities and differences for these 2 euthanasia techniques. As such, this study provides relevant and previously unavailable information about time to loss of brain activity and death; however, the study was not designed to investigate the aspects of pain or distress associated with either treatment. Since it is now established that the times to death with TC are similar to those for the use of pentobarbital, further studies could evaluate biomarkers of distress in birds that had not been anesthetized.

The variations in times from initiation of euthanasia by IPT or TC for the variables of interest in this study might have been affected by several factors such as the sizes of birds, depth of anesthesia at the time of euthanasia, variability in dose and rate of pentobarbital injections, or investigator who performed TC. Another variable among birds was the period of time required after discontinuation of inhalation anesthesia until titration to a light plane of anesthesia with propofol, because the determining factor for initiation of euthanasia was maintenance of a continuous EEG pattern free of burst suppression. A fixed volume of pentobarbital was delivered to sparrows because the birds were similar in body size, which created a small range of doses. In contrast, starlings received a dose calculated according to body weight,[12] but the dose was increased from the lower dose of 100 mg/kg (delivered to the first 2 starlings) to the higher dose of 220 mg/kg, both within the recommended range, because 1 bird given the lower dose had a prolonged time to cessation of pulse, despite a rapid conversion to isoelectric EEG. All TC procedures were performed by 2 investigators (AE and IEE) who used the same technique and had similar levels of experience with the method, and we consider it unlikely that differences in application of TC contributed to the variations in time intervals.

Because the study required death of the birds, the sample size was chosen with the goal of minimizing the number of animals used to obtain meaningful results. To evaluate the risk of type II error due to low sample size or data variability, confidence intervals were calculated for the difference in medians between treatment groups for each end point that did not have a significant effect in the rank sum test (data not shown). A true effect size of zero falls within the confidence interval for any test in which the null hypothesis cannot be rejected. For time to apnea and time to onset of isoelectric EEG in sparrows, the confidence intervals were asymmetric around 0, such that a moderate decrease in the interval width could reveal a significant effect. This asymmetry did not prove a type II error but suggested that these variables might warrant further study. For the time to onset of abnormal ECG in sparrows, time to apnea in starlings, and time to onset of isoelectric EEG in starlings, the confidence intervals were symmetric around zero, and the risk of type II error was considered to be low.

When used in a field setting, TC is performed without supplemental oxygen administration (ie, at an F_{IO_2} of 0.21). In the present study, anesthesia was induced with anesthetic gas in oxygen, and a ventilator was used as needed for respiratory support. Because of concerns that artificially high F_{IO_2} and, therefore, blood oxygenation would affect the time when vital signs would be sustained after euthanasia, F_{IO_2} was lowered after induction of anesthesia. As a result of variability in anesthetic time, an F_{IO_2} of 0.21 was not reached for all birds prior to initiation of euthanasia. However, for both sparrows and starlings, there was no significant difference in the time from initiation of euthanasia to any of the defined monitoring end points between birds that had an $F_{IO_2} \leq 0.40$ and those that had an $F_{IO_2} \geq 0.70$ at the time TC was initiated.

During the first 45 seconds of TC, feather erection and relaxation were observed in 3 of 6 sparrows and 2 of 6 starlings but did not occur in any of the birds (6 birds/species) that had IPT. Sudden feather erection has been anecdotally associated with cardiac arrest or reduced cardiac blood flow of anesthetized birds.[16] It has also been noted to occur in poultry killed with CO_2 and in euthanasia of turkeys during the tonic phase of convulsions,[16–18] and the implication of feather erection during euthanasia needs further exploration. Beak gaping and nonpurposeful body movements have been noted to occur in other avian euthanasia studies. These are considered signs of distress when they occur prior to recumbency but are considered reflexive when EEG activity and brainstem reflexes are absent.[19–21] In the present study, the occurrence of beak movements relative to the EEG pattern was inconsistent in the 3 birds for which it was observed, and the small numbers made interpretation difficult.

The most common postmortem finding unique to birds euthanized by TC was hemorrhage in the coelomic cavity, primarily between the heart and the cranial margin of the right liver lobe. Dissection of formalin-fixed specimens confirmed the source of hemorrhage was from a rupture of the right atrium or vena cava, often at the junction of the caudal vena cava and the right atrium. Microscopic examination of tissues from 4 sparrows euthanized by TC revealed pericardial hemorrhages that dissected under the serosal surfaces of the caudal vena cava and beneath the liver capsule, supporting the conclusion that rupture of the caudal vena cava or right atrium had occurred. Slides of tissues from 2 of the 4 sparrows included sections through avulsions of the right atrium or the proximal segment of the vena cava. The margins of the defects had frayed edges, with attenuation of myofibers and small amounts of intramural hemorrhage. Three of the 17 birds necropsied after TC did not have gross evidence of coelomic hemorrhage, indicating that, although rupture of the atria or vena cava frequently occured secondary to compression of the heart, it was not essential for euthanasia. The TC technique involved direct pressure application over the heart, leading to obstruction of venous return and stopping cardiac output. The rapid cessation of pulse and apnea that occurred during TC of birds in the study was consistent with this interpretation.

The results of TC in birds of this study provided information to support that this commonly used term is misleading and that the terminology promotes a misconception that suffocation is the cause of death.[22,23] The appropriate TC technique involves digital pressure directly over the location of the heart, with 2 fingers placed on either side of the body, dorsal to the pectoral muscles, where the thoracic body wall is thin and very pliable. Postmortem evidence in combination with physiologic events indicated that the heart was directly compressed, often leading to rupture in the thin-walled regions of the vena cava or atrium. For these reasons, the authors contend that the technique would be more appropriately described as rapid cardiac compression.

In addition to the comparison of physiologic end points for euthanasia by TC and IPT, the gross and histologic postmortem findings provided insights into the physical cause of death following TC. The knowledge gained in this study can be used to assist institutional animal care and use committees and researchers in further assessment of the appropriate methods of euthanasia for small birds.

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Footnotes

a. Kaytee Products Inc, Chilton, Wis.
b. Piramal Healthcare Ltd, Bethlehem, Penn.
c. BD Insyte, Franklin Lakes, NJ.
d. Teleflex Medical, Research Triangle Park, NC.
e. Hallowell EMC, Pittsfield, Mass.
f. GE Healthcare BioSciences, Pittsburgh, Penn.
g. Parks Medical Electronics Inc, Aloha, Ore.
h. Nihon Kohden Inc, Irvine, Calif.

i. Ives EEG Solutions Inc, Newburyport, Mass.
j. AstroNova, West Warwick, RI.
k. Stata, version 13.1, StataCorp, College Station, Tex.

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COMMENTARY

Rapid cardiac compression: An effective method of avian euthanasia

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ABSTRACT
Proper methods for euthanasia are critical for research with birds. Training in these methods is generally required by institutional animal care and use committees for any research that involves handling of birds, even if the intent is for birds to be released into the wild. Rapid cardiac compression (RCC) remains a preferred method for quick euthanasia in field settings but has not been described adequately in the literature. We describe proper application of RCC for euthanasia of small birds. We also provide external cues for a bird as it progresses toward death as well as other considerations when using RCC. Note that RCC is also known as "thoracic compression," but that term is not biologically accurate and should be abandoned.

Keywords: bird, euthanasia, method, rapid cardiac compression, thoracic compression

Compresión cardíaca rápida: Un método efectivo para la eutanasia de aves

RESUMEN
Los métodos adecuados de eutanasia son críticos para la investigación con aves. Generalmente se requiere entrenamiento en estos métodos por parte de los comités institucionales de bienestar y uso animal para cualquier investigación que involucre la manipulación de aves, incluso si la intención es la liberación de todas las aves al medio silvestre. La compresión cardíaca rápida (CCR) sigue siendo un método recomendado para una rápida eutanasia en condiciones de campo pero no ha sido descripta adecuadamente en la literatura. Describimos una adecuada aplicación de la CCR para la eutanasia de aves pequeñas. También describimos los signos externos de un ave en el proceso de muerte así como otras consideraciones cuando se usa CCR. Se conoce también a la CCR como compresión torácica, pero este término no es biológicamente preciso y debería abandonarse.

Palabras clave: ave, compresión cardíaca rápida, compresión torácica, eutanasia, método

Ornithologists working in the field are faced with many challenges in ensuring that their research is conducted both safely and ethically. Proficiency in euthanasia is fundamental to all field studies in which birds are handled, regardless of whether death is an endpoint of the fieldwork (e.g., museum collections or specimen-based research) or the bird is to be released unharmed. The need for euthanasia in the former cases is self-evident, but euthanasia may also be required where death is not the endpoint (e.g., banding, tissue collection, attachment of devices) because accidents can happen. Various methods of euthanasia are suitable for birds, but only a few are practical in a field setting.

Two federal animal welfare laws regulate research involving animals in the United States. First, the Animal Welfare Act of 1966 (and corresponding regulations) dictates the treatment of animals in research, exhibition, and transport; it requires that methods of euthanasia be humane. Second, the Public Health Service Policy on Humane Care and Use of Laboratory Animals requires the use of euthanasia methods classified by the American Veterinary Medical Association (AVMA) as acceptable or acceptable with conditions. In practice, the AVMA guidelines are the standard upon which institutional animal care and use committees (IACUCs) rely when reviewing research protocol applications. The primary experience of IACUC members involves reviewing protocols where death is an endpoint, typically for captive small mammals. Fewer proposals are evaluated for research with wild birds. In addition, AVMA-approved methods superficially address the challenges faced by scientists and museum collectors working in remote areas or over extended periods. The availability, transport, shelf life, and use of euthanasia drugs or syringes are problematic in many field situations.

For the past century, thoracic compression (TC) has been the preferred method for euthanizing small birds in field studies throughout the world (Winker 2000, Fair et al. 2010). This method has been handed down from experienced researchers to mentored biologists for decades. Experienced field researchers attest that TC is quick, humane, and yields the highest-quality specimens for museums or research requiring an intact carcass. Other AVMA-approved physical methods of euthanasia, such as cervical dislocation or decapitation, render a specimen unusable for many needs. The most recent AVMA Guidelines for the Euthanasia of Animals (AVMA 2013) reclassified TC from "acceptable with conditions" to "unacceptable until data could be produced in a clinical study clarifying the rapidity (time to loss of consciousness) and cause of death from TC." The AVMA decision jeopardized specimen-based research for all disciplines that rely on TC in field studies. This decision led to a study assessing the efficacy of TC at the University of California at Davis, where clinicians at the School of Veterinary Medicine collaborated with museum specialists at the Museum of Wildlife and Fish Biology (Paul-Murphy et al. 2017).

TC was previously considered controversial because of concern that death was caused by suffocation (Bennett 2001, Ludder 2001, AVMA 2011). In fact, TC uses direct application of pressure over the heart, leading to obstruction of venous return and stoppage of cardiac output, and in many cases results in rapid rupture of the thin-walled regions of the vena cava or atrium and near-instantaneous cessation of brain and pulse activity (Paul-Murphy et al. 2017). Furthermore, when TC was compared to the interosseous injection of pentobarbital, which the AVMA (2013) states is the quickest and most reliable method, there was no significant difference in either time to loss of consciousness or time to death in small birds (Paul-Murphy et al. 2017). Because the term *thoracic compression* has been associated in the literature with suffocation, the method can be viewed unfavorably. More importantly, thoracic compression is not a biologically accurate description of the cause of death. We suggest that the term should be abandoned and replaced with *rapid cardiac compression* (RCC).

RCC has not been adequately described, peer-reviewed, or illustrated (Winker 2000, Fair et al 2010). The Ornithological Council (2013) created a peer-reviewed position paper on the method, which many researchers have used for IACUC authorization. Our goal here is to follow the clinical study of Paul-Murphy et al. (2017), including details appropriate for practitioners and IACUCs. The authors' (A.E. and I.E.E.) experience in the use of RCC is extensive, including more than 35 years using RCC in field studies.

In most field cases when RCC is used, the bird has been captured by mist net or wounded by firearms. In all cases, euthanization should be rapid and humane; this is particularly true for wounded birds. Many IACUCs currently require that a wounded bird be shot again to euthanize it quickly. Gunshot is an approved method of euthanasia (AVMA 2013), but a second shot from close range can be damaging to the carcass. RCC provides a better alternative to shooting a bird a second time. For example, a bird may be shot and dropped into heavy cover; if it is found alive and capable of escape, there is risk of losing the bird during the time necessary to establish proper distance for a second shot. This delay would also prolong the bird's suffering. Therefore, quickly capturing the bird and applying RCC can minimize the bird's pain and stress. Birds inadvertently injured during handling or found injured can also be euthanized most rapidly using RCC.

When a bird is captured in a mist net and death is the determined endpoint, it should be euthanized directly in the net to eliminate the additional stress of extraction from the net. In these cases RCC can be quickly applied to the bird while it remains in the net. Alternatively, when a bird is to be removed alive from the mist net for procedures prior to euthanasia, it should be placed in a cloth handling bag following bird-banding protocols. Extraction of birds from a mist net and a description of the bander's grip are detailed in the *North American Banders' Study Guide* (North American Banding Council 2001). The bander's grip is appropriate prior to RCC because the bird can be easily manipulated into the three-point method described below, which minimizes stress.

There are several variants of RCC. The three-point method recommended here ensures proper euthanasia. RCC requires two hands. The dominant hand is used for the compression position while the nondominant hand supports the keel and prevents the bird from twisting out of position (Figure 1). The bird is rotated from the bander's grip into position for RCC, and then the dominant hand is used to access the cardiothoracic area dorsally by placing the thumb under one wing and the index or middle finger under the other (Figure 1A, 1B). The heart is located under a "triangle" formed by the coracoid, ribs, and scapula. This triangle is marked by a soft, indented space between the first rib and the coracoid (Figure 2). The forefinger and thumb are placed over this triangle on both sides of the bird, with the tips of the fingers touching the coracoid (Figure 1C, 1D; Figure 3). Cardiac contractions can sometimes be felt when fingers are correctly placed. Once fingers are in position, the fingers of the nondominant hand are placed on the keel to stabilize the bird and keep it from twisting out of position (Figure 1E). It takes only a few seconds to place the fingers properly. With minimal practice on birds that will not be euthanized, a researcher can prepare to administer RCC. Once fingers are in proper position, the forefinger and thumb of the dominant hand are rapidly squeezed together with an anterior ventral motion that ensures the heart is between the fingertips. Pressure is applied rapidly, with fingertips nearly touching

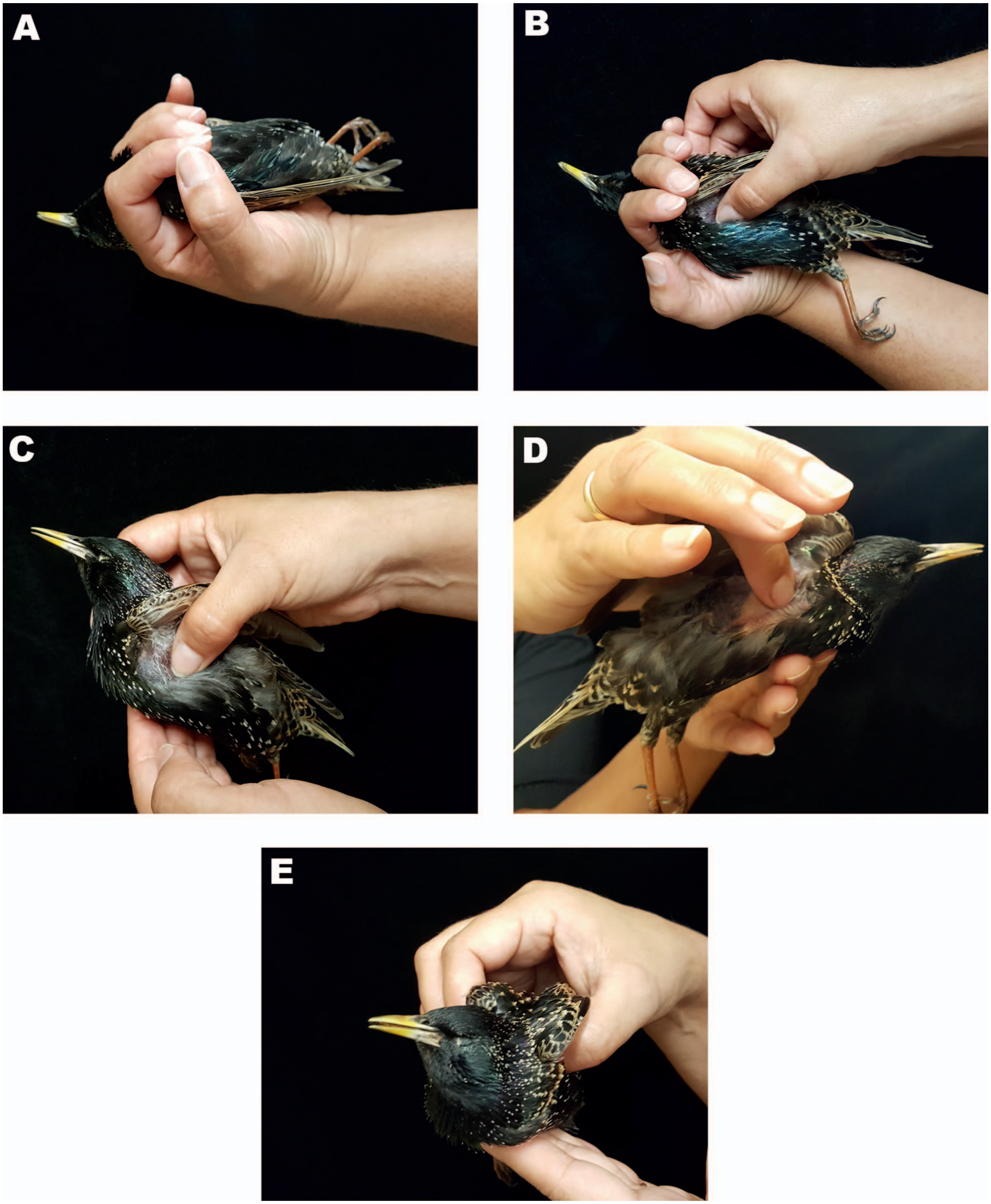

FIGURE 1. Proper handling of a bird (here, a European Starling) during rapid cardiac compression (RCC). A dead bird was used for these photographs. Flank and downy feathers were removed to better show finger placement. (**A**) Bird in bander's grip. (**B**) Maneuvering bird from bander's grip to RCC position. (**C**) Correct position of thumb. (**D**) Correct position of forefinger. (**E**) Anterior view of correct position of both hands during RCC.

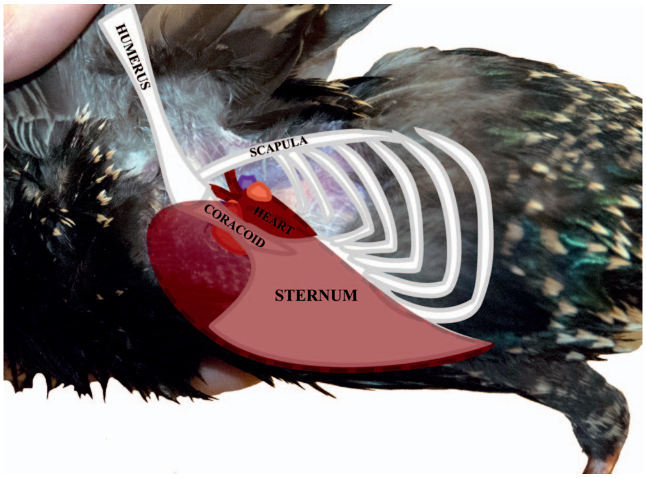

FIGURE 2. Lateral view of European Starling anatomy relevant to rapid cardiac compression.

through the body cavity, compressing the heart between the fingertips. The continuous pressure immediately restricts the heart from beating, and the fingers of the nondominant hand are held steady, thus keeping the bird firmly in place. The pressed fingers are held together and not released until external cues indicate that the bird has expired. For a bird ranging in size from a House Sparrow (*Passer domesticus*) to a European Starling (*Sturnus vulgaris*), death occurs in 25–30 s (Paul-Murphy et al. 2017). However, clinical trials found variation within species to warrant application of RCC for an additional 30 s beyond perceived death.

External Mortality Cues

When applied correctly, compression over the heart stops contractions, thereby stopping the pulse instantaneously. Birds in the study were under light anesthesia, and EEG activity became flat (isoelectric) in approximately 19–88 s for sparrows and starlings, respectively (Paul-Murphy et al. 2017). Death from RCC is presumed to have a similar time course in a conscious bird of similar size (Paul-Murphy et al. 2017). External behavioral cues that indicate progression toward death include the following: (1) The bird will shudder and stiffen, can show an initial shaking of the head, and the eyes may close; (2) agonal gaping will sometimes occur at $\sim$15 s; (3) feather erection, particularly on the head and dorsal neck region, will sometimes occur, followed by rapid relaxation and limp neck as the bird expires; (4) upon death, pupillary dilation (miosis) occurs. Continuing the cardiac compression for an additional 20–30 s beyond the neck going limp will ensure the bird is dead. Almost all birds will defecate upon application of RCC; thus, we recommend holding the bird with the vent toward the ground, with the tail pulled back so that droppings do not soil feathers.

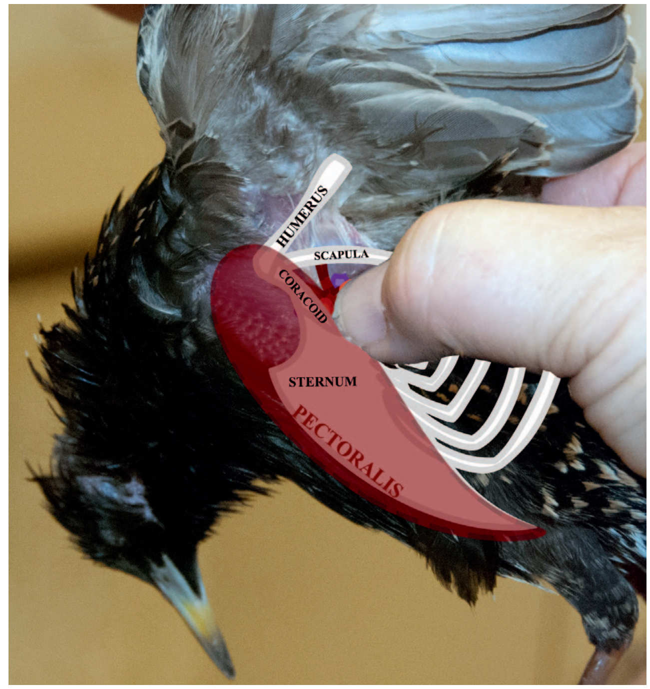

FIGURE 3. Proper placement of the dominant (compression) hand on a European Starling. To show the position of the dominant hand, the nondominant hand is not in position for this photograph (see Figure 1).

Other Considerations

The most common deviation from the method described above is a ventral approach to placing fingers in the coracoid triangle, thereby encircling the pectoral muscles. However, the ventral approach can be more challenging for correct finger placement, and thus the heart may not be properly compressed. Therefore, we do not recommend the ventral approach. Another technique is to approach the bird from the dorsum as described, but without using the second hand to stabilize the bird's sternum. In this position, a larger bird can twist out of position during RCC. Thus, using the second hand to prevent the bird from twisting out of position, working in unison with the hand applying compression, ensures the most reliable and repeatable results.

The size of the bird is an important consideration. If it is too large, then the heart cannot be compressed in a manner that ensures RCC. The body-mass limit for effective RCC is variable, based on the taxon and the experience of the researcher. Paul-Murphey et al. (2017) considered only two species, with average weights of 26 g (House Sparrow) and 71 g (European Starling). Studies have not been undertaken to assess the effectiveness of the method on larger or smaller birds. RCC is a standard technique for passerines, which rarely exceed 300 g, but the procedure has also been effectively used for birds up to 500 g such as gallinaceous birds, doves, and shorebirds

(A.E. personal observation; K. Winker and B. K. Schmidt personal communication). The size and strength of the researcher's hands may limit the application of the technique for birds exceeding 500 g.

The AVMA has approved guidelines for the use of RCC conditional upon the bird first being anesthetized (see "Adjunctive methods" in AVMA 2013:83). Anesthesia may be suitable for laboratory animals but is not practical in the field and frequently contaminates specimens. Moreover, if RCC is deemed humane (as argued here), there is no reason to require anesthesia. Euthanasia by RCC reduces the time a bird is handled, thus minimizing discomfort and stress during the time necessary to prepare and administer anesthesia. This advantage is directly supported by the AVMA (2013:84): "When properly used by skilled personnel with well-maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane, and practical than other forms of euthanasia."

Training, Institutional Support, and Approved Use Recommendations

Performing a physical method of euthanasia, whether it is RCC, cervical dislocation, or decapitation, requires proper training to ensure humane euthanasia. The *AVMA Guidelines on Euthanasia* require training for other physical methods, and this requirement should be expected for RCC. Training oversight is the responsibility of the IACUC and may come from another investigator, veterinarian, or technician with substantial RCC experience.

Advantages of Rapid Cardiac Compression

(1) RCC has been proven to result in rapid loss of consciousness and death (Paul-Murphy et al. 2017). (2) RCC yields carcasses in optimal condition for use as specimens for research collections and other purposes because the carcass remains intact and there is no chemical contamination. (3) RCC does not require drugs or chemicals, and it can be applied in any field setting.

Disadvantages of Rapid Cardiac Compression

(1) RCC may be discomforting to personnel performing or observing the method. (2) RCC requires mastering technical skills to ensure rapid loss of consciousness. (3) RCC is limited to smaller birds (although it is suitable for the overwhelming majority of bird species).

General Recommendations

Rapid cardiac compression is acceptable for euthanasia of smaller birds when performed by trained individuals. The applicant to the IACUC is responsible for ensuring that personnel performing RCC have been properly trained.

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Fact sheet: Rapid cardiac compression

Introduction

Rapid cardiac compression (formerly known as thoracic compression) is a method of euthanasia[1] widely used by ornithologists when collecting small birds for museum specimens and tissue samples. On occasion, rapid cardiac compression is also used to euthanize small birds that have been inadvertently injured during research manipulations or that have been found injured by ornithologists when working in the field, when veterinary care is not available and first aid is unlikely to result in the bird's survival. Ornithologists use rapid cardiac compression because it causes very rapid loss of consciousness and death and because it has long been recognized, based on decades of experience, that the method is humane and certainly the most humane method available in many field situations.

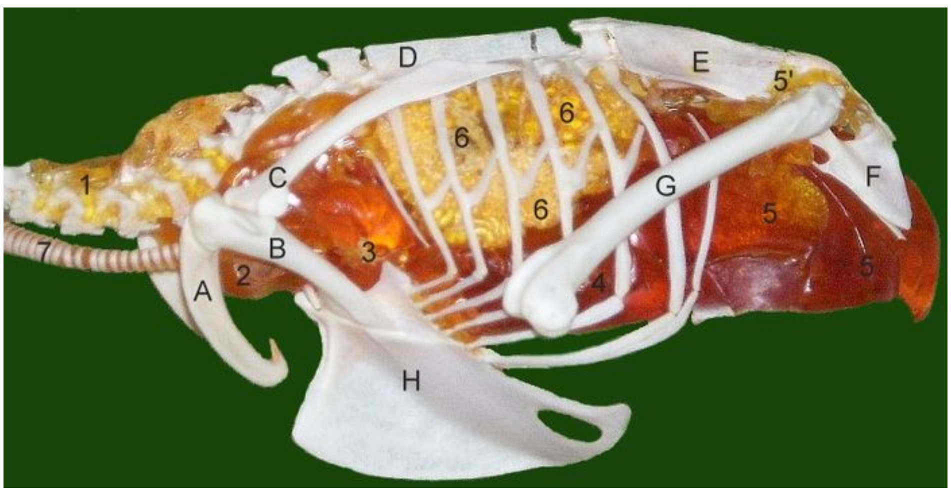
The lack of studies that measure brain activity to assess loss of consciousness resulting from rapid cardiac compression caused some in the veterinary medical community to raise concerns about the method. They also believed – mistakenly – that the method entailed the crushing of the thorax and the suffocation of the bird, based in part, no doubt on the terminology. These concerns caused the American Veterinary Medical Association (AVMA) to reclassify it as unacceptable in the 2013 revision of the euthanasia guidelines. That reclassification led some Institutional Animal Care and Use Committees (IACUCs) to require extraordinary justification for approving its use. After consulting with the AVMA as to the type of data it would consider sufficient to reconsider the classification, the Ornithological Council in late 2012 requested a research proposal from a leading research veterinarian to generate data that measures brain activity to determine time to loss of consciousness and death. That study has now been published (Paul-Murphy et al. 2017). The study compared intraosseous pentobarbital treatment (IPT) and thoracic compression (TC) on time to circulatory arrest and an isoelectric electroencephalogram (EEG) in anesthetized passerine birds. The study was designed to compare time to circulatory arrest, time to isoelectric EEG, and other end points of interest between the 2 euthanasia methods among birds within each species. It was determined that there was no significant difference in time from initiation of the euthanasia method to isoelectric EEG between TC and IPT groups for either sparrows ($P = 0.199$) or starlings ($P = 0.873$; **Figure 4**). Further, the time from initiation of euthanasia to cessation of arterial pulse detection by the Doppler method was significantly shorter for the TC group, compared with that for the IPT group, among sparrows ($P = 0.003$) and starlings ($P = 0.004$; Figure 4). In the discussion, the investigators explained that "cessation of pulse is an essential component for determination of death. In both sparrows and starlings" and that TC resulted in a more rapid cessation of pulse than did IPT.

Also significant were the necropsy results, which supported the contention that the term "thoracic compression" is misleading and that the terminology promotes a misconception that suffocation is the cause of death. The paper confirmed that the appropriate TC technique involves digital pressure directly over the location of the heart, with 2 fingers placed on either side of the body, dorsal to the pectoral muscles, where the thoracic body wall is thin and very pliable. Postmortem evidence in combination with physiologic events indicated that the heart was directly compressed, often leading to rupture in the thin-walled regions of the vena cava or atrium. For these reasons, the authors contend that the technique would be more appropriately described as rapid cardiac compression.

The Ornithological Council will ask the AVMA to reclassify the method in light of this research. In the meantime, for those who might find it necessary to use this method and for the IACUC members who must decide if it is scientifically justified, this fact sheet is intended to provide information about rapid cardiac compression – including reports of observations of behavioral and physiological changes that support the contention that rapid cardiac compression results in the rapid loss of consciousness and a rapid death.

Description of the method as used for birds

Rapid cardiac compression involves holding the bird between the thumb and forefinger of one hand. The researcher's thumb and forefinger are positioned under the bird's wing, from the posterior, and below the spine. Two fingertips are positioned between the spine and the coracoid, and above the anterior edge of the pectoral muscle, in the space indicated by the numeral 3 on this image:



The forefinger of the other hand is placed against the ventral edge of the sternum, just below the furculum. Squeezing the fingers together rapidly with the force of a hard pinch in the space above the coracoid prevents air from entering the air sacs and causes the heart to stop (Winker 2000). The pressure placed against the sternum results from the position in which the bird is held. It is slight pressure relative to the force placed against the soft tissue above the coracoid, because the need for an intact specimen, including an undamaged skeleton, precludes the use of force that would be sufficient to break the sternum or ribs.

The bird loses consciousness within a few seconds. Continued pressure is maintained on the thorax to ensure that the heart won't restart. Death follows quickly thereafter. That corporal trauma is minimal is easily and immediately verified during preparation of the corpse by the fact that there is often no evidence of hemorrhage inside the bodies of birds euthanized in this way and the absence of broken bones or crushed organs.

This method requires only seconds of handling, unconsciousness occurs extremely quickly, and, in the hands of an experienced researcher, the method is relatively full-proof to error; the sensitivity of one's own hands allows for a degree of monitoring not possible by any other method.

Additional evidence of rapidity

One ornithologist (Bostwick, 2010 pers. comm. to the American Veterinary Medical Association) measured the interval between the application of pressure and the loss of consciousness as determined by relaxation of the feathers, loss of body tension, and reduction in eye "clarity" (Erasmus *et al.* 2009). Sudden feather erection was assumed to indicate time of death; this same observation that has been made in studies to determine behavioral reactions of poultry to carbon dioxide (Gerritzen *et al.* 2007). In some of the 35 small passerines studied, loss of consciousness appeared to occur virtually simultaneously with the application of pressure. It has long been thought that rapid cardiac compression can cause a sudden and significant increase in hydrostatic pressure pulse to the brain, resulting in virtually immediate loss of consciousness. Dissection of the brains of these birds immediately after death reveals small amounts of blood in the brain, which would be consistent with this mechanism. In other cases, loss of consciousness occurred in 5-10 seconds, during which time the birds gaped (opened their bills) for air.

Five highly experienced field ornithologists - each having used rapid cardiac compression on at least
~500->1000 birds over many years of field collecting – reported their observations on the length of time between the initiation of rapid cardiac compression and the loss of consciousness:

The consensus among the five researchers was that birds weighing less than 100 g were typically unconscious within 5 seconds after beginning rapid cardiac compression and dead within 15-20 seconds. Birds between 100-250 g were unconscious within 10-20 seconds and verifiably dead within 20-60 seconds. More confidence was associated with the time estimates for smaller birds, and less confidence in estimates and greater variation in bird response were described for larger birds.

These rates, as well as those reported by Paul-Murphy et al. (2017) compare favorably to those reported for loss of consciousness resulting from the use of carbon dioxide. According to the AVMA's 2013 guidelines, which classify CO_2 as "acceptable with conditions," "time to unconsciousness with CO_2 is dependent on the displacement rate, container volume, and concentration used. In rats, unconsciousness is induced in approximately 12 to 33 seconds with 80 to 100% CO_2 and 40 to 50 seconds with 70% CO_2 (citation omitted)[2]. Similarly, a rapidly increasing concentration (flow rate > 50% of the chamber volume per minute) induces unconsciousness in only 26 to 48 seconds (citations omitted). Leake and Waters (citation omitted) found that dogs exposed to 30% to 40% CO_2 were anesthetized in 1 to 2 minutes. For cats, inhalation of 60% CO2 results in loss of consciousness within 45 seconds, and respiratory arrest within 5 minutes (citation omitted). For pigs, exposure to 60 to 90% CO_2 causes unconsciousness in 14 to 30 seconds (citations omitted) with unconsciousness occurring prior to onset of signs of excitation (citations omitted)."

Shorter times to unconsciousness reduce stress and pain to an animal. During the time an animal remains conscious, a number of painful or distressful reactions to CO_2 have been documented, including "(1) pain due to formation of carbonic acid on respiratory and ocular membranes, (2) production of "air hunger" and a feeling of breathlessness, and (3) direct stimulation of ion channels within the amygdala associated with the fear response."[2]

Why rapid cardiac compression is used in ornithological research

The purposes for scientific collecting of birds entail very different concerns than those resulting from the need to euthanize an animal at the end of an experimental procedure, which entails a desire to end suffering or simply a means to dispose of an animal that is not suitable for future research. In ornithological research, birds are collected in the field for specific purposes. In some cases, they will become museum specimens (either skins, fluid preserved, whole specimens, skeletons, or some combination of these) and are stored in research and teaching collections. In other cases, birds are collected to obtain tissue samples that are used for stable isotope analysis, disease or contaminant assessment, and genetic analysis. A given specimen or sample may be used decades or centuries after the specimen is collected; it is not possible to know all the analyses to which a sample may be eventually subjected. The goal is to maximize the usefulness of every bird collected.

Given the importance of maintaining the physical integrity of the specimens for museum collections and research, the method chosen to kill or euthanize a bird specimen is equally important. Compromising the morphological, histological, or molecular integrity of the specimens is not acceptable. Chemical methods of killing are considered unacceptable unless it can be shown that an agent will not compromise or bias potential tissue analysis. Cervical dislocation – which can easily tear the head from a small bird – and decapitation are simply not appropriate as the carcass would not be useable for museum collections and most studies. Shotguns, historically were recognized as an acceptable means to collect birds for museums and scientific research. However, shotguns require permits and extensive training and may destroy tissue samples or wound birds. Birds that have been wounded by gunshot would have to be euthanized by other means (e.g., rapid cardiac compression). Rapid cardiac compression is an important research tool available to field ornithologists to humanely kill or euthanize birds.

Euthanasia in the field setting

Rapid cardiac compression is used because in the field setting, no other humane methods are available in many cases.

In the veterinary clinics and hospitals, zoos, or other facilities where animals are held in captivity, all methods of euthanasia are or should be readily available. However, most of these methods of euthanasia are not possible, practical, or appropriate for use in ornithological field research, which most commonly takes place at some distance from a traditional research facility and often takes place in remote field locations.

If available, an inhalant can be a useful and practical method of euthanasia when research is conducted near a field station or in a situation where supplies can be stored or replenished. However, inhalants are not practical in situations where field research will be conducted over a period of weeks in very remote areas or when all equipment and supplies are carried in on foot.

Inhalants such as isoflurane can be difficult to obtain. Although isoflurane is not a controlled substance to which access is limited by the Drug Enforcement Agency, state licensing requirements in the United States and in most countries limit access to inhalants to licensed veterinarians. Thus, a veterinarian must be willing to obtain it and provide it to the ornithologist for use in field research though the veterinarian is not likely to be available to supervise its use and assure that it will not be acquired by others who do not have authorization to possess or use the substance. Most states restrict the use of substances by licensees to situations where a Veterinary-Client-Patient Relationship (VCPR) exists. According to the AVMA, this relationship is established only when "the veterinarian has sufficient knowledge of the animal(s) to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s). This means that the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of an examination of the animal(s), or by medically appropriate and timely visits to the premises where the animal(s) are kept. The veterinarian is readily available, or has arranged for emergency coverage, for follow-up evaluation in the event of adverse reactions or the failure of the treatment regimen." Of course, these conditions are essentially inapplicable to most field research or to the methods of euthanasia used in the context of field research, but as it is a legal restriction in some states, veterinarians in those states may be unwilling to provide it to field researchers.

In some states, the license restricts the use of the substance to a particular building, making it impossible to use the substance legally at a field site. In some countries, inhalants are not available to anyone but licensed physicians and veterinarians, who are not permitted to supply it to others. Some inhalants, including isoflurane, cannot be carried on aircraft or are highly restricted. Researchers who use CO_2 may face similar obstacles. Both U.S. domestic and international air transport shipping regulations consider CO_2-filled cylinders to be a dangerous good requiring specialized training, packaging, and labeling; pilots are given the discretion to refuse to allow this material on board the aircraft.

The unpredictability of field research can also make the use of isoflurane impractical. For instance, investigators are presented with opportunities to capture small animals that represent important specimens in the course of conducting other research. In these instances the investigators are usually without euthanasia equipment or supplies of any kind. Also, inhalants may not readily vaporize in cold weather or at high elevations.

Veterinarians often refuse to give controlled substances to researchers, particularly for off-label use, due to the Animal Medicinal Drug Use Clarification Act restrictions and out of concern for potential abuse. Some IACUCs and universities will refuse to allow the use of controlled substances unless a veterinarian is present, but few veterinarians are willing and available to accompany researchers into the field on a regular basis. These substances frequently cannot be carried legally into other countries. In fact, the Food and Drug Administration now requires the use of a separate registration for each location where veterinarians store, distribute, or dispense controlled substances. This rule places an even greater burden on veterinarians and a virtual barrier for wildlife biologists, who rarely work at fixed locations. The Veterinary Medicine Mobility Act allows veterinarians to use controlled substances at locations other than the registered location but the exemption applies only to veterinarians. Only rarely does a veterinarian accompany an ornithologist to the field, and even then, there is no VCPR because the veterinarian has not assumed the responsibility for making clinical judgments regarding the health of the animal. The veterinarian has never before seen the animal and has no knowledge of its health or condition, which of course is not an issue where the intent is to euthanize the bird for research purposes. Even in cases where the bird has been injured, it is unlikely that the veterinarian will be able to provide follow-up care, unless the bird is removed from the wild.

Limitations on use

Although there is some variation based on the size and strength of the hands of individual researchers, rapid cardiac compression for birds over 250 g is not recommended because it can be more difficult to perform, slower, generally undesirable, and possibly inhumane.

Training is essential

Ornithologists practicing rapid cardiac compression routinely train the next generation of practitioners. Today's ornithologists are well attuned to the need to minimize animal suffering, and the IACUC process further encourages this and enforces needed oversight. There is no reason why training – using captured birds that would have been euthanized for research or teaching or that were to be euthanized as the planned endpoint of a study - cannot take place in a controlled environment. In such cases, isoflurane or other inhalant or injectable to induce loss of consciousness could be used prior to the use of rapid cardiac compression.

Conclusion

Ornithologists use rapid cardiac compression because it results in very rapid loss of consciousness, and death of the bird follows rapidly thereafter. Of the many methods that have been tried, it is among the most humane. It is easy to learn, so with proper training there is little risk that it will be performed incorrectly. It maximizes the scientific utility of specimens, and thereby helps to minimize the number of individuals collected for scientific research. Given the expertise and cumulative decades of experience of ornithologists and their careful observations, and given the absence of any evidence – observational or measured by instrumentation such as an EEG – to the contrary, there is a sufficient basis to continue to accept the use of rapid cardiac compression as a humane means of euthanasia given adequate training. It is particularly important that rapid cardiac compression be permitted where circumstances such as the inability to obtain a reliable and legal supply of inhalants or pharmaceutical agents and associated equipment preclude the use of these methods.

[1] Euthanasia literally means "good death." The Animal Welfare Act regulations state that, "*Euthanasia* means the humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness

and subsequent death" [9 CFR 1.1]. This legal definition does not qualify or limit the term euthanasia to the taking of the animal's life for any particular purpose. However, the AVMA 2013 guidelines make a distinction based on the purpose for ending life, regarding euthanasia as both a means to end suffering and a matter of humane technique. In wildlife research, euthanasia may sometimes be used to end suffering but many studies entail the killing of a healthy animal for research purposes, including taxonomic studies that require an intact carcass for a museum specimen and studies of wildlife disease, nutrition, parasitology, and toxicology that require intact tissues for necropsy and analysis. We disagree with the construct employed by the AVMA and assert that the Animal Welfare Act definition, which represents the legal standard, is the appropriate definition. The purpose for ending life is irrelevant both legally and biologically. It matters not to the animal why its life is to be taken; it matters only that the death is humane. Therefore, we use the term euthanasia to refer to humane technique without regard to the purpose, for under the AVMA definition, no method used by ornithologists would ever constitute euthanasia, no matter how humane, except in the relatively rare instances where the purpose is to end suffering. Moreover, because the use of rapid cardiac compression in small birds produces a speedy and humane death without evidence of pain or distress, it is entirely compatible with the AVMA directive to end a life with a humane technique.

[2] Quoted directly and in entirety from the draft AVMA 2013 guidelines (citations omitted):

> Carbon dioxide may cause pain due to the formation of carbonic acid when it contacts moisture on the respiratory and ocular membranes. In humans, rats and cats most nociceptors begin to respond at CO_2 concentrations of approximately 40% (citations omitted). Humans report discomfort begins at 30 to 50% CO_2, and intensifies to overt pain with higher concentrations (citations omitted).
>
> Inhaled irritants are known to induce a reflex apnea and heart rate reduction, and these responses are thought to reduce transfer of harmful substances into the body (citation omitted). In rats, 100% CO_2 elicits apnea and bradycardia, but CO_2 at concentrations of 10, 25 and 50% do not (citation omitted), suggesting gradual displacement methods are less likely to produce pain prior to unconsciousness in rodents.
>
> Carbon dioxide has a key role as a respiratory stimulant, and elevated concentrations are known to cause profound effects on the respiratory, cardiovascular and sympathetic nervous systems (citations omitted). In humans, air hunger begins at concentrations as low as 8% and this sensation intensifies with higher concentrations, becoming severe at approximately 15% (citations omitted). With mild increases in inspired CO_2, increased ventilation results in a reduction or elimination of air hunger, but there are limits to this compensatory mechanism such that air hunger may reoccur during spontaneous breathing with moderate hypercarbia and hypoxemia (citations omitted). Adding O_2 to CO_2 may or may not preclude signs of distress (citations omitted). Supplemental O_2 will, however, prolong time to hypoxemic death and may delay onset of unconsciousness.

Although CO_2 exposure has the potential to produce a stress response, interpretation of the subjective experiences of animals is complicated. Borovsky (1998) found an increase in norepinephrine in rats following 30 seconds of exposure to 100% CO_2. Similarly, Reed (2009) exposed rats to 20 to 25 seconds of CO_2, which was sufficient to render them recumbent, unconscious, and unresponsive, and observed 10-fold increases in vasopressin and oxytocin concentrations. Indirect measures of sympathetic nervous system activation, such as elevated heart rate and blood pressure, have been complicated by the rapid depressant effects of CO_2 exposure. Activation of the hypothalamic pituitary axis has also been examined during CO_2 exposure. Prolonged exposure to low concentrations of CO_2 (6 to 10%) has been found to increase corticosterone in rats (Raff, 1988; Marotta, 1976) and cortisol in dogs (Raff, 1983).

In humans, a single breath of 35% CO_2 was found to result in elevated cortisol concentrations and exposure was associated with an increase in fear (citation omitted). It has been suggested that responses to systemic stressors associated with immediate survival, such as hypoxia and hypercapnia, are likely directly relayed from brainstem nuclei and are not associated with higher order CNS processing and conscious experience (citation omitted). In fact, Kc et al. (citation omitted) found that hypothalamic vasopressin-containing neurons are similarly activated in response to CO_2 exposure in both awake and anesthetized rats. As stated previously, assessment of the animal's response to inhaled agents, such as CO_2, is complicated by continued exposure during the period between loss of consciousness and death.

Distress during CO_2 exposure has also been examined using behavioral assessment and aversion testing. Variability in behavioral responses to CO_2 has been reported for rats and mice (citations omitted), pigs (citations omitted), ducks (citations omitted) and poultry (citations omitted). While signs of distress have been reported as occurring in animals in some studies, other researchers have not consistently observed these effects. This may be due to variations in methods of gas exposure and types of behaviors assessed, as well as strain variability.

Using preference and approach-avoidance testing, rats and mice show aversion to CO_2 concentrations sufficient to induce unconsciousness (citations omitted), and are willing to forgo a palatable food reward to avoid exposure to CO_2 concentrations of approximately 15% and higher (citations omitted) after up to 24 hours of food deprivation (citation omitted). Mink will avoid a chamber containing a desirable novel object when it contains 100% CO_2 (citation omitted). In contrast to other species, a large proportion of chickens and turkeys will enter a chamber containing moderate concentrations of CO_2 (60%) to gain access to food or social contact (citations omitted). However, following incapacitation and prior to loss of consciousness, birds in these studies show behaviors that may be indicative of distress such as open-beak breathing and head-shaking. Regardless, it appears that birds are more willing than rodents and mink to tolerate CO_2 at concentrations that are sufficient to induce loss of posture, and that loss of consciousness follows shortly afterwards."

[3] Citations have been omitted for brevity and because we do not question the underlying sources cited by the AVMA in support of its statement. We quote the text from the AVMA guidelines to delineate the metrics upon which the AVMA classifications are based and to demonstrate that the conclusions reached are inconsistent. The full text and citations can be obtained from the AVMA Guidelines on Euthanasia.

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About the Ornithological Council

The founding premise of the Ornithological Council is that the ability to make sound policy regarding the scientific study of birds requires the application of impartial scientific data and the continued collection of such data. The Council works to support this important mission. The Council was founded in 1992 and proudly counts as its members twelve ornithological societies in the Western Hemisphere: American Ornithologists' Union, Association for Field Ornithology, Cooper Ornithological Society, Pacific Seabird Group, Raptor Research Foundation, Waterbird Society, the Wilson Ornithological Society, the Society of Canadian Scientists, the Society for the Conservation and Study of Caribbean Birds, the Neotropical Ornithological Society, CIPAMEX, and the North American Crane Working Group.