AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

Members of the Panel on Euthanasia

Steven Leary, DVM, DACLAM (Chair); Washington University, St. Louis, Missouri
Wendy Underwood, DVM (Vice Chair); Eli Lilly and Company, Indianapolis, Indiana
Raymond Anthony, PhD (Ethicist); University of Alaska Anchorage, Anchorage, Alaska
Samuel Cartner, DVM, MPH, PhD, DACLAM (Lead, Laboratory Animals Working Group); University of Alabama at Birmingham, Birmingham, Alabama
Douglas Corey, DVM (Lead, Equine Working Group); Associated Veterinary Clinic, Walla Walla, Washington
Temple Grandin, PhD (Lead, Physical Methods Working Group); Colorado State University, Fort Collins, Colorado
Cheryl Greenacre, DVM, DABVP (Lead, Avian Working Group); University of Tennessee, Knoxville, Tennessee
Sharon Gwaltney-Brant, DVM, PhD, DABVT, DABT (Lead, Noninhaled Agents Working Group); ASPCA Poison Control Center, Urbana, Illinois
Mary Ann McCrackin, DVM, PhD, DACVS (Lead, Companion Animals Working Group); Virginia Polytechnic Institute and State University, Blacksburg, Virginia
Robert Meyer, DVM, DACVA (Lead, Inhaled Agents Working Group); Mississippi State University, Mississippi State, Mississippi
David Miller, DVM, PhD, DACZM (Lead, Reptiles, Zoo and Wildlife Working Group); Loveland, Colorado
Jan Shearer, DVM, MS, DACAW (Lead, Animals Farmed for Food and Fiber Working Group); Iowa State University, Ames, Iowa
Roy Yanong, VMD (Lead, Aquatics Working Group); University of Florida, Ruskin, Florida

AVMA Staff Consultants

Gail C. Golab, PhD, DVM, MANZCVS, DACAW; Director, Animal Welfare Division
Emily Patterson-Kane, PhD; Animal Welfare Scientist, Animal Welfare Division

---

The following individuals contributed substantively through their participation in the Panel's Working Groups and their assistance is sincerely appreciated.

Inhaled Agents—Scott Helms, DVM, DABVP; Lee Niel, PhD; Daniel Weary, PhD
Noninhaled Agents—Virginia Fajt, DVM, PhD, DACVCP; Don Sawyer, DVM, PhD, DACVA, DABVP
Physical Methods—Rose Gillesby, DVM; Jeff Hill, PhD; Jennifer Woods, BSc
Aquatics—Craig Harms, DVM, PhD, DACZM; Helen Roberts, DVM; Nick Saint-Erne, DVM; Michael Stoskopf, DVM, PhD, DACZM
Avian—Laurel Degernes, DVM, MPH, DABVP; Laurie Hess, DVM, DABVP; Kemba Marshall, DVM, DABVP; James Morrisey, DVM, DABVP; Joanne Paul-Murphy, DVM, DACZM, DACAW
Companion Animals—Kathleen Cooney, MS, DVM; Stacey Frick, DVM; John Mays; Rebecca Rhoades, DVM
Equids—Fairfield Bain, DVM, MBA, DACVIM, DACVP, DACVECC; Midge Leitch, VMD, DACVS; Thomas R. Lenz, DVM, MS, DACT; Nathaniel Messer, DVM, DABVP; Hayden Sears, DVM; Stuart Shoemaker, DVM, ACVS
Food and Fiber Animals—Eric Benson, PhD; C. Scanlon Daniels, DVM, MBA; John Deen, DVM, PhD, DABVP, DACAW; Robert Evans, PhD, DVM, DACPV; Jerome Geiger, DVM, MS; Dee Griffin, DVM, MS; Christa Goodell, DVM; Glen Johnson, DVM; Richard Reynnells, PhD; James Reynolds, DVM, MVPM, DACAW; Bruce Webster, PhD
Laboratory Animals—James Artwhol, MS, DVM, DACLAM; Larry Carbone, DVM, PhD, DACLAM; Paul Flecknell, VetMB, MRCVS, PhD, DECVA, DECLAM, DACLAM, FRCVS; David P. Friedman, PhD; Kathleen Pritchett-Corning, DVM, DACLAM, MRCVS
Reptiles, Zoo and Wild Animals—Scott Citino, DVM, DACZM; Mark Drew, DVM, MS, DACZM; Julie Goldstein, DVM; Barry Hartup, DVM, PhD; Gregory Lewbart, MS, VMD, DACZM; Douglas Mader, MS, DVM, DABVP, FRSM; Patrick Morris, DVM, DACZM

ISBN 978-1-882691-21-0

Version 2013.0.1

I1. PREFACE

Animal issues are no longer socially invisible. During the past half-century, efforts to ensure the respectful and humane treatment of animals have garnered global attention.[1,2] Concern for the welfare of animals is reflected in the growth of animal welfare science and ethics. The former is evident in the emergence of academic programs, scientific journals, and funding streams committed either partially or exclusively to the study of how animals are impacted by various environments and human interventions. The latter has seen the application of numerous ethical approaches (eg, rights-based theories, utilitarianism, virtue ethics, contractarianism, pragmatic ethics) to assessing the moral value of animals and the nature of the human-animal relationship.[1,3–9] The proliferation of interest in animal use and care, at the national and international levels, is also apparent in recent protections accorded to animals in new and amended laws and regulations, institutional and corporate policies, and purchasing and trade agreements. Changing societal attitudes toward animal care and use have inspired scrutiny of some traditional and contemporary practices applied in the management of animals used for agriculture, research and teaching, companionship, and recreation or entertainment and of animals encountered in the wild. Attention has also been focused on conservation and the impact of human interventions on terrestrial and aquatic wildlife and the environment. Within these contexts, stakeholders look to veterinarians to provide leadership on how to care well for animals, including how to relieve unnecessary pain and suffering.

In creating the 2013 edition of the AVMA Guidelines for the Euthanasia of Animals (Guidelines), the Panel on Euthanasia (POE) made every effort to identify and apply the best research and empirical information available. As new research is conducted and more practical experience gained, recommended methods of euthanasia may change. As such, the AVMA and its POE have made a commitment to ensure the Guidelines reflect an expectation and paradigm of continuous improvement that is consistent with the obligations of the Veterinarian's Oath.[10] As for other editions of the document, modifications of previous recommendations are also informed by continued professional and public sensitivity to the ethical care of animals.

While some euthanasia methods may be utilized in slaughter and depopulation, recommendations related to humane slaughter and depopulation fall outside the purview of the Guidelines and will be addressed by separate documents that are under development.

The Guidelines set criteria for euthanasia, specify appropriate euthanasia methods and agents, and are intended to assist veterinarians in their exercise of professional judgment. The Guidelines acknowledge that euthanasia is a process involving more than just what happens to an animal at the time of its death. Apart from delineating appropriate methods and agents, these Guidelines also recognize the importance of consider-der free-ranging conditions, where the needs of animals and the challenges faced by veterinarians and other personnel may be quite different from those in domestic environments. And, where possible, appropriate flowcharts, illustrations, tables, and appendices have been used to clarify recommendations. Appendices 1 through 3 also may be useful as a quick reference guide, but those performing euthanasia are strongly advised to refer to the full text of the document for important additional information. Section labels have been included in Appendix 1 to assist readers in locating related text for particular species.

Collection of animals for scientific investigations, euthanasia of injured or diseased wildlife, and removal of animals causing damage to property or threatening human safety are addressed. Recognizing that veterinary responsibilities associated with euthanasia are not restricted to the process itself, additional information about confirmation of death and disposal of animal remains has been included.

One area identified as needing additional guidance in the last iteration of the Guidelines was depopulation (ie, the rapid destruction of large numbers of animals in response to emergencies, such as the control of catastrophic infectious diseases or exigent situations caused by natural disasters). Depopulation may employ euthanasia techniques, but not all depopulation methods meet the criteria for euthanasia. Because they do not always meet the criteria for euthanasia, these techniques will be addressed in a separate document, the AVMA Guidelines for the Depopulation of Animals. Similarly, because methods used for slaughter or harvest may also not meet all the conditions necessary to be deemed euthanasia, these techniques will be addressed by a third document, the AVMA Guidelines for the Humane Slaughter of Animals.

I2.3 STATEMENT OF USE

The Guidelines are designed for use by members of the veterinary profession who carry out or oversee the euthanasia of animals. As such, they are intended to apply only to nonhuman species.

The species addressed by the practice of veterinary medicine are diverse. A veterinarian experienced with the species of interest should be consulted when choosing a method of euthanasia, particularly when little species-specific research on euthanasia has been conducted. Methods and agents selected will often be situation specific, as a means of minimizing potential risks to the animal's welfare and personnel safety. Given the complexity of issues that euthanasia presents, references on anatomy, physiology, natural history, husbandry, and other disciplines may assist in understanding how various methods may impact an animal during the euthanasia process.

Veterinarians performing or overseeing euthanasia must assess the potential for animal distress due to physical discomfort, abnormal social settings, novel physical surroundings, pheromones or odors from nearby or previously euthanized animals, the presence of humans, or other factors. In addition, human safety and perceptions, availability of trained personnel, potential infectious disease concerns, conservation or other animal population objectives, regulatory oversight that may be species specific, available equipment and facilities, options for disposal, potential secondary toxicity, and other factors must be considered. Human safety is of utmost importance, and appropriate safety equipment, protocols, and knowledge must be available before animals are handled. Advance preparation includes protocols and supplies for addressing personnel injury due to animal handling or exposure to drugs and equipment used during the process. Once euthanasia has been carried out, death must be carefully verified. All laws and regulations pertaining to the species being euthanized, the methods employed, and disposal of the animal's remains and/or water containing any pharmaceuticals used for euthanasia must be followed.

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. While every effort has been made to identify and recommend appropriate approaches for common species encountered under common conditions, the POE recognized there will be less than perfect situations in which a recommended method of euthanasia may not be possible and a method or agent that is best under the circumstances will need to be applied. For this reason, although the Guidelines may be interpreted and understood by a broad segment of the general population, a veterinarian should be consulted in their application.

I3. WHAT IS EUTHANASIA?

Euthanasia is derived from the Greek terms *eu* meaning good and *thanatos* meaning death. The term is usually used to describe ending the life of an individual animal in a way that minimizes or eliminates pain and distress. A good death is tantamount to the humane termination of an animal's life.

In the context of these Guidelines, the veterinarian's prima facie duty in carrying out euthanasia includes, but is not limited to, (1) his or her humane disposition to induce death in a manner that is in accord with an animal's interest and/or because it is a matter of welfare, and (2) the use of humane techniques to induce the most rapid and painless and distress-free death possible. These conditions, while separate, are not mutually exclusive and are codependent.

Debate exists about whether *euthanasia* appropriately describes the killing of some animals at the end of biological experiments[11] and of unwanted shelter animals. The Panel believes that evaluating the social acceptability of various uses of animals and/or the rationale for inducing death in these cases is beyond its purview; however, current AVMA policy supports the use of animals for various human purposes,[12] and also recognizes the need to euthanize animals that are unwanted or unfit for adoption.[13] Whenever animals are used by humans, good animal care practices should be implemented and adherence to those good practices should be enforced. When evaluating our responsibilities toward animals, it is important to be sensitive to the context and the practical realities of the various types of human-animal relationships. Impacts on animals may not always be the center of the valuation process, and there is disagreement on how to account for conflicting interspecific interests. The Panel recognizes these are complex issues raising concerns across a large number of domains, including scientific, ethical, economic, environmental, political, and social.

I3.1 A GOOD DEATH AS A MATTER OF HUMANE DISPOSITION

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. Thus, euthanasia as a matter of humane disposition can be either intent or outcome based.

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. When animals are plagued by disease that produces insurmountable suffering, it can be argued that continuing to live is worse for the animal than death or that the animal no longer has an interest in living. The humane disposition is to 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. As an example, when treating a companion animal that is suffering severely at the end of life due to a debilitating terminal illness, a veterinarian may recommend euthanasia, because the loss of life (and attendant natural decline in physical and psychological faculties) to the animal is not relatively worse compared with a continued existence that is filled with prolonged illness, suffering, and duress. In this case, euthanasia does not deprive the animal of the opportunity to enjoy more goods of life (ie, to have more satisfactions fulfilled or enjoy more pleasurable experiences). And, these opportunities or experiences are much fewer or lesser in intensity than the presence or intensity of negative states or affect. 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.

Veterinarians may also be motivated to bring about the best outcome for the animal. Often, veterinarians face the difficult question of trying to decide (or helping the animal's owner to decide) when euthanasia would be a good outcome. In making this decision many veterinarians appeal to indices of welfare or quality of life. Scientists have described welfare as having three components: that the animal functions well, feels well, and has the capacity to perform behaviors that are innate or species-specific adaptations[14–16] (an alternative view is also available[17]). Anhaviors (-)Tj -0.003 Tw e. hav9life.tfect. Death, in this credible arguments on how to approach the ethically important issue of the death of an animal. In so doing, it hopes to promote greater understanding regarding the contexts or settings involving euthanasia and the complexity of end-of-life issues involving animals.

While not a regulatory body, the AVMA also hopes to offer guidance to those who may apply these Guidelines as part of regulatory structures designed to protect the welfare of animals used for human purposes. By creating and maintaining these Guidelines, the AVMA hopes to ensure that when a veterinarian or other professional intentionally kills an animal under his or her charge, it is done with respect for the interests of the animal and that the process is as humane as possible (ie, that it minimizes pain and distress to the animal and that death occurs as rapidly as possible).

The AVMA does not take the death of nonhuman animals lightly and attempts to provide guidance for its members on both the morality and practical necessity of the intentional killing of animals. Veterinarians, in carrying out the tenets of their Oath, may be compelled to bring about the intentional death of animals for a variety of reasons. The finality of death is, in part, what makes it an ethically important issue; death forever cuts off future positive states, benefits, or opportunities.[19] In cases where an animal no longer has a good life, however, its death also extinguishes permanently any and all future harms associated with poor welfare or quality of life.[18]

reduction of stage II or postmortem activity that could be distressing to personnel is strongly encouraged to reduce animal distress and improve personnel safety. This is particularly important for prey species, nondomesticated species, and animals enduring painful conditions.

Personnel who perform euthanasia must demonstrate proficiency in the use of the technique in a closely supervised environment. Each facility or institution where euthanasia is performed (whether a clinic, laboratory, or other setting) is responsible for training its personnel adequately to ensure the facility or institution operates in compliance with federal, state, and local laws. Furthermore, experience in the humane restraint of the species of animal to be euthanized is important and should be expected, to ensure that animal pain and distress are minimized. Training and experience should include familiarity with the normal behavior of the species being euthanized, an appreciation of how handling and restraint affect that behavior, and an understanding of the mechanism by which the selected technique induces loss of consciousness and death. Euthanasia should only be attempted when the necessary drugs and supplies are available to ensure a smooth procedure.

Selection of the most appropriate method of euthanasia in any given situation depends on the species and number of animals involved, available means of animal restraint, skill of personnel, and other considerations.

ward rotation of the eyeballs, and tonic spasm changing to clonic spasm, with eventual muscle flaccidity.[49,50]

Decapitation and cervical dislocation as physical methods of euthanasia require separate comment. The interpretation of brain electrical activity, which can persist for up to 30 seconds following these methods,[51–54] has been controversial.[55] As indicated previously, EEG methods cannot provide definitive answers as to onset of unconsciousness. Other studies[56–59] indicate such activity does not imply the ability to perceive pain and conclude that loss of consciousness develops rapidly.

Once loss of consciousness occurs, subsequently 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.

Compelling recent evidence indicates finfish possess the components of nociceptive processing systems similar to those found in terrestrial vertebrates,[55–70] though debate continues based on questions of the impact of quantitative differences in numbers of specific components such as unmyelinated C fibers in major nerve bundles. Suggestions that finfish responses to pain merely represent simple reflexes[71] have been refuted by studies[72,73] demonstrating forebrain and midbrain electrical activity in response to stimulation and differing with type of nociceptor stimulation. Learning and memory consolidation in trials where finfish are taught to avoid noxious stimuli have moved the issue of finfish cognition and sentience forward[74] to the point where the preponderance of accumulated evidence supports the position that finfish should be accorded the same considerations as terrestrial vertebrates in regard to relief from pain. The POE was not able to identify similar studies of Chondrichthyes (cartilaginous finfish), amphibians, reptiles, and invertebrates, but believes that available information suggests that efforts to relieve pain and distress for these taxa are warranted, unless further investigation disproves a capacity to feel pain or distress.

While there is ongoing debate about finfishes', amphibians', reptiles', and invertebrate animals' ability to feel pain or otherwise experience compromised welfare, they do respond to noxious stimuli. Consequently, the Guidelines assume that a conservative and humane approach to the care of any creature is warranted, justifiable, and expected by society. Euthanasia methods should be employed that minimize the potential for distress or pain in all animal taxa, and these methods should be modified as new taxa-specific knowledge of their physiology and anatomy is acquired.

I5.3 STRESS AND DISTRESS

An understanding of the continuum that represents stress and distress is essential for evaluating techniques that minimize any distress experienced by an animal being euthanized. Stress has been defined as the effect of physical, physiologic, or emotional factors (stressors) that induce an alteration in an animal's homeostasis or adaptive state.

struggling, attempts to escape, and defensive or redirected aggression. In cattle and pigs, vocalization during handling or painful procedures is associated with physiologic indicators of stress.[97–99]

or argon (Ar), or by exposure to carbon monoxide (CO) to block uptake of O_2 by red blood cells. Exsanguination, an adjunctive method, is another method of euthanized using barbiturates. This places renderers and those wishing to employ rendering as a means of disposal for animals euthanized using pentobarbital in a difficult position, and may result in renderers being reluctant to accept more animal remains than they can reasonably manage without creating residue concerns. Alternatives for disposal of animal remains must be considered in advance, in case the renderer cannot or will not accept animal remains containing barbiturate residues.

Composting is another means of disposing of animal remains that is becoming increasingly common. Studies examining the persistence of barbiturate residues in composted animal remains are few, but those that do exist suggest the persistence of the drugs in composted material. While the implications of this are still unclear, it does raise questions about potential environmental impacts in the case of animal health emergencies or mass mortality events.

Alternatives to the use of pentobarbital that may reduce the risk of secondary toxicity include general anesthesia followed by nontoxic injectable agents such as potassium chloride, or the application of physical methods such as penetrating captive bolt or gunshot. These alternatives, however, are not risk free. For example, pharmaceutical residues in animal remains other than barbiturates (eg, xylazine) may affect scavengers and can reduce the acceptability of the animal remains for renderers. Unfortunately, specific guidance from regulators regarding the use of such alternatives is limited.

The persistence of antimicrobials in animal remains presents parallel concerns, particularly for animal remains that will be rendered. While many antimicrobials may be inactivated or destroyed through the rendering process, public health concerns associated with antimicrobial resistance, coupled with the enhanced sensitivity of chemical assays and limited regulatory guidance for renderers, further complicate veterinarians' responsibilities for safe remediation.

Safe handling and disposal of the resulting animal remains are also critically important when zoonotic diseases, foreign animal diseases, or diseases of concern to population health are suspected. Appropriate diagnostic samples should be collected for testing, regulatory authorities must be contacted, and the animal remains must be incinerated (if possible). Personal protective equipment and precautions for handling biohazardous materials are recommended. Animals that have injured humans may require specific actions to be taken depending on local and state laws.

Part II—Methods of Euthanasia

M1. INHALED AGENTS

M1.1 COMMON CONSIDERATIONS

Inhaled vapors and gases require a critical concentration within the alveoli and blood for effect; thus, 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). Although SNS and HPA activation are well accepted as markers of a stress response, these systems are activated in response to both physical and psychological stressors and are not necessarily associated with higher-order CNS processing and conscious experience by the animal. 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.

Distress during administration of inhaled agents has been evaluated by means of both behavioral assessment and aversion testing. While overt behavioral signs of distress have been reported in some studies, others have not consistently found these effects. Through preference and approach-avoidance testing, all inhaled agents currently used for euthanasia have been identified as being aversive to varying degrees. Aversion is a measure of preference, and while aversion does not necessarily imply that the experience is painful, forcing animals into aversive situations creates stress. The conditions of exposure used for aversion studies, however, may differ from those used for stunning or killing. In addition, agents identified as being less aversive (eg, Ar or N_2 gas mixtures, inhaled anesthetics) can still produce overt signs of behavioral distress (eg, open-mouth breathing) in some species under certain conditions of administration (eg, gradual displacement). As previously noted in the section on consciousness, one of the characteristics of anesthesia in people is feeling as if one is having an out-of-body experience, suggesting a disconnection between one's sense of self and one's awareness of time and space.[140] Although we cannot know for certain the subjective experiences of animals, one can speculate similar feelings of disorientation may contribute to the observed signs of distress.

As for physical methods, the conditions under which inhaled agents are administered for euthanasia can have profound effects on an animal's response and, thus, agent suitability. Simply placing Sprague-Dawley rats into an unfamiliar exposure chamber containing room air produces arousal, if not distress.[141] Pigs are social animals and prefer not to be isolated from one another; consequently, moving them to the CO_2 stunning box in groups, rather than lining them up single file as needed for electric stunning, improves voluntary forward movement, reduces handling stress and electric prod use, and improves meat quality.[142]

That inhaled agents can produce distress and aversion in people raises concerns for their use in animals, in that the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training[143] state "Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals." Interestingly, more than 40% of human children 2 to 10 years old display distress behaviors during sevoflurane induction, with 17% displaying significant distress and more than 30% physically resisting during induction.[144] Fear in children undergoing anesthesia may be due to odor, feel of the mask, or a true phobia of the mask.[145] Despite evidence of distress and aversion, inhaled anesthetics continue to be administered because the benefits associated with their use greatly outweigh any distress and/or aversion they may cause.

The suitability of any particular inhaled agent for euthanasia therefore depends largely on distress and/or pain experienced prior to loss of consciousness. Distress can be caused by handling, specific agent properties, or method of administration, such that a one-size-fits-all approach cannot be easily applied. Suffering can be conceptualized as the product of severity, incidence, and duration. As a general rule, a gentle death that takes longer is preferable to a rapid, but more distressing death[25]; 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. Our goal is to identify best practices for administering inhaled agents, defining the optimal conditions for transport, handling, and agent selection and delivery to produce the least aversive and distressing experience for each species.

The following contingencies are common to all inhaled euthanasia agents:

(1) Time to unconsciousness with inhaled agents is dependent on the displacement rate, container volume, and concentration. An understanding of the principles governing delivery of gases or vapors into enclosed spaces is necessary for appropriate application of both prefill and gradual displacement methods.

(2) Loss of consciousness will be more rapid if animals are initially exposed to a high concentration of the agent. However, for many agents and species, forced exposure to high concentrations can be aversive and distressing, such that gradual exposure may be the most pragmatic and humane option.

(3) Inhaled agents must be supplied in purified form without contaminants or adulterants, typically from a commercially supplied source, cylinder, or tank, such that an effective displacement rate and/or concentration can be readily quantified. The direct application

of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate.

(4) The equipment used to deliver and maintain inhaled agents must be in good working order and in compliance with state and federal regulations. Leaky or faulty equipment may lead to slow, distressful death and may be hazardous to other animals and to personnel.

(5) Most inhaled agents are hazardous to animal workers because of the risk of explosions (eg, ether, CO), narcosis (eg, halocarbon anesthetics, CO_2, asphyxiating gases), hypoxia (eg, asphyxiating gases, CO), addiction or physical abuse (eg, nitrous oxide [N_2O], halocarbon anesthetics), or health effects resulting from chronic exposure (eg, N_2O, CO, possibly halocarbon anesthetics).

(6) In sick or depressed animals where ventilation is decreased, agitation during induction is more likely because the rise in alveolar gas concentration is delayed. A similar delayed rise in alveolar gas concentration can be observed in excited animals having increased cardiac output. Suitable premedication or noninhaled methods of euthanasia should be considered for such animals.

(7) Neonatal animals appear to be resistant to hypoxia, and because all inhaled agents ultimately cause hypoxia, neonatal animals take longer to die than adults.[146] Inhaled agents can be used alone in unweaned animals to induce loss of consciousness, but prolonged exposure time or a secondary method may be required to kill the unconscious animal.

(8) Reptiles, amphibians, and diving birds and mammals have a great capacity for holding their breath and for anaerobic metabolism. Therefore, induction of anesthesia and time to loss of consciousness when inhaled agents are used may be greatly prolonged. Noninhaled methods of euthanasia should be considered for these species and a secondary method is required to kill the unconscious animal.

(9) Rapid gas flows can produce noise or cold drafts leading to animal fright and escape behaviors. If high flows are required, equipment should be designed to minimize noise and gas streams blowing directly on the animals.

(10) When possible, inhaled agents should be administered under conditions where animals are most comfortable (eg, for rodents, in the home cage; for pigs, in small groups). If animals need to be combined, they should be of the same species and compatible cohorts, and, if needed, restrained or separated so that they will not hurt themselves or others. Chambers should not be overloaded and need to be kept clean to minimize odors that might cause distress in animals subsequently euthanized.

(11) Because some inhaled agents may be lighter or heavier than air, layering or loss of agent may permit animals to avoid exposure. Mixing can be maximized by ensuring incoming gas or vapor flow rates are sufficient. Chambers and containers should be as leak free as possible.

(12) Death must be verified following administration of inhaled agents. This can be done either by examination of individual animals or by adherence to validated exposure processes proven to result in death.[147] If an animal is not dead, exposure must be repeated or followed with another method of euthanasia.

M1.2 PRINCIPLES GOVER
idated exp275(ons wher)20(e animals ar)20(e most)]TJ -0.0
idated exp275(ons 53.53601)12uGmals are most -
noor89em 3s 40 lcaghoa)istiT1gelt ioccura,868 gaoveTm

()istiT1g319 unc29 Tw 1.8 65.5551Tm 6d ea death.).zzi3(

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[162–164] 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. Makowska and Weary[165] also reported halothane and isoflurane to be aversive to male Wistar rats, but less so than CO_2.

Anesthetic vapor is inhaled until respiration ceases and death ensues. Because the liquid state of most inhaled anesthetics is irritating, animals should be exposed only to vapors. With inhaled anesthetics, animals can be placed in a closed receptacle containing cotton or gauze soaked with an appropriate amount of liquid anesthetic[166] or anesthetic vapor can be introduced from a precision vaporizer.[167] Precision anesthetic vaporizers typically are limited to 5% to 7% maximum output between 0.5 and 10 L/min O_2 flow rate. Induction time will be influenced by dial setting, flow rate, and size of the container; time to death may be prolonged because O_2 is commonly used as the vapor carrier gas. The amount of liquid anesthetic required to produce a given concentration of anesthetic vapor within any closed container can be readily calculated[168]; in the case of isoflurane, a maximum of 33% vapor can be produced at 20°C. Sufficient air or O_2 must be provided during the induction period to prevent hypoxia.[166] In the case of small rodents placed in a large container, there will be sufficient O_2 in the chamber to prevent hypoxia. Larger species placed in small containers may initially need supplemental air or O_2.[166]

Nitrous oxide is the least potent of the inhalation anesthetics. In humans, the minimum alveolar concentration (defined as the median effective dose) for N_2O is 104%; its potency in other species is less than half that in humans (ie, approx 200%). Because the effective dose for N_2O is above 100% it cannot be used alone at 1 atmosphere of pressure in any species without producing hypoxia prior to respiratory or cardiac arrest. As a result, animals may become distressed prior to loss of consciousness. Up to 70% N_2O may be combined with other inhaled gases to speed the onset of anesthesia; however, the anesthetic contribution of N_2O will be only half (20% to 30%) of that expected in humans due to its reduced potency in animals.[169]

Effective procedures should be in place to reduce animal worker exposure to anesthetic vapors.[170] Human workplace recommended exposure limits were issued in 1977 by the National Institute of Occupational Safety and Health (NIOSH); concentrations for halogenated inhaled anesthetics are not to exceed 2 ppm (1-hour ceiling) when used alone, or 0.5 ppm for halogenated anesthetics combined with 25-ppm N_2O (time-weighted average during use). The American Conference of Government Industrial Hygienists has assigned a threshold limit value time-weighted average of 50 ppm for N_2O, 50 ppm for halothane, and 75 ppm for enflurane for an 8-hour time-weighted exposure. These concentrations were established because they were found to be attainable utilizing clinical scavenging techniques and there are no controlled studies proving exposure at these concentrations are safe. No NIOSH recommended exposure limits exist for the three most currently used anesthetics (isoflurane, desflurane, and sevoflurane), and, at present, the Occupational Safety and Health Administration has no permissible exposure limits regulating these specific agents.

Advantages—(1) Inhaled anesthetics are particularly useful for euthanasia of smaller animals (< 7 kg [15.4 lb]) or for animals in which venipuncture may be difficult. (2) Inhaled anesthetics can be administered by several different methods depending on the circumstances and equipment available (eg, face mask, open drop where the animal is not permitted to directly contact the anesthetic liquid, precision vaporizer, rigid or nonrigid containers). (3) Halothane, enflurane, isoflurane, sevoflurane, desflurane, methoxyflurane, and N_2O are nonflammable and nonexplosive under usual clinical conditions. (4) Inhaled anesthetics can be useful as the sole euthanasia agent or as part of a 2-step process, where animals are first rendered unconscious through exposure to inhaled anesthetic agents and subsequently killed via a secondary method.

Disadvantages—(1) Inhaled anesthetics are aversive to rabbits and laboratory rodents and the same may be true for other species. Animals may struggle and become anxious during induction of anesthesia, with some animals exhibiting escape behaviors prior to onset of unconsciousness. Should apnea or excitement occur, time to loss of consciousness may be prolonged. (2) Ether is irritating, flammable, and explosive. Explosions have occurred when animals, euthanized with ether, were placed in an ordinary (not explosion-proof) refrigerator or freezer and when bagged animals were placed in an incinerator. (3) Induction with methoxyflurane is unacceptably slow in some species. (4) Because of design limits on vapor output, precision anesthetic vaporizers may be associated with a longer wash-in time constant and, thus, longer induction time; time to death may be prolonged as O_2 is commonly used as the vapor carrier gas. (5) Nitrous oxide used alone will create a hypoxic atmosphere prior to loss of consciousness and will support combustion. (6) Personnel and animals may be injured by exposure to these agents. There is recognized potential for human abuse of inhaled anesthetics. (7) Because large amounts of inhaled anesthetics are absorbed and substantial amounts remain in the body for days,[171]use of inhaled anesthetics for euthanasia is challenging for food-producing animals due to potential for tissue residues.

General recommendations—Inhaled anesthetics are acceptable with conditions for euthanasia of small animals (< 7 kg) where the following contingencies can be met: (1) In those species where aversion or overt escape behaviors have not been noted, exposure to high concentrations resulting in rapid loss of consciousness is preferred. Otherwise, gradual fill methods can be used, keeping in mind the effect that chamber volume, flow rate, and anesthetic concentration will have on the time

must be well lighted and must allow personnel direct observation of animals. (5) The CO flow rate should be adequate to rapidly achieve a uniform CO concentration of at least 6% after animals are placed in the chamber, except for those species (eg, neonatal pigs) where it has been shown that less agitation occurs with a gradual rise in CO concentration.[187] (6) If the chamber is inside a room, CO monitors must be placed in the room to warn personnel of hazardous concentrations. (7) It is essential that CO use be in compliance with state and federal occupational health and safety regulations. (8) Carbon monoxide must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate. As gas displacement rate is critical to the humane application of CO, an appropriate pressure-reducing regulator and flow meter combination or equivalent equipment with demonstrated capability for generating the recommended displacement rate for the size container being utilized is absolutely necessary.

M1.5 NITROGEN, ARGON

Nitrogen and Ar are odorless, colorless and tasteless gases that are inert, nonflammable, and nonexplosive. Nitrogen normally comprises 78% of atmospheric air, whereas Ar comprises less than 1%. These gases function in the current context by displacing air (and the O_2 it contains), causing anoxia. Exposure of Sprague-Dawley rats to severe hypoxic conditions (< 2% O_2) using either gas leads to unconsciousness around 90 seconds and death after 3 minutes using Ar or 7 minutes using N_2[141]; similar findings have been reported for dogs, rabbits, and mink.[181,182,189,190] Male Sprague-Dawley rats become hyperpneic, but can survive for more than 20 minutes in Ar or N_2 at an O_2 concentration of 4.9%.[191]

Rats are sensitive to even small changes in the concentration of O_2, and are able to detect concentrations both lower and higher than the 20.9% normally found in air.[192] Rats and mice allowed to travel between chambers containing different gases spent most of their time in the control chamber (containing air), but preferred a hypoxic chamber (containing Ar) to a chamber containing CO_2; however, the animals stayed only a few seconds in either gas.[162–164] Even when rats were trained to enter a chamber for a food reward they typically refused to enter, or left immediately after entering, when the atmosphere was hypoxic (< 2% O_2, 90% Ar).[193] When rats were exposed to gradually decreasing concentrations of O_2 and increasing concentrations of Ar, they always left the chamber before losing consciousness (typically when O_2 declined to about 7%).[194] With N_2 flowing at a rate of 39% of chamber volume/min (τ = 2 minutes 34 seconds), rats collapsed

es time to onset of unconsciousness to 156 seconds at a CO_2 concentration of 21%.[195] For poultry, immersion into relatively low concentrations or exposure to CO_2 concentrations producing a gradual induction of unconsciousness reduces convulsions compared with immersion into N_2 or Ar.[204,269] Carbon dioxide may invoke involuntary (unconscious) motor activity in birds, such as flapping of the wings or other terminal movements, which can damage tissues and be disconcerting for observers[248,270]; wing flapping is less with CO_2 than with N_2 or Ar.[269]

Due to respiratory adaptations in immature animals, reptiles, amphibians, and some burrowing and diving species (eg, lagomorphs, mustelids, aquatic birds, nonhatched birds, newly hatched chicks), high CO_2 concentrations, combined with extended exposure times, follow-up exposure to hypoxemia, or a secondary euthanasia method, may be required to ensure unconsciousness and death. High CO_2 concentrations (> 60%) and extended exposure times (> 5 minutes) are required for effective euthanasia of newly hatched chickens.[201,271] On the day of birth, rats and mice exposed to 100% CO_2 required exposure times of 35 and 50w 10 0 0oxm01.0aand required f5rTd0ong sa0(ed f5rT0(equir)202)i6tpii55 T2 T*[(ar)2ge,(expo)]Te times of arto 1etract. The primary routes of their administration are parenteral injection, topical application, and immersion. When it is being determined whether a particular drug and route of administration are appropriate for euthanasia, consideration needs to be given to the species involved, the pharmacodynamics of the chemical agent, degree of physical or chemical restraint required, potential hazards to personnel, consequences of intended or unintended consumption of the animal's remains by humans and other animals, and potential hazards to the environment from chemical residues. Many noninhaled euthanasia agents can induce a state of unconsciousness during which minimal vital functions are evident but from which some animals may recov7 TwhiFmmay r as for any euthanasia method, death must be confirmed prior to final disposition of the animal's remains.

M2.1.1 Compounding

While several euthanasia agents (eg, barbiturates, barbiturate combinations, Tributame[b] [not currently being manufactured in the United States due to concerns with the manufacturing process, although the approved New Animal Drug Application has been retained], and T-61[c] [withdrawn from the market in the United States in 1991; consequently, it is no longer commercially available in this country]) have been approved or are in review by the FDA Center for Veterinary Medicine (FDA-CVM), some commonly used injectable euthanasia drugs are not approved, but are compounded from bulk drugsFmmayse include chloral hydrate, magnesium sulfate, and some formulations of potassium chloride. The FDA-CVM's most recent version of the Compliance Policy Guide on compounding of drugs for use in animals states that compounding from bulk drugs, except those specifically addressed for regulatory discretion by the FDA, raises concerns and may result in regulatory oversight.[276] Use of compounded euthanasia drugs that may create human or animal health risks (eg, unintentional ingestion by other animals) is of concern.

M2.1.2 Residue/Disposal Issues

Animals euthanized by chemical means must never enter the human food chain and should be disposed of in acc0 s
euthanized animals has become increasingly problematic because most rendering facilities will no longer take animals euthanized with agents that pose residue hazards (eg, barbiturates)Fmmay potential for ingestion of euthanasia agents is an important consideration in the euthanasia of animals that are disposed of in outdoor settings way r [277] or wayn
euthanized animals are fed to zoo and exotic animals.yT* [(malmay 009 25(.isposed)1(of)1(by)]TJ 46 ons aprioasiaer anuseaies ing manbi20(esidsia ofpote

euthanasia agent, and other strategies that may reduce discomfort should be used where possible when administering euthanasia agents through pre-existing intraosseous catheters.[287] Placement of intraosseous (greater trochanter of the femur, greater tubercle of the humerus, medial aspect of the proximal tibia) catheters for administration of euthanasia agents and intracardiac, intrahepatic, intrasplenic, or intrarenal injections are acceptable only when performed on anesthetized or unconscious animals (with the exception of intrahepatic injections in cats as discussed in the Companion Animals section of the text). These routes are not acceptable in awake mammals and birds due to the difficulty and unpredictability of performing the techniques accurately with minimal discomfort. In some poikilotherms for which intracardiac puncture is the standard means of vascular access (eg, some snakes and other reptiles), intracardiac administration of euthanasia solutions in awake animals is acceptable. With the exceptions of IM delivery of ultrapotent opioids (ie, etorphine and carfentanil) and IM delivery of select injectable anesthetics, IM, SC, intrathoracic, intrapulmonary, intrathecal, and other nonvascular injections are not acceptable routes of administration for injectable euthanasia agents in awake animals.

M2.2.2 Immersion

Euthanasia of finfish and some aquatic amphibians and invertebrates must take into account the vast differences in metabolism, respiration, and tolerance to cerebral hypoxia among the various aquatic species. Because aquatic animals have diverse physiologic and anatomic characteristics, optimal methods for delivery of euthanasia agents will vary. In many situations, the immersion of aquatic animals in water containing euthanasia agents is the best way to minimize pain and distress. The response of aquatic animals to immersion agents can vary with species, concentration of agent, and quality of water; consideration of these factors should be made when selecting an appropriate euthanasia agent. Immersion agents added to water may be absorbed by multiple routes, including across the gills, *General recommendations*—The advantages of using barbiturates for euthanasia in dogs and cats far outweigh the disadvantages. Intravenous injection of a barbituric acid derivative is the preferred method for euthanasia of dogs, cats, other small animals, and horses. Barbiturates are also acceptable for all other species of animals if circumstances permit their use. Intraperitoneal or intracoelomic injection may be used in situations when an IV injection would be distressful, dangerous, or difficult due to small patient size. Intracardiac (in mammals and birds), intrasplenic, intrahepatic, and intrarenal injections must only be used if the animal is unconscious or anesthetized (with the exception of intrahepatic injections in cats as discussed in the Companion Animals section of the text).

M2.4 PENTOBARBITAL COMBINATIONS

Several euthanasia products combine a barbituric acid derivative (usually sodium pentobarbital) with local anesthetic agents, other CNS depressants (eg, phenytoin, ethanol), or agents that metabolize to pentobarbital. Although some of the additives are slowly cardiotoxic, euthanasia makes this pharmacologic effect inconsequential. These combination products are listed by the DEA as schedule III drugs, making them somewhat simpler to obtain, store, and administer than schedule II drugs such as sodium pentobarbital. The pharmacologic properties and recommended use of euthanasia products that com-been voluntarily withdrawn from the market by the manufacturer and is no longer manufactured or commercially available in the United States, although it is available in Canada and other countries. T-61 should be administered only IV and at carefully monitored rates of injection to avoid dysphoria during injection.

Advantages—(1) T-61 has a rapid onset of action and has been used to euthanize dogs, cats, horses, laboratory animals, birds, and wildlife. (2) Terminal (agonal) gasps that can occur in animals euthanized by IV barbiturates are not seen with use of T-61.

Disadvantages—(1) T-61 is currently not being manufactured in the United States. (2) Slow IV injection is necessary to avoid dysphoria prior to unconsciousness. (3) Each animal must be appropriately restrained and the agent must be administered by trained personnel. (4) Secondary toxicosis may occur in animals that consume remains of animals euthanized with T-61. (5) Because T-61 contains embutramide, a schedule III controlled drug, it is subject to the same restrictions in acquisition, storage, and use as other schedule III agents.

General recommendations—T-61 is acceptable as an agent of euthanasia provided it is administered appropriately by trained personnel. Routes of administration of T-61 other than IV are not acceptable.

M2.7 ULTRAPOTENT OPIOIDS

Etorphine hydrochloride and carfentanil citrate are ultrapotent opioids (10,000 times as potent as morphine sulfate) that are FDA approved for the immobilization of wildlife.[296] These opioids have been used as immobilization and extralabel euthanasia drugs primarily for large animals, particularly wildlife. Carfentanil has been used transmucosally in a lollipop form to euthanize captive large apes.[297] These drugs act on µ opioid receptors to cause profound CNS depression, with death secondary to respiratory arrest.

Advantages—(1) Etorphine and carfentanil can be delivered IM or transmucosally in situations where IV administration is unfeasible or dangerous. (2) These drugs have a rapid onset of action.

Disadvantages—(1) These drugs are strictly regulated, require special licensing to obtain and use, and are not FDA approved for use as agents of euthanasia. (2) There is substantial risk for humans handling the drugs, which can be absorbed through broken skin or mucous membranes. (3) These opioids may pose a risk of secondary toxicosis if the remains of euthanized animals are ingested; therefore proper disposal of animal remains is essential.

General recommendations—Etorphine or carfentanil is acceptable with conditions for euthanasia only in situations where use of other euthanasia methods is impractical or dangerous. Personnel handling the drugs must be familiar with their hazards, and a second person should be standing by and be prepared to summon medical support and administer first aid in case of accidental human exposure.

M2.8 DISSOCIATIVE A

cardiac arrest and death. The potassium ion is cardiotoxic, and rapid IV or intracardiac administration of 1 to 2 mmol/kg (0.5 to 0.9 mmol/lb) of body weight (1 to 2 mEq K^+/kg; 75 to 150 mg/kg [34.1 to 68.2 mg/lb] of potassium chloride) will cause cardiac arrest.[299] This is an injectable technique for euthanasia of livestock or wildlife species that may reduce the risk of toxicosis for predators or scavengers in situations where the remains of euthanized animals may be consumed.[300,301] Potassium chloride injected IV at 3 mEq/kg (1.4 mEq/lb) into parrots anesthetized with isoflurane caused mild vocalization in 1 of 6 birds and resulted in asystole in 68 seconds.[302] Use of 10 mEq/kg (4.5 mEq/lb) IV in anesthetized parrots resulted in involuntary muscle tremors in 5 of 6 birds and caused asystole in 32.8 seconds. Neither dosage resulted in histologic artifacts.

Magnesium salts may also be mixed in water for use as immersion euthanasia agents for some aquatic invertebrates. In these animals, magnesium salts induce death through suppression of neural activity.[134]

Advantages—(1) Potassium chloride and magnesium salts are not controlled substances and are easily acquired, transported, and mixed in the field. (2) Potassium chloride and magnesium salt solutions, when administered after rendering an animal unconscious, result in animal remains that are potentially less toxic for scavengers and predators and may be a good choice in cases where proper disposal of animal remains (eg, rendering, incineration) is impossible or impractical.

Disadvantages—(1) Rippling of muscle tissue and clonic spasms may occur upon or shortly after injection. (2) Potassium chloride and magnesium salt solutions are not approved by the FDA for use as euthanasia agents. (3) Saturated solutions are required to obtain suitable concentrations for rapid injection into large animals.

General recommendations—Personnel performing this technique must be trained and knowledgeable in anesthetic techniques, and be competent in assessing the level of unconsciousness that is required for administration of potassium chloride and magnesium salt solutions IV. Administration of potassium chloride or magnesium salt solutions IV requires animals to be in a surgical plane of anesthesia characterized by loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli. Use in unconscious animals (made recumbent and unresponsive to noxious stimuli) is acceptable in situations where other euthanasia methods are unavailable or not feasible. Although no scavenger toxicoses have been reported with potassium chloride or magnesium salts in combination with a general anesthetic, proper disposal of animal remains should always be attempted to prevent possible toxicosis by consumption of animal remains contaminated with general anesthetics.

M2.10 CHLORAL HYDRATE AND α CHLORALOSE

Chloral hydrate (1,1,1-trichloro-2,2,-dihydroxyethane) was once used in combination with magnesium sulfate and sodium pentobarbital as an economical anesthesia and euthanasia agent for large animals, but now is rarely used for this application in veterinary medicine. α Chloralose is a longer-acting derivative of chloral hydrate that has been used for anesthesia of laboratory animals, particularly for study of cerebrovasculature.[303,304] When administered IV, these agents have almost immediate sedative action, but unless agents. (4) Alcohols are not FDA approved as euthanasia agents. (5) Tribromoethanol is not commercially available as a pharmaceutical-grade product and must be compounded.

General recommendations—Ethanol in low concentrations is an acceptable secondary means of euthanasia in finfish rendered insensible by other means and as a primary or secondary means of euthanasia of some invertebrates. Immersion in high concentrations (eg, 70%) of ethanol is not acceptable. Ethanol may be acceptable with conditions as an agent of euthanasia for mice in specific situations, but is unacceptable as an agent of euthanasia for larger species. Tribromoethanol is acceptable with conditions as a method for euthanasia of laboratory

The application of benzocaine hydrochloride gel to the ventral abdomen of amphibians (20% concentration; 2.0-cm X 1.0-mm application) is an effective means of anesthesia and euthanasia for some species.[312,314,315] Following application of the gel to the ventrum of *X laevis* and placement in a wet bucket, righting and withdrawal reflexes subsided within 7 minutes, and death occurred within 5 hours.[312] No evidence of dermal injury, loss of dermal hydration, or difficulty breathing was associated with topical application of benzocaine hydrochloride gel to amphibians. A recent investigation on euthanasia of adult *X laevis* describes a dose of 182 mg/kg (82.7 mg/lb) of benzocaine hydrochloride gel as effective.[312] A comparison of benzocaine hydrochloride application with ice-slurry immersion for euthanasia of bony bream (*Nematalosa erebi*) indicated that, for certain warm water finfish species, an ice-slurry elicits less motor response than benzocaine overdose as a method of euthanasia, but additional work is needed to determine the most humane method.[316]

Advantages—(1) Benzocaine hydrochloride is a relatively fast-acting and effective euthanasia agent for finfish and amphibians. (2) Benzocaine hydrochloride is not a controlled substance. (3) Benzocaine hydrochloride has low toxicity for humans at concentrations used to euthanize finfish. (4) Benzocaine hydrochloride poses little environmental risk as it is readily filtered by use of activated carbon and breaks down in water within approximately 4 hours.

Disadvantages—(1) Benzocaine hydrochloride is not FDA approved for use as an agent of euthanasia. (2) Benzocaine hydrochloride may be cost prohibitive for euthanasia of larger finfish, amphibians, and reptiles or large populations. (3) Benzocaine hydrochloride solutions must be carefully buffered to avoid tissue irritation. (4) The impact of benzocaine residues in euthanized finfish on the environment or scavenger species has not been determined.

General recommendations—Benzocaine hydrochloride gel and solutions are acceptable agents for euthanasia for finfish and amphibians. Benzocaine hydrochloride is not an acceptable euthanasia agent for animals intended for consumption.

M2.14 CLOVE OIL, ISOEUGENOL, AND EUGENOL

Cloves contain a number of essential oils, including eugenol, isoeugenol, and methyleugenol.[317] Eugenol comprises 85% to 95% of the essential oils in cloves, and has been used as a food flavoring and a local anesthetic for human dentistry. It is also classified as an exempted minimum-risk pesticide active ingredient by the US EPA. Eugenol exhibits antifungal, antibacterial, antioxidant, and anticonvulsant activity. Some other components of clove oil, such as isoeugenol, are equivocal carcinogens based on studies in rodents.[318] Clove oil and its extracts have become popular as anesthetic agents for freshwater and marine finfish because of their wide availability, low cost, and shorter induction times when compared with MS 222.[319,320] When compared with MS 222 as an anesthetic agent, eugenol was found to have a more rapid induction, prolonged recovery, and narrow margin of safety, as it can cause rapid onset of ventilatory failure at high concentrations (> 400 mg/L).[321]

The anesthetic mechanism of clove oil and its derivatives has been poorly studied, but they appear to act similarly to other local anesthetics by inhibition of voltage-sensitive sodium channels within the nervous system.[296] Studies[322–324] of rodents indicate this class of agents may cause paralysis in addition to their anesthetic effects.

Advantages—(1) Clove oil and its derivatives are widely available, are relatively inexpensive, and are not controlled substances. (2) These agents have a short induction time. (3) Clove oil and its derivatives are effective at a wide range of water temperatures.

Disadvantages—(1) Clove oil and its derivatives are not FDA approved for use as an agent of euthanasia. (2) Animals euthanized with clove oil products are not approved for human consumption. (3) Some clove oil derivatives are potential carcinogens. (4) The impact of clove oil residues in euthanized finfish on the environment or scavenger species has not been determined.

General recommendations—Clove oil, isoeugenol, and eugenol are acceptable agents of euthanasia for finfish. It is recommended that, whenever possible, products with standardized, known concentrations of essential oils be used so that accurate dosing can occur. These agents are not acceptable means of euthanasia for animals intended for consumption.

M2.16 QUINALDINE (2-METHYLQUINOLINE, QUINALIDINE SULFATE)

Quinaldine has low solubility in water and therefore must first be dissolved in acetone or alcohol and then buffered with bicarbonate.[309] The potency of quinaldine varies with species, water temperature, water pH, and mineral content of water. Quinaldine acts through depression of sensory centers of the CNS.

Advantages—(1) Quinaldine can be used in a 1-step immersion method for euthanasia of finfish. (2) Quinaldine is not a controlled substance.

Disadvantages—(1) Quinaldine is not FDA approved for use as an agent of euthanasia. (2) The impact of quinaldine residues in euthanized finfish on the environment or scavenger species has not been determined.

General recommendations—Quinaldine is an acceptable method of euthanasia for finfish under certain circumstances. Quinaldine is not an acceptable means of euthanasia in animals intended for consumption.

M2.17 METOMIDATE

Metomidate is a highly water-soluble, nonbarbiturate hypnotic that acts by causing CNS depression. It is currently listed in the Index of Legally Marketed Unapproved New Animal Drugs for Minor Species by the FDA for use in sedation and anesthesia. While it is a rapidly acting euthanasia compound for certain species when used at 10 times the upper limit of the recommended anesthetic dose, its listing in the Index makes extralabel use (eg, its use for euthanasia) illegal. Should the index status of metomidate change to inclue euthanasia, or should FDA approval be obtained (thereby allowing extralabel use under AMDUCA), metomidate would be considered an acceptable agent of euthanasia for some species of finfish under certain circumstances.

Metomidate is not an acceptable means of euthanasia in animals intended for consumption.

M2.18 SODIUM HYPOCHLO

method of euthanasia. However, pneumatic purpose-built nonpenetrating captive bolt guns have been used successfully to euthanize suckling pigs,[c] neonatal ruminants,[130] and turkeys.[339]

M3.4 MANUALLY APPLIED BLUNT FORCE TRAUMA TO THE HEAD

Euthanasia by manually applied blunt force trauma to the head must be evaluated in terms of the anatomic features of the species on which it is to be performed, the skill of those performing it, the number of animals to be euthanized, and the environment in which it is to be conducted. Manually applied blunt force trauma to the head can be a humane method of euthanasia for neonatal animals with thin craniums if a single sharp blow delivered to the central skull bones with sufficient force can produce immediate depression of the CNS and destruction of brain tissue. When properly performed, loss of consciousness is rapid. Personnel performing manually applied blunt force trauma to the head must be properly trained and monitored for proficiency with this method of euthanasia, and they must be aware of its aesthetic implications.

Manually applied blunt force trauma to the head has been used primarily to euthanize small laboratory animals with thin craniums.[334,340,341] It has also been applied for euthanasia of young piglets. The anatomic features of neonatal calves make manually applied blunt force trauma to the head unacceptable as a method of euthanasia for this species.

Personnel who have to perform manually applied blunt force trauma to the head often find it displeasing and soon become fatigued. Fatigue can lead to inconsistency in application, creating humane concerns about its efficacious application to large numbers of animals. For this reason, the AVMA encourages those using manually applied blunt force trauma to the head as a euthanasia method to actively search for alternate approaches.

Advantages—(1) Blunt force trauma applied manually to the head is inexpensive and effective when performed correctly. (2) Blunt force trauma does not chemically contaminate tissues.

Disadvantages—(1) Manually applied blunt force trauma is displeasing for personnel who have to perform it. (2) Repeatedly performing manually applied aboutcT(ce)]TJ 0.13 ma7.ifTJ 01osing for p1(becom, Tw Tive whe
perI(few T* [ilternate)]TJ 0pubcio e hew Ts. For thstriccldgive when 7iyrfogunshma can le w hh can1(It)10(forhar

Lighter-weight, higher-velocity bullets can have high muzzle energy, but decreased penetration, which can be an issue when penetrating thick bones.

Whereas most euthanasia using firearms is conducted at close range, calculations of muzzle energy are useful for determining which firearms are appropriate for euthanasia of animals of varying sizes. As the bullet travels beyond the muzzle of the firearm its energy gradually begins to decrease. While this is not a concern for the use of firearms in close proximity to the animal, when attempting to euthanize an animal from a distance, to ensure accuracy and that an acceptable level of muzzle energy is achieved, a high-powered rifle may be the better choice for conducting euthanasia. In all cases, the most important factors in ensuring successful euthanasia are the experience and skill of the shooter.

M3.5.2 Muzzle Energy Requirements

For euthanasia, the combination of firearm and ammunition[350] selected must achieve a muzzle energy of at least 300 ft-lb (407 J) for animals weighing up to 400 lb (180 kg). For animals larger than 400 lb, 1,000 ft-lb (1,356 J) is required.[130] As demonstrated by Table 1, handguns do not typically achieve the muzzle energy required to euthanize animals weighing more than 400 lb (180 kg), and therefore rifles must be used to euthanize these animals.

Some would argue that the muzzle energies recommended are well beyond what is necessary to achieve satisfactory results. Anecdotal comment suggests that the .22 LR is one of the most frequently used firearms for euthanasia of livestock with varying degrees of success. There is little doubt that success or failure is partially related to firearm and bullet characteristics, but probably more so to selection of the ideal anatomic site (ie, a site more likely to affect the brainstem) for conducting the procedure. The Humane Slaughter Association lists multiple firearms for euthanasia of livestock, including shotguns (12, 16, 20, 28, and .410 gauges), handguns (.32 to .45 caliber), and rifles (.22, .243, .270, and .308). In general, when comparing handguns with rifles, the longer the barrel, the higher the muzzle velocity. Therefore, if a .22 is used for euthanasia it is best fired from a rifle. The .22 should never be used on aged bulls, boars, or rams.[351]

M3.5.3 Bullet Selection

While much of the emphasis in euthanasia by gunshot is placed on choice of the most appropriate firearm, it should be remembered that the gun is only the means of delivery. Bullet selection is quite possibly the most important consideration for euthanasia of livestock by gunshot. There are three basic types of bullets pertinent to this discussion: solid points, hollow points, and full metal jacketed bullets. Solid-point bullets are preferred for euthanasia since they are designed for greater penetration of their targets. Under ideal conditions this type of bullet will also undergo moderate expansion to a mushroom shape that increases its destructive characteristics. Hollow-point bullets are designed with a hollowed-out tip that causes rapid expansion and fragmentation of the bullet on impact. The hollow-point design allows maximum transfer of energy without risk of overpenetration. For applications where it may be desirable to control or reduce the degree of bullet penetration, hollow-point bullets are preferred. Howevere ie fooj 0At grams. These programs offer training in firearm safety and also provide information on rules and regulations for firearm use.

Firearms should never be held flush to an animal's body. The pressure within the barrel when fired may cause the barrel of the gun to explode, placing the shooter and observers at great risk of injury. Ideally, the muzzle of the firearm should be held within 1 to 2 feet of the animal's forehead and perpendicular to the skull with the intended path of the bullet roughly in the direction of the foramen magnum. This will reduce the potential for ricochet while directing the bullet toward the cerebrum, midbrain, and medulla oblongata, which will assure immediate loss of consciousness and rapid death.

Advantages—(1) Loss of consciousness is instantaneous if the projectile destroys most of the brain. (2) Given the need to minimize stress induced by handling and human contact, gunshot may be the most practical and logical method of euthanasia for wild or free-ranging species.

Disadvantages—(1) Gunshot may be dangerous for personnel. (2) It is aesthetically unpleasant for many. (3) Under field conditions, it may be difficult to hit the vital target area. (4) Brain tissue may not be able to be examined for evidence of brain diseases (eg, rabies infection, chronic wasting disease) when the head is targeted. (5) Skill in application of firearms and species-specific knowledge of appropriate target sites is required. In some states, firearm use is not permitted if the operator has been convicted of a felony.

General recommendations—When other methods cannot be used, an accurately delivered gunshot is acceptable with conditions for euthanasia.[344,352] When an animal can be appropriately restrained, the penetrating indicate this activity does not imply that pain is perceived, and in fact conclude that loss of consciousness develops rapidly. Visually evoked potentials in mice were reduced more quickly after cervical dislocation compared with decapitation.[51]

Guillotines designed to accomplish decapitation of adult rodents and small rabbits in a uniformly instantaneous manner are commercially available. Guillotines are not commercially available for neonatal rodents, but sharp blades can be used for this purpose.

Advantages—(1) Decapitation appears to induce rapid loss of consciousness.[56–58] (2) It does not chemically contaminate tissues. (3) It is rapidly accomplished.

Disadvantages—(1) Handling and restraint required to perform decapitation may be distressful for animals.[358] (2) The interpretation of the presence of electrical activity in the brain following decapitation has created controversy, and its importance may still be open to debate.[56–59] (3) Personnel performing this method should recognize the inherent danger of the guillotine and take precautions to prevent personal injury. (4) Decapitation may be aesthetically displeasing to personnel performing or observing the method.

General recommendations—This method is acceptable with conditions if performed correctly, and it may be used in research settings when its use is required by the experimental design and approved by the IACUC. Decapitation is justified for studies where undamaged and uncontaminated brain tissue is required. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal. Decapitation of amphibians, finfish, and reptiles is addressed elsewhere in the Guidelines. Those responsible for the use of this method must ensure that personnel who perform decapitation have been properly trained to do so and are monitored for competence.

M3.8 ELECTROCUTION

Alternating current has been used to euthanize dogs, cattle, sheep, goats, swine, chickens, foxes, mink, and finfish.[45,54,342,345,359–366] Fifty- or 60-cycle electrical current is more effective than higher frequencies.[367,368] Electrocution induces death by cardiac fibrillation, which causes cerebral hypoxia.[365,366,369] However, animals do not lose consciousness for 10 to 30 seconds or more after onset of cardiac fibrillation. It is imperative that animals be unconscious and insensible to pain before being electrocuted. Unconsciousness can be induced by any method that is acceptable or acceptable with conditions, including passing a current through the brain.[370]

Parameters for use of electricity to induce unconsciousness are readily available.[342,371] When electricity is used to induce unconsciousness, a current is passed through the brain, which will induce a grand mal epileptic seizure.[106,363,366,372] Signs of effective induction of the seizure are extension of the limbs, opisthotonus, downward rotation of the eyeballs, and a tonic (rigid) spasm changing to a clonic (paddling) spasm with eventual muscle flaccidity.

There are three approaches to the use of electricity for euthanasia. They are head only, 1-step head to body, and 2-step head and body. To be effective for euthanasia all three of these methods must induce a grand mal epileptic seizure.

For the head-only procedure, an electrical current is passed through the head to induce a seizure. This causes a temporary loss of consciousness of 15 to 30 seconds' duration,[106,372,373] but does not induce cardiac fibrillation. For this reason, head-only application must be immediately followed by a secondary procedure to cause death. When the head-only procedure is applied, the grand mal seizure is easily observable. Electrically induced cardiac fibrillation, exsanguination, or other appropriate adjunctive methods may be used to achieve death and should be performed within 15 seconds of when the animal becomes unconscious.

In the 1-step head-to-body approach an electrical current is simultaneously passed through both the brain and the heart. This simultaneously induces a grand mal seizure and electrocutes the animal by inducing cardiac arrest.[106,359,374–376] Because electricity passes through the spinal column, clinical signs of the grand mal seizure may be masked; however, it is usually possible to see a weak tonic phase and weak clonic phase after a 3-second application. If current is applied for more than 3 seconds, tonic and clonic spasms may be blocked. The 1-step approach must be used with amperage settings that have been scientifically verified to induce a seizure. Recommended amperages are 1.25 amps for pigs, 1 amp for sheep, and 1.25 amps for cattle.[341,376] Denicourt et al[377] report that 110 V at 60 Hz applied for 3 seconds was effective for pigs up to 125 kg (275 lb).

In the 2-step method an electrical current is passed through the head to induce unconsciousness, then a second current is passed through either the side of the body or the brisket to induce cardiac arrest.[378,379] Applying the second current by an electrode placed on the side of the body behind the forelimb has been reported to be effective.[49]

A common cause of failure to induce unconsciousness is incorrect placement of the electrodes.[374] Experiments with dogs revealed that electrode positions where the brain is bypassed do not cause instantaneous unconsciousness. When electricity passes only between the forelimbs and hind limbs or neck and feet, it causes the heart to fibrillate but does not induce sudden loss of consciousness.[369] The animal will be electrocuted, but will remain conscious until it dies from cardiac fibrillation.

Three options are available for correct electrode placement for the head-only method, including on both sides of the head between the eye and ear, the base of the ear on both sides of the head, and diagonally below one ear and above the eye on the opposite side of the head. For the 1-step (head-to-back) method, the head electrode may be placed on the forehead or immediately behind the ear. The head electrode should never be placed on the neck because the brain will be bypassed.[100] Diagonal movement of the electrical current through the body can be accomplished by placing the head electrode behind one ear and the body electrode on the opposite side. When the 2-step procedure is used, placement of the body electrode behind the forelimb is effective.[49] Electrodes consisting of a metal band or chain around the nose and a band or chain around the thorax appear to be effective for pigs weighing up to 125 kg.[377]

When electrical methods of euthanasia are used, the following signs of return to consciousness must be absent: rhythmic breathing, righting reflex, vocalization, eyeblink, and tracking of a moving object.[49] Gasping and nystagmus may be present in animals that have been successfully rendered unconscious with electricity. Gasping should not be confused with rhythmic breathing, and nystagmus (a rapid vibrating or fluttering of the eye) should not be confused with eyeblink (complete closure and then complete opening of the eye, which occurs without touching).

Advantages—(1) Electrocution is humane if the animal is first rendered unconscious. (2) It does not chemically contaminate tissues. (3) It is economical.

Disadvantages—(1) Electrocution may be hazardous to personnel. (2) It is not useful for dangerous, intractable animals that are difficult to restrain. (3) It is aesthetically objectionable because of violent extension and stiffening of the limbs, head, and neck. (4) It may not result in death in small animals (< 5 kg [11 lb]) because ventricular fibrillation and circulatory collapse do not always persist after cessation of current flow. (5) Sometimes it is not effective in dehydrated animals.[371] (6) Personnel must be familiar with appropriate placement of electrodes and use of equipment. (7) Purpose-built equipment must be used.

General recommendations—Euthanasia by electrocution is acceptable with conditions. It requires special skills and equipment that will ensure passage of sufficient current through the brain to induce loss of consciousness and induce tonic and clonic epileptic spasms. Unconsciousness must be induced before cardiac fibrillation or simultaneously with cardiac fibrillation. Cardiac fibrillation must never occur before the animal is rendered unconscious. Methods that apply electric current from head to tail, head to foot, or head to moistened metal plates on which the animal stands are unacceptable. The 2-step method should be used in situations where there may be questions about sufficient current to induce a grand mal seizure with tonic and clonic spasms. This approach enables observation of tonic and clonic spasms before a second current is applied to induce cardiac arrest. Although acceptable with conditions if the aforementioned requirements are met, the method's disadvantages outweigh its advantages in most applications. Electroimmobilization that paralyzes an animal without first inducing unconsciousness is extremely aversive and is unacceptable.[370,371] For both humane and safety reasons, the use of household electrical cords is not acceptable.

M3.9 KILL TRAPS

Mechanical kill traps are used for the collection and killing of small, free-ranging mammals for commercial purposes (fur, skin, or meat), scientific purposes, to stop property damage, and to protect human safety. Their use remains controversial and kill traps do not always render a rapid or stress-free death consistent with the criteria established for euthanasia by the POE.[380] For this re witd kila70(, he ent thatechanicalthis r)2

Tw T*(POE.)Tj 5.83hat T*[(efeith)]Td(overp)]TJ 0 p

svTj 0(not alwaof toTasms. Th0(diacsmall, fr)20(ee-rtect hu0

substance are not acceptable for euthanasia, but may be

S1. COMPANION ANIMALS

Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method can be met.

S1.1 GENERAL CONSIDERATIONS

Companion animals for which euthanasia is determined to be necessary are usually encountered in 4 main environments: individually owned animals; breeding animals (from dams, sires, and single litters to colonies of breeding animals); populations of animals maintained in animal control facilities, shelters and rescues, and pet shops; and animals maintained in research laboratories. Examples of less common venues in which companion animals might be euthanized include quarantine stations and Greyhound racetracks. Aquatic companion animals are considered in Section S6, Finfish and Aquatic Invertebrates, of the Guidelines. As indicated previously in this document (see Section I5.5, Human Behavior), the relationships between companion animals and their owners or caretakers vary and should be carefully considered and respected when selecting an approach to euthanasia for these species.

Euthanasia of companion animals is best conducted in quiet, familiar environments when practical. The species being euthanized, the reason for euthanasia, and the availability of equipment and personnel will all contribute to decisions about the most appropriate location. The professional judgment of the veterinarian conducting or providing oversight for euthanasia is paramount in making appropriate decisions about euthanasia (eg, location, agent, route of administration) in species kept as companions and in the specific environments where they are encountered. Personnel conducting euthanasia must have a complete understanding of and proficiency in the euthanasia method to be used.

For individually owned companion animals, euthanasia will often be conducted in a private room in a veterinary clinic or in the home, to minimize animal and owner distress.[421] Factors leading to the decision to euthanize should be discussed openly,[109] and the animal's owner should be permitted to be present during euthanasia whenever feasible. Owners should be fully informed about the process they are about to observe, including the potential for excitation during anesthesia and other possible complications.[421,422] If one euthanasia method is proving difficult, another method should be tried immediately. Euthanasia should only be attempted when the necessary drugs and supplies are available to ensure a smooth procedure and, upon verification of death, owners should be verbally notified.[110]

In animal control, shelter, and rescue situations; research laboratories; and other institutional settings, trained technical personnel rather than veterinarians often perform euthanasia. Training and monitoring of these individuals for proficiency vary by setting and state (eg, animal control officers, animal care technicians in laboratories, certified euthanasia technicians in shelters in some states), as does the amount of veterinary supervision required. Euthanizing large numbers of animals on a regular basis can be stressful and may result in symptoms of compassion fatigue.[123] To minimize the stress and demands of this duty, trained personnel must be assured that they are performing euthanasia in the most humane manner possible. This requires an organizational commitment to provide ongoing professional training on the latest methods and materials available for euthanasia and effective management of compassion fatigue for all personnel.[121] In addition, personnel should be familiar with methods of restraint and euthanasia for all species likely to be encountered in their facility.

Areas where euthanasia is conducted in institutional settings should be isolated from other activities, where possible, to minimize stress on animals and to provide staff with a professional and dedicated work area. A well-designed euthanasia space provides good lighting with the ability to dim or brighten as required, ventilation, adaptable fixtures, and adequate space for at least two people to move around freely in different types of animal-handling situations.[121,423] Attempts should be made to minimize smells, sights, and sounds that may be stressors for animals being euthanized. Basic equipment for handling and restraint, a scale, clippers, tourniquets, stethoscope, cleaning supplies, a variety of needles and syringes, and body bags should be readily available to accommodate the needs of potentially diverse animal populations. In addition, a first-aid kit should be available to address minor human injuries, and medical attention should always be sought for bite injuries and more serious human injuries.

Euthanasia protocols for companion animals (usually dogs and cats) in institutional settings (eg, shelters, large breeding facilities, research facilities, quarantine facilities, racetracks) may differ from those applied in traditional companion animal clinical practices due to situation-specific requirements, including variable access to pharmaceuticals and other equipment, diagnostic and research needs (eg, postmortem tissue samples), and the number of animals to be euthanized. For this reason, general recommendations about euthanasia methods applicable to companion animals are followed by more specific information as to their applicability in frequently encountered environments. While protocols may differ, the interests of the animal must be given equal consideration whether the animal is individually owned or not.

S1.2 ACCEP

ure 7][136,428,g]) may be used as an alternative to IV or IP injection of barbiturates when IV access is difficult.[428] Intra-organ injections may speed the rate of barbiturate uptake over standard IP injections, and when an owner is present, this approach may be preferred over the IP route.[429]

Penetrating captive bolt—Use of a penetrating captive bolt by trained personnel in a controlled laboratory setting has been described as an effective and humane method of euthanasia for rabbits and dogs.[331] The bolt must be placed directly against the skull; therefore, safe and effective application of the technique may be facilitated by pre-euthanasia sedation or anesthesia. Penetrating captive bolt is not recommended as a routine approach to the euthanasia of dogs, cats, or other small companion animals, and should not be used when other methods are available and practicable.

S1.4 ADJUNCTIVE METHODS

Potassium chloride—Potassium chloride (1 to 2 mmol/kg, 75 to 150 mg/kg, or 1 to 2 mEq K^+/kg) administered IV or intracardially may be used to euthanize companion animals when they are unconscious (unresponsive to noxious stimuli) or under general anesthesia. Use of potassium chloride in awake animals is unacceptable.

Nitrogen or argon—Gradual displacement methods using N_2 or Ar, alone or with other gases, in awake dogs and cats may result in hypoxia prior to loss of consciousness (see Inhaled Agents section of the Guidelines for details). Therefore, administration of N_2 or Ar (< 2% O_2) should only be used as an adjunctive method for unconscious or anesthetized dogs and cats; prolonged exposure may be necessary to ensure death. Alternate methods with fewer conditions and disadvantages are recommended whenever feasible.

Electrocution—Electrocution using alternating current in dogs rendered unconscious by an acceptable means (eg, general anesthesia) may be used for euthanasia (see Section M3.8 of the Guidelines for details). The disadvantages of electrocution outweigh its advantages; therefore it is not recommended for routine use in companion animals. Alternate methods with fewer conditions and disadvantages should be used whenever feasible.

S1.5 UNACCEPTABLE METHODS

With the exception of IM delivery of select injectable anesthetics, the SC, IM, intrapulmonary, and intrathecal routes of administration are unacceptable for administration of injectable euthanasia agents because of the limited information available regarding their effectiveness and high probability of pain associated with injection in awake animals.

Household chemicals, disinfectants, cleaning agents, and pesticides are not acceptable for administration as euthanasia agents.

Other unacceptable approaches to euthanasia include hypothermia and drowning.

S1.6 SPECIAL CONSIDERATIONS

S1.6.1 Dangerous or Fractious Animals

Animals that are unable to be safely and humanely restrained should be sedated by means of drugs delivered orally (eg, gelatin capsules for delivery of drugs in food,[91] liquid formulations squirted into mouths[92]) or remotely (eg, darts, pole syringes) before administration of euthanasia agents. Doing so will assist in relieving anxiety and pain for the animal, in addition to reducing safety risks for personnel. There is a variety of pre-euthanasia drugs that can be administered PO, SC, or IM, alone or in combination, to render animals unconscious with minimal handling in preparation for euthanasia.[431]

S1.6.2 Disposal of Animal Remains

Residues of injectable agents commonly used for euthanasia of companion animals (eg, sodium pentobarbital) tend to persist in the remains and may cause sedation or even death of animals that consume the body. For this reason safe handling and appropriate disposal of the remains are critically important. Additional information is available in Section I8, Disposal of Animal Remains.

S1.7 FETUSES AND NEONATES

Scientific data[432] indicate that mammalian embryos and fetuses are in a state of unconsciousness throughout pregnancy and birth. For dogs and cats, this is in part due to moderate neurologic immaturity, with sentience being achieved several days after birth. The precocious young of guinea pigs remain insentient and unconscious until 75% to 80% of the way through pregchemical inhibitors (eg, adenosine, allopregnanolone, pregnanolone, prostaglandin D_2, placental peptide neuroinhibitor) and hypoxic inhibition of cerebrocortical activity.[432] As a consequence, embryos and fetuses cannot consciously experience feelings such as breathlessness or pain. Therefore, they also "cannot suffer while dying in utero after the death of the dam, whatever the cause."[432] Information about developing nonmammalian eggs is available in the S5, Avians; S6, Finfish and Aquatic Invertebrates; and S7, Captive and Free-Ranging Nondomestic Animals sections of the Guidelines.

Euthanasia of dogs, cats, and other mammals in mid- or late-term pregnancy should be conducted via an injection of a barbiturate or barbituric acid derivative (eg, sodium pentobarbital) as previously described. Fetuses should be left undisturbed in the uterus for 15 to 20 minutes after the bitch or queen has been confirmed dead. This guidance is also generally applicable to nonmammalian species, with euthanasia of eggs per guidance provided in the S5, Avians; S6, Finfish and Aquatic Invertebrates; and S7, Captive and Free-Ranging Nondomestic Animals sections of the Guidelines. Intraperitoneal injections of pentobarbital should be avoided whenever possible during the later stages of pregnancy due to the likelihood of inadvertently entering the uterus, rendering the injection ineffective.

Altricial neonatal and preweanling mammals are relatively resistant to euthanasia methods that rely on hypoxia as their mode of action. It is also difficult, if not impossible, to gain venous access. Therefore, IP injection of pentobarbital is the recommended method of euthanasia in preweanling dogs, cats, and small mammals. Intraosseous injection may also be used, if strategies are used to minimize discomfort from injection by using intraosseous catheters that may be in place (see Section M2, NonInhaled Agents, of the Guidelines), or if the animal is anesthetized prior to injection.

During ovariohysterectomy of pregnant dogs and cats and small mammals with altricial neonates, ligation of the uterine blood vessels with retention of the fetuses inside the uterus will result in death of the fetuses. The resistance of altricial neonates (eg, cats, dogs, mice, rats) to euthanasia methods whose mechanisms rely on hypoxia suggests that the uterus should not be opened for substantially longer periods than for precocial neonates,[433] perhaps 1 hour or longer. In the case of caesarian section in late-term pregnancy, IP injection of pentobarbital is recommended for fetuses that must be euthanized for congenital deformities or illness and that have been removed from the uterus (creating the potential that successful breathing may have occurred).

S1.8 EUTHANASIA IN SPECIFIC ENVIRONMENTS

S1.8.1 Individual Animals

mandatory veterinary input and considers animal welfare, requirements for postmortem tissue specimens, and interference of euthanasia agents or methods with study results. Scientific and husbandry staff form strong emotional bonds with companion animals in scientific settings, so sensitivity to grief and compassion fatigue is necessary.

S2. LABORATORY ANIMALS

Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application

S2.2.4 Fetuses and Neonates

Rodents with altricial young, such as mice and rats, must be differentiated from rodents with precocial young, such as guinea pigs. Precocial young should be treated as adults.

S2.2.4.1 Acceptable Methods

Euthanasia of the dam and fetuses—Rodent fetuses along with other mammals are unconscious in utero mals already under anesthesia may be euthanized by an overdose of anesthetic.

Carbon dioxide—While CO_2 is an effective method of euthanasia, its use as the sole agent in rabbits results in apparent distress to the rabbit. Premedication with sedative agents will allow for the administration of CO_2 for euthanasia.

S2.4.3.2 Physical Methods

Cervical dislocation—Cervical dislocation is acceptable with conditions for rabbits when performed by individuals with a demonstrated high degree of technical proficiency. The need for technical competency is great in heavy or mature rabbits in which the large muscle mass in the cervical region makes manual cervical dislocation more difficult. Commercial devices designed to aid in rabbit cervical dislocation are available and should be evaluated for their effectiveness.

Penetrating captive bolt—The use of rabbit-sized penetrating captive bolts to euthanize rabbits in laboratory or production facilities is acceptable with conditions. The captive bolt must be maintained in clean working order, positioned correctly, and operated safely by trained personnel.

S2.4.4 Special Cases

When rabbits to be euthanized are in a surgical plane of anesthesia, adjunctive methods such as delivery of potassium chloride, exsanguination, or bilateral thoracotomy are acceptable.

S2.5 LABORATORY FINFISH, AQUATIC INVERTEBRATES, AMPHIBIANS, AND REPTILES

Recommending euthanasia methods for finfish, aquatic invertebrates, amphibians, and reptiles used in biomedical research is challenging due to the enormous number of species and variations in biological and physiologic characteristics. Methods for euthanizing species commonly used in research are discussed in detail in the relevant sections of the Guidelines. See these sections for additional information.

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 operculum movements and subsequent holding times in ice-chilled water, specific to finfish size and age.[316,461,462] Adult zebrafish should be exposed for a minimum of 10 minutes and fry 4 to 7 days after fertilization (dpf) for at least 20 minutes following loss of operculum movement. Rapid chilling (as well as MS 222) has been shown to be an unreliable euthanasia method for embryos < 3 dpf. To ensure embryonic lethality these methods should be followed with another agent such as diluted sodium or calcium hypochlorite solution.[462] If necessary to ensure death of other life stages, rapid chilling may be followed by either an approved adjunctive euthanasia method or a humane killing method. Until further research is conducted, rapid chilling is acceptable with conditions for other small-bodied tropical and subtropical stenothermic species.

Amphibian species commonly used in research include the African clawed frog (*X laevis*) and leopard and bull (*Rana* spp) frogs. These species are best euthanized via a physical method while fully anesthetized.

S3. ANIMALS FARMED FOR FOOD AND FIBER

Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method are met.

3.1 GENERAL CONSIDERATIONS

While some methods of slaughter and depopulation might meet the criteria for euthanasia identified by the POE, others will not and comments in this document are limited to methods used for euthanasia. The following section relates to species of animals domesticated for agricultural purposes, specifically cattle, sheep, goats, swine, and poultry, regardless of the context in which that animal is being kept or the basis for the decision to euthanize it.

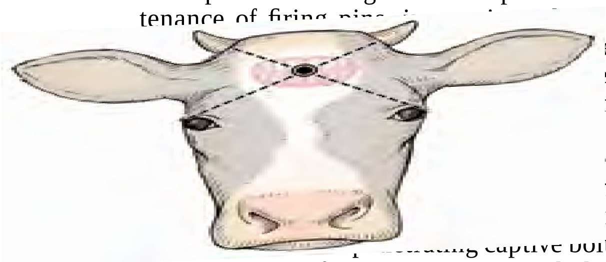
Handling of animals prior to euthanasia should be as stress free as possible. This is facilitated by ensuring that facilities are well designed, appropriate equipment is available, and animal handlers are properly trained and their performance monitored.[101,105–108]

Regardless of the method of euthanasia used, death must be confirmed before disposal of the animal's remains. The most important indicator of death is lack of a heartbeat. However, because this may be difficult to evaluate or confirm in some situations, animals can be observed for secondary indicators of death, which might include lack of movement over a period of time (30 minutes beyond detection of a heart beat) or the presence of rigor mortis.

S3.2 BOVIDS AND SMALL RN

excess energy of the bolt. Depending upon model, the bolt may automatically retract or require manual placement back into the barrel through the muzzle. Accurate placement over the ideal anatomic site, energy (ie, bolt velocity), and depth of penetration of the bolt determine effectiveness of the device to cause a loss of consciousness and death. Bolt velocity is dependent on maintenance of the captive bolt gun (cleaning and replacement of worn parts), as well as proper storage of the cartridge charges. Bolt velocities of 55 to 58 m/s are desirable for effective captive bolt use in slaughter plants.[332,333,465,466] Recommended minimum bolt velocities proposed for shooting bulls are as high as 70 m/s. In slaughter plants where bolt velocity is of particular concern, bolt velocity is routinely monitored to assure proper function of these devices.[467]

In general, captive bolt guns, whether penetrating or nonpenetrating, induce immediate loss of consciousness, but death is not always assured with the use of this device alone. In a study of 1,826 fed steers and heifers only 3 (0.16%) had signs of a return to sensibility or consciousness.[336] Results were similar in observations of 692 bulls and cows where 8 (1.2%) animals had signs consistent with a return to consciousness.[336] Failure to achieve a 100% loss of consciousness with no return to a conscious mental state was attributed to storage of the captive bolt charges in a damp location, poor maintenance of firing pin... sonnel oper- ...ect anatomic ...r on the cap- ...nd bulls with



...thod such as ...n of a saturat- ...mmended to ...penetrating captive bolt is used.[347] A newer version of penetrating captive bolt has emerged in recent years.[130]

-echniqueT -1scattposed flt ghobolng t) ous-
prophe use of -—Is

Firearms should be positioned so that the muzzle is perpendicular to the skull to avoid ricochet. Proper positioning of the firearm or penetrating captive bolt is necessary to achieve the desired results.

Use of the poll (bony protuberance on the top of the skull) for application of the penetrating captive bolt in slaughter plants is not allowed by regulations in the European Union because the depth of concussion in this region is less than that observed in frontal sites.[468] Conversely, for large bulls and water buffalo use of the frontal site for administration of a captive bolt is not always effective because of the thickness of the hide and skull in this region. Use of the poll position veins are cut, bleeding may persist at variable rates for several minutes. Severing these vessels closer to the thoracic inlet where the vessels are larger will increase blood flow rate. Some evidence suggests that restricted blood flow may be caused by the formation of false aneurysms in the severed ends of arteries in cattle.[474]

Pithing—Pithing is a technique designed to cause death by increasing destruction of brain and spinal cord tissue. It is performed by inserting a pithing rod through the entry site produced in the skull by a bullet or penetrating captive bolt. The operator manipulates the pithing tool to destroy brainstem and spinal cord tissue to ensure death (see Physical Methods). Muscular activity during the pithing process is often quite violent, but is followed by quiescence that facilitates exsanguination or other procedures.[420]

S3.2.2 Sheep and Goats

Euthanasia of small ruminants may be necessary for reasons ranging from traumatic injury to incurable disease. Methods include barbiturate overdose, gunshot, or captive bolt followed by an adjunctive method such as exsanguination, IV administration of potassium chloride or magnesium sulfate, or pithing. Electrocution is another option, but this method requires specialized equipment to restrain the animal for proper placement of the electrodes. Because electricity and the necessary equipment are unlikely to be available for euthanasia under field conditions, electrocution is not considered to be practical for routine use.

S3.2.2.1 Acceptable Methods

S3.2.2.1.1 Noninhaled Agents

Barbiturates and barbituric acid derivatives—Barbiturates act by depression of the CNS, which progresses from a state of consciousness to unconsciousness, deep anesthesia, and eventually death. Although use of these agents requires restraint and involves mild discomfort (ie needle placement) for administration, observers generally find this a more acceptable method of euthanasia because death comes about more peacefully. In the companion animal setting, these attributes are highly desirable. In production settings, concerns for cost and disposal of animal remains make this method a less attractive euthanasia option.

S3.2.2.2 Acceptable With Conditions Methods

S3.2.2.2.1 Physical Methods

Gunshot—Firearms recommended for euthanasia of adult small ruminants include the .22 LR rifle; .38 Special, .357 Magnum, and 9 mm or equivalent handguns; and shotguns. Some prefer hollow-point bullets to increase brain destruction and reduce the chance of ricochet. However, operators are reminded that bullet fragmentation may substantially reduce the potential for brain destruction because of reduced penetration, particularly when used in large-horned adult rams. Shotguns or higher-caliber firearms loaded with solid-point bullets are preferred in these conditions. When firearms are used for euthanasia it is important that the gun never be held flush with the skull. Instead, the muzzle of the gun should be aimed in the desired direction and held no closer than 6 to 12 inches from the target.

Penetrating and nonpenetrating captive bolts—The principal anatomic sites for application of captive bolts in small ruminants are the frontal and poll positions (Figure 11). In sheep with horns, the poll position is often preferred. Use of a captive bolt in the poll position was evaluated, using 8 anesthetized sheep.[468] Projection of the shot was on a line running between the bases of the ears and aiming toward the throat. Cortical visual because their skulls are too hard to achieve immediate destruction of brain tissue leading to unconsciousness and death. Manually applied blunt force trauma is also difficult if not impossible to apply consistently because of the degree of restraint required and complications in positioning calves, lambs, and kids for conducting this procedure.

Barbiturate overdose may be used for euthanasia of neonatal calves, lambs, and kids. In noncommercial tempts would include impaired brain function caused by anoxia occurring during the rescue attempt, compromised respiratory function and body heat production resulting from fetal immaturity, and greater risk of infection as a consequence of failure of passive transfer of immunity.[432,484,485] When the value of the fetus justifies the effort to secure a successful live delivery, the preferred approach to assure fetal health and welfare is by caesarian section using standard surgical procedures.

Barbiturates and barbituric acid derivatives—Pentobarbital readily crosses the placenta resulting in fetal depression in pregnant animals. However, death of the dam normally precedes the death of the fetus. In one study[486] cardiac arrest in lambs was delayed for as long as 25 minutes beyond the death of the dam. Similar observations in mice demonstrated that death of the fetuses could only be achieved by the use of doses well in excess of those normally required for euthanasia.[487] Based on these observations, one could offer a similar recommendation to that provided previously for death by exsanguination whereby fetuses should be retained within the uterus for at least 15 to 20 minutes after maternal death has occurred to prevent the delivery of viable fetuses.

S3.3 SWINE

Methods of euthanasia commonly applied to swine include CO_2, Ar, N_2, gas mixtures, gunshot, nonpenetrating and penetrating captive bolts, overdose of an anesthetic administered by a veterinarian, electrocution, and blunt force trauma (in suckling piglets only). Selection of the most appropriate method for each situation is dependent upon size and weight of the animal, availability of equipment and facilities, operator skill and experience with the procedure, aesthetic concerns, human safety, and options for disposal of remains. Certain physical methods of euthanasia may require adjunctive methods such as exsanguination or pithing to ensure death. A brief description of each method and appropriate candidates for it are described. Detailed information on inhaled, noninhaled, and physical methods of euthanasia may be found in the respective sections of this document.

S3.3.1 Mature Sows, Boars, and Grower-Finisher Pigs

Methods usually used for euthanasia of sows, boars, and grower-finisher pigs include gunshot, penetrating captive bolt, electrocution, and barbiturate overdose.

Use of physical methods of euthanasia requires direct contact with the animal, and therefore restraint is necessary. Use of a snare is the most common form of restraint for adult swine. Studies[488–495] demonstrate varying degrees of stress associated with restraint by snaring techniques. To minimize stress associated with snaring, personnel conducting euthanasia of swine are advised to make advance preparations (eg, prepare the site, load the gun or captive bolt) so that the time during which the animal must be restrained is minimized.

S3.3.1.1 Acceptable Methods

S3.3.1.1.1 Noninhaled Agents

Barbiturates and barbituric acid derivatives—Mature sows, boars, and grower-finisher pigs may be euthanized by IV administration of euthanasia solutions containing barbiturates.[496] A dosage of 1 mL/5 kg (0.45 mL/2.3 lb) up to 30 kg (66 lb), then 1 mL/10 kg (0.45 mL/4.5 lb) thereafter, has been recommended.[497] This method may not cause death if a lethal dose is not administered IV. Barbituates are not commonly used in field conditions, but may be applicable in some settings. Because these drugs are controlled substances they must be administered by personnel who are registered with the US DEA, and extralebel use requires administration by or under the supervision of a veterinarian. Strict record keeping is required of all who use and store these drugs.

Many find euthanasia by the IV administration of a barbituate less displeasing than gunshot, captive bolt, or electrocution. Therefore, it is preferred in some settings. A disadvantage of this method of euthanasia is that tissues from animals euthanized with barbiturates may not be suitable for diagnostic evaluation. Furthermore, options for disposal of animals euthanized with

Figure 12—There are three possible sites for conducting euthanasia in swine: frontal, temporal and from behind the ear toward the opposite eye. The frontal site is in the center of the forehead slightly above a line drawn between the eyes. The bolt or bullet should be directed toward the spinal canal. The temporal site is slightly anterior and below the ear. The ideal target location and direction of aim may vary slightly according to breed and the age of the animal (due to growth of the frontal sinuses). (Adapted with permission from Shearer JK, Nicoletti P. Anatomical landmarks. Available at: www.vetmed.iastate.edu/vdpam/extension/dairy/programs/humane-euthanasia/anatomical-landmarks. Accessed Jun 24, 2011.)

energies of 300 ft-lb or more are required for euthanasia of adult sows, boars, and growing-finishing pigs. When the alternate site behind the ear is chosen, a .22 caliber firearm loaded with a solid-point bullet may be used. Wadcutters and fragmenting bullets should not be used for euthanasia of adult swine. Potential for ricochet is reduced when euthanasia by gunshot can be conducted outdoors where bullets that pass through the animal may be captured in an earthen surface. Shotguns may be used at short range and offer the advantage of less potential for bullet ricochet. Twelve-, 16- or 20-gauge shotguns are recommended for mature pigs. The muzzle should never be held flush to the skull.

Gunshot is an effective, low-cost method of euthanasia when properly performed. Firearms are readily available in most areas. Human safety is the primary concern with the use of gunshot for euthanasia. Proper training on firearm safety and use is imperative and gunshot should only be performed by personnel who have had appropriate training.

Penetrating captive bolt—Use of well-maintained penetrating captive bolt guns with ammunition appropriately selected for the size of the animal is acceptable with conditions as a method of euthanasia for growing and adult swine.[499,500] Proper application of the penetrating captive bolt requires restraint of the animal because the device must be held firmly against the forehead over the site described for gunshot (Figure 12). When performed correctly, the pig drops to the floor immediately, exhibiting varying amounts of tonic and clonic muscle movements. Confirmation that the animal has been rendered insensible includes observation of the following: rhythmic breathing stops, no righting reflex is observed, vocalization is absent, and no palpebral reflexes or responses to noxious stimuli are present. All pigs should be observed for evidence of these responses until death has been confirmed.

Death following use of the penetrating captive bolt is commonly achieved, but is not assured depending upon bolt length and depth of the frontal sinus in mature sows and boars. Therefore, secondary steps to ensure death (eg, a second application of the penetrating captive bolt, exsanguination, pithing) should be applied as necessary. Breed differences result in variable skull shapes making determination of the best anatomic site for conducting euthanasia in mature sows and boars difficult.[352]

Penetrating captive bolts offer safety advantages compared with firearms. Properly applied, the method is very effective and costs associated with its use are minimal. However, it is important that penetrating captive bolt guns be maintained regularly (cleaning and replacement of worn parts) and that cartridge charges be stored properly to ensure appropriate bolt velocity. Bolt length and ammunition requirements for effective single-step euthanasia vary for different sizes and maturities of pigs. Using a captive bolt of inappropriate length or with insufficient charge reduces effectiveness. Personnel must be trained in the proper use of penetrating captive bolts to ensure effective euthanasia.

Electrocution—Electrocution as a sole method of euthanasia can achieve death via 2-step or single-step processes.[359,373,501–508] Electrical current must pass through the brain to achieve loss of consciousness, but then must cross the heart to cause fibrillation and cardiac arrest. As a 2-step process, electrode placements are head-head, followed by head to flank, for the appropriate time. For a single-step process for euthanasia, head to opposite flank is an example of appropriate placement.

Head-only electrocution induces a grand mal seizure and immediate unconsciousness, but death does not occur unless followed by head-to-heart electrocution or the application of an adjunctive method to ensure death such as exsanguination[373,509] or pithing. The secondary step, whether head-to-heart electrocution or another method, must be performed within 15 seconds of onset of unconsciousness; otherwise, the animal may regain consciousness. Head-only electrocution is performed by placement of the electrodes in one of three positions: between the eyes and base of the ears on either side of the head; below the base of the ears on either side of the head; or diagonally, below one ear to above the opposite eye. Placement of electrodes for head-to-heart electrocution is on the head in front of the brain (some use the base of the ear) with a secondary electrode attached to the body behind the heart on the opposite side. This assures diagonal movement of current through the animal's body. With specific electrode placement, current of 110 V at a minimum frequency of 60 Hz applied for a minimum of 3 seconds is sufficient for euthanasia of pigs up to 125 kg.[510] Systems used for electrocution must be capable of meeting minimum current requirements to ensure insensibility in the head-only method, and insensibility and cardiac fibrillation in the head-heart method.

Electrocution is effective as a single-step process with appropriate tong or clamp placement. However, proper training and special equipment must be used to ensure adequate and safe euthanasia. While electrocution is commonly used to render animals insensible in slaughter plants and safety precautions in that environment are routine, for implementation on-farm where use of the method is less common, extra precautions may need to be taken to ensure human safety. Agonal gasping may be evident after current is withdrawn and may be aesthetically unacceptable for observers and operators.

S3.3.1.3 Adjunctive Methods

Exsanguination—While not appropriate as a sole method of euthanasia, exsanguination may be performed as a secondary step to ensure death when necessary.

Pithing—While not appropriate as a sole method of euthanasia, pithing may be performed as a secondary step to ensure death when necessary.

More information about these methods is available in the Physical Methods section of the Guidelines.

S3.3.2 Nursery Pigs (70 lb or Lighter)

Nursery pigs may be euthanized by use of CO, CO_2, gunshot, penetrating captive bolt, purpose-built nonpenetrating captive bolt, electrocution, or anesthetic overdose. Descriptions of the use of CO_2 and nonpenetrating captive bolt for euthanasia of young pigs follow. For details on other methods please see the preceding information in this section or the Physical Methods section of the Guidelines.

S3.3.2.1 Acceptable Methods

S3.3.2.1.1 Noninhaled Agents

Bartiburates and barbituric acid derivatives—Nursery pigs may be euthanized by IV administration of euthanasia solutions containing barbiturates. Because these drugs are controlled substances they must be administered by personnel who are registered with the US that may be induced with this method. Some interpret these movements as indications of aversion. While this may be the case in systems that are not functioning properly, there is evidence that such reactions may be normal for pigs in an unconscious state.[214,515,516] Small or incapacitated piglets have low tidal volumes and will not die as rapidly as larger, more viable pigs. Carbon dioxide euthanasia in chamber settings has not been extensively studied for larger pigs. Meyer and Morrow[148] recommend that chamber volume be exchanged at least 2.5 times to accommodate the wash-in–washout principle regardless of the size of swine to be euthanized. Monitoring of equipment and gas must be routine and consistent to ensure there is always sufficient gas to accomplish the objective of euthanasia. Carbon dioxide containers should never be placed in an unventilated area due to risks associated with an overdose of gaseous CO_2 for humans.

S3.3.2.2.2 Physical Methods

Nonpenetrating captive bolt—A purpose-built nonpenetrating captive bolt may be used for euthanasia of young pigs. The concussive impact of the bolt induces an immediate loss of consciousness that when followed by an adjunctive method to ensure death meets the criteria for euthanasia. The nonpenetrating captive bolt works best in younger pigs before the frontal bones are fully developed and hardened.

Use of a proper functioning nonpenetrating captive bolt with appropriate charges offers the advantage of delivering a uniform concussive force to the skull (controlled blunt force trauma). This reduces the potential for ineffective stunning and euthanasia that may occur more often with the use of manually applied blunt force trauma. However, this method requires immediate application of an adjunctive method to ensure euthanasia.

Electrocution—Electrocution is acceptable with conditions for swine weighing more than 10 lb. Details are provided earlier in this section and in the Physical Methods section of the Guidelines.

S3.3.3 Suckling Pigs

Options for the euthanasia of suckling pigs include CO_2; Ar, N_2 and CO_2 mixtures; CO; inhaled anesthetics; purpose-built nonpenetrating captive bolt; electrocution (for pigs over 10 lb); anesthetic overdose; and blunt force trauma. Described are the application of barbituates, nonpenetrating captive bolt, manually applied blunt force trauma, and CO_2. See previous sections of the Guidelines for more detailed information on the application of other euthanasia techniques.

S3.3.3.1 Acceptable Methods

S3.3.3.1.1 Injectable Agents

Bartiburates and barbituric acid derivatives—Suckling pigs may be euthanized by IV administration of tively search for alternatives to ensure that criteria for euthanasia can be consistently met.

S3.4 POULTRY

Euthanasia methods for poultry (domesticated birds used for egg, meat, or feather production [eg, chickens, turkeys, quail, pheasants, ducks, geese]) include gas inhalation, manually applied blunt force trauma, cervical dislocation, decapitation, electrocution, gunshot, captive bolt, and injectable agents. Where appropriate, additional comments are included to address physiologic differences among avian species, variations in environment, and the size or age of birds.

S3.4.1 Acceptable Methods

S3.4.1.1 Noninhaled Agents

Overdoses of injectable anesthetics, including barbiturates and barbituric acid derivatives—Poultry may be euthanized by IV injection of overdoses of anesthetics, including barbiturate and barbituric acid derivatives. Because these drugs are controlled substances they must be administered by personnel who are registered with the US DEA, and extralabel use requires administration by or under the supervision of a veterinarian. Strict record keeping is required of all who use and store these drugs.

Many find administration of an anesthetic less displeasing than administration of CO_2, CO, captive bolt, manually applied blunt force trauma, cervical dislocation, decapitation, or electrocution. Therefore, it may be preferred in some settings. A disadvantage of this method is that tissues from animals euthanized with barbiturates may not be used for food and may not be suitable for diagnostic evaluation. Furthermore, options for disposal of animals euthanized with barbiturates are complicated by concerns for residues that create risks for scavengers, other domesticated animals that may consume portions of the animal's remains, and humans.

S3.4.2 Acceptable With Conditions Methods

S3.4.2.1 Inhaled Agents

Inhaled gases may be used satisfactorily for euthanasia of poultry, and detailed information about the various types of inhaled gases is available in the Inhaled Agents section of the Guidelines. When inhaled gases are used for euthanasia, birds should be checked to verify death because they may appear dead but can regain consciousness if the exposure time or the concentration of the agent is insufficient. Gases must be supplied in purified forms without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The gas-dispensing system should have sufficient capacity and control to maintain the necessary gas concentrations in the container being utilized, and the container itself should be sufficiently airtight to hold the gas at appropriate levels.

Carbon dioxide—The most common gas used for euthanasia of poultry is CO_2, and its application has been extensively studied for chickens, turkeys, and ducks with information available about behavioral responses, times to collapse, unconsciousness, death, loss of somatosensory evoked potentials, loss of visually evoked responses, and changes in EEG and ECG (see Inhaled Agents section of the Guidelines). Carbon dioxide has successfully been applied for euthanasia of nonhatched eggs (pips), newly hatched poultry in hatcheries, and adult birds (including routine euthanasia of large commercial laying hen flocks[356,522]) and on farms keeping birds for research or elite genetics. Because neonatal birds may be more accustomed to high concentrations of CO_2 (incubation environments typically include more CO_2), concentrations necessary to achieve rapid euthanasia of pipped eggs or newly hatched chicks may be substantially greater (as high as 80% to 90%) than for adults of the same species.

Carbon dioxide may invoke involuntary (unconscious) motor activity in birds, such as flapping of the wings or other terminal movements, which can damage tissues and be disconcerting for observers.[248,270] Slower induction of euthanasia in hypercapnic atmospheres reduces the severity of convulsions after loss of consciousness.[204,205] Death normally occurs within minutes, depending on the species and the concentration of CO_2 present in the closed chamber.

Carbon monoxide—Carbon monoxide may also be used for euthanasia of poultry. More convulsions may be observed in the presence of CO than normally occur when CO_2 is used for euthanasia.[188] The CO flow rate should be sufficient to rapidly achieve a uniform concentration of at least 6% after birds are placed in the chamber (see Inhaled Agents section). Only pure, commercially available CO should be used. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and or displacement rate. Appropriate precautions must be taken to ensure human safety because CO has a cumulative effect in binding hemoglobin.

Nitrogen or argon—Nitrogen or Ar, mixed or used alone, with approximately 30% CO_2 is acceptable with

availability. For these reasons, chloral hydrate is an unacceptable means of euthanizing equids.

S4.3 SPECIAL CASES AND EXCEPTIONS

In emergency situations, such as euthanasia of an equid with a serious injury at a racetrack or another equestrian event, it may be difficult to restrain a dangerous equid for IV injection. While administration of a sedative might be desirable, in some situations it is possible the equid could injure itself or bystanders before a sedative could take effect. In such cases, a neuromuscular blocking agent (eg, succinylcholine) may be administered to the equid IM or IV, but the equid must be euthanized via an appropriate method as soon as the equid can be controlled. Succinylcholine alone or without sufficient anesthetic is not acceptable for euthanasia.

S5. AVIANS

Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method are met.

S5.1 GENERAL CONSIDERATIONS

The following comments and recommendations pertain to pet, aviary, falconry, racing, research, and zoo birds. Information about appropriate euthanasia methods for wild birds can be found in the Captive and Free-Ranging Nondomestic Animals section of the Guidelines, whereas euthanasia of poultry and other birds used for food is addressed in the Animals Farmed for Food and Fiber section.

Few peer-reviewed reports are available in the scientific literature about euthanasia of individual or small groups of birds. The information that does exist comprises anecdotal accounts in book chapters, guidelines from various associations, and journal roundtable dis-fear or distress. Wild, fearful, or excited birds may require a sedative or anesthesia before IV injection can be performed. When IV injection is impossible, injectable euthanasia agents can be administered via intracoelomic, intracardiac, or intraosseous routes only if a bird is unconscious or anesthetized. If the intracoelomic route is used for birds, injection into the air sacs must be avoided, because of the potential for respiratory compromise, irritation of the respiratory system, and there is little scientific information available regarding the effect of various physical methods on electrical activity in the brain of birds, which makes evaluation of the humaneness of these procedures difficult.

Cervical dislocation—Cervical dislocation has generally been used for small birds (< 200 g) when no other method is available, but the procedure has been performed on birds as large as 2.3 kg (5.1 lb). It should only be performed by well-trained personnel who are regularly monitored to ensure proficiency. Skilled individuals have been able to humanely perform cervical dislocation in poultry. There is limited research specific to birds concerning electrical activity in the brain following cervical dislocation. Cervical dislocation of chickens (average weight of 2.3 kg) did not result in loss of visually evoked responses in 90% of cases when compared with use of a percussion bolt pistol, suggesting that fewer than 10% of cervical dislocations resulted in concussion.[354] In 3-week-old turkeys (average weight of 1.6 kg [3.5 lb]) time to insensibility (based on nictitating membrane movement) was longer, but time to death (based on cessation of movement) was shorter after cervical dislocation compared with use of a nonpenetrating captive bolt and blunt force trauma.[337] Whether pain is perceived is not known. Consciousness and perception of pain are not necessarily concurrent.

Decapitation—Based on information currently available, decapitation is considered to be acceptable with conditions for euthanasia of small (< 200 g) birds. The AAZV Guidelines for Euthanasia of Nondomestic Animals[416] also lists decapitation as acceptable with conditions, and suggests the method may be preferred over cervical dislocation under certain field conditions due to clear evidence of a successful procedure. One study[54] indicated that several methods of partial, mechanical decapitation of chickens (weighing 2.1 to 3.5 kg [4.6 to 7.7 lb]) did not result in the loss of visually evoked responses in 90% of cases when compared with use of a percussion bolt pistol and concluded that fewer than 10% of cervical dislocations resulted in concussion. In another study decapitation applied to anesthetized chickens resulted in visually evoked responses up to 30 seconds following decapitation, but because the responses were obtained from anesthetized chickens it is not possible to conclude any association with cognitive processes.[52–54] As indicated previously (see discussion of Consciousness and Unconsciousness in the Guidelines), at some level between behavioral unresponsiveness and the induction of a flat EEG, consciousness must vanish; however, EEG data cannot provide definitive answers as to onset of unconsciousness.

Gunshot—Gunshot is not recommended as a method for captive birds, where restraint is feasible. Its use for wild birds is addressed in the Captive and Free-Ranging Nondomestic Animals section of the Guidelines.

S5.2.3 Adjunctive Methods

Potassium chloride—Although administration of potassium chloride to a conscious, unanesthetized bird is considered to be an unacceptable method of euthanasia, potassium chloride may be administered via the IV or intracardiac routes if a bird is unconscious or completely anesthetized prior to the injection.

Exsanguination—Although exsanguination of a conscious, unanesthetized bird is an unacceptable approach to euthanasia, exsanguination may be used for euthanasia of unconscious or anesthetized birds. This approach may be appropriate if blood samples are needed for diagnostic or research purposes.

Thoracic compression—Although thoracic compression of a conscious, unanesthetized bird is an unacceptable approach to euthanasia, it may be used as an adjunctive method for animals that are insentient.

S5.2.4 Unacceptable Methods

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.[413–416] 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. Details are available in the Physical Methods section of the Guidelines.

S5.3 EGGS, EMBRYOS, AND NEONATES

Bird embryos that have attained > 50% incubation have developed a neural tube sufficient for pain per-

cent0 Td (-; Guietized059 ETt.2936 Tm 14.029 0 Td (O)T.021e)
sufjatee ilong[(B(1.8 -1.053 Td [(have)1(developed)1(a).053 Td(20 (dsumin))]TJ 0.01ent)1(knoexposu6 Tm 11.o

Water quality should be similar to that of the environment from which the finfish originated, or optimized for that species and situation, for the duration of euthanasia. If of acceptable quality for finfish health, water in which they have been house or captured should be used, and supplemental aeration and temperature control may be necessary. Either the immersion euthanasia solution is prepared with water from the finfish housing system and the finfish are transferred into it or a concentrated form of the anesthetic agent as a solution (containing buffering agent if appropriate) is introduced directly into the container of finfish to minimize stressors. If euthanizing a large population of finfish, it is important to monitor the anesthetic bath water quality (temperature, dissolved O_2, and organic loading, in particular). The euthanasia agent may need to be supplemented or replaced periodically. Euthanasia methods should be tested in one animal or a small group of animals prior to use in a large population for an unfamiliar species.[325] If handling is required, appropriate equipment (nets, gloves) should be used to minimize stressors.

S6.1.4 Indicators of Death in Finfish and Aquatic Invertebrates

Because the thousands of species of finfish and aquatic invertebrates vary greatly in anatomic and physiologic characteristics, reliable indicators of death may not be available for some. However, there are some standard approaches that can be useful for many of the more commonly encountered species. Loss of movement, loss of reactivity to any stimulus, and initial flaccidity (prior to rigor mortis) may serve as indicators of death for finfish and some aquatic invertebrates. More useful indicators for many finfish include respiratory arrest (cessation of rhythmic opercular activity) for a minimum of 10 minutes and loss of eyeroll (vestibulo-ocular reflex, the movement of the eye when the finfish is rocked from side to side). The latter is no longer present in finfish that have been deeply anesthetized or euthanized.[557] The heart can continue to contract even after brain death or removal from the bodies of finfish,[558] so the presence of a heartbeat is not a reliable indicator of life, but sustained absence of heartbeat is a strong indicator of death. For more sessile, less active organisms, or those with specific anatomic or physiologic adaptations that prevent use of these indicators, it may be more difficult to assess loss of consciousness and death, and consultation with species experts is recommended.

S6.1.5 Disposition of Euthanized Animals

Any euthanized finfish or invertebrate should be promptly removed from its aquarium, pond, or other vessel and disposed of according to all pertinent federal, state, and local regulations, in a manner that will reduce the risk of disease spread, prevent pests and other nontarget species from gaining access to animal remains, and ensure human and environmental safety. Preventing environmental contamination by any life stage of finfish that could hatch and/or survive outside an acceptable, enclosed body of water is an important consideration in confirmation of death and disposal of the animal's remains.

S6.1.6 Finfish and Aquatic Invertebrates Intended for Human Consumption

As previously indicated, the term *slaughter* is used primarily to refer to the killing of animals intended for human consumption (eg, agricultural harvest, commercial fisheries) and these Guidelines are not intended to address that activity. However, when euthanasia of animals intended for human consumption is desired, tissue residues from the use of drugs and other chemicals will make many methods unacceptable unless they have been approved by the FDA for this purpose and appropriate withdrawal periods are followed. Use of any unapproved chemicals for euthanasia prohibits entry of the finfish into the food chain, either by rendering, as fish meal, or as directly consumed product.[549] With that said, currently there are no drugs approved for euthanasia of finfish or aquatic invertebrates. Carbon dioxide is a drug of low regulatory priority[317] that avoids unacceptable residues, but it is not an FDA-approved method for killing aquatic animals used for food. Physical methods that are acceptable with conditions include manually applied blunt force trauma to the head, decapitation, and pithing.

S6.2 FINFISH

Common methods used to euthanize finfish include noninhaled methods (ie, immersion and injection) and physical methods. Because of general differences in anatomy and application seen between finfish and terrestrial animals (especially with regard to primary respiratory organs, and aqueous vs air environment), techniques involving addition of drugs to the finfish's environment (ie, the water), for purposes of this document, are considered noninhaled methods.

Descriptions of methods used to euthanize finfish follow and include 1-step and 2-step procedures. Each method is further classified as acceptable, acceptable with conditions, or unacceptable considering characteristics of the methods and the environments in which euthanasia is conducted, including veterinary private practice (eg, companion and ornamental [display] finfish), ornamental (aquarium) finfish wholesale and retail

(2) Carbon dioxide. Immersion in CO_2-saturated water causes narcosis and loss of consciousness after several minutes.[63,325] Some species may exhibit hyperactivity prior to loss of consciousness.[559] Purity and concentration of CO_2 are important for effectiveness. Only CO_2 from a source that allows for careful regulation of concentration, such as from cylinders, is acceptable. Care must be taken when using CO_2 to prevent exposure to personnel (ie, euthanasia must be conducted in well-ventilated areas).

(3) Ethanol. Ethanol has been suggested as an acceptable alternative method for finfish.[306] The depressive effects of ethanol on the CNS are well described,[562] and exposure of zebrafish via immersion has become a model for behavioral and molecular responses to alcohol, at concentrations from 10 to 30 mL of 95% ethanol/L.[563–565] At this dose, alcohol induces anesthesia, and prolonged immersion produces death via respiratory depression causing anoxia. This is not equivalent to immersing finfish directly into preservative concentrations of ethanol (70%), which is not acceptable as a euthanasia method.

(4) Eugenol, isoeugenol, and clove oil. Whenever possible, products with standardized, known concentrations of essential oils should be used so that accurate dosing is possible. Concentrations required for anesthesia will vary depending on species and other factors, but may be as low as 17 mg/L for some species. Greater concentrations will be required for euthanasia.[566–568] Finish should be left in the anesthetic solution for a minimum of 10 minutes after cessation of opercular movement. These compounds are equivocal or known carcinogens according to the National Toxicology Program.[318] Some studies in rodents indicate this group of anesthetics may cause paralysis in addition to having anesthetic effects, and analgesic properties are unknown.[321–324] Because some clove oil products may contain or include either methyleugenol or isoeugenol, or both, FDA has expressed concern that the use of clove oil or its components in finfish may adversely affect human food safety and animal food safety. In addition, because clove oil and its components have not been evaluated for target animal safety, FDA is also concerned that the use of any of these compounds may adversely affect finish, including endangered aquatic species.

blow of sufficient energy to the cranium with an appropriate-sized club) can cause immediate unconsciousness and potentially death, but should be followed by pithing to ensure death. The finfish's size, species, and anatomy and characteristics of the blow (including its accuracy, speed, and club mass) will determine the efficacy of manually applied blunt force trauma. This procedure requires training and monitoring for proficiency. Anatomic features, such as the location of the eyes, can help serve as a guide to the location of the brain.[570,571]

(4) Captive bolt (most commonly nonpenetrating; 1 step). This is a method usually applied to large finfish species.[570]

(5) Maceration (1 step). When applied correctly, using a well-maintained macerator specifically designed for the size of finfish being euthanized, death is nearly instantaneous.[572] The process is aesthetically unpleasant for some operators and observers.

(6) Rapid chilling (hypothermic shock; 1 step or 2 step). It is acceptable for zebrafish (*D rerio*) to be euthanized by rapid chilling (2° to 4°C) until loss of orientation and operculum movements[316,461,462] and subsequent holding times in ice-chilled water, specific to finfish size and age. Zebrafish adults (approx 3.8 cm long) can be rapidly killed (10 to 20 seconds) by immersion in 2° to 4°C (36° to 39°F) water. Adult zebrafish should be exposed for a minimum of 10 minutes and fry 4 to 7 dpf for at least 20 minutes following loss of operculum movement. Use of rapid chilling and use of buffered MS 222 alone have been shown to be unreliable euthanasia methods for zebrafish embryos < 3 dpf. To ensure embryonic lethality these methods should be followed with an adjunctive method such as use of diluate sodium or calcium hypochlorite solution at 500 mg/L.[327,462] If necessary to ensure death of other life stages, rapid chilling may be followed by either an approved adjunctive euthanasia method or a humane killing method. Until further research is conducted, rapid chilling is acceptable with conditions for other small-bodied, similarly sized tropical and subtropical stenothermic species. Species-specific thermal tolerance and body size will determine the appropriateness and effectiveness of rapid chilling for euthanasia of finfish. Finfish size is important because the rate of heat loss via thermal conduction from a body is proportional to its surface area. Based on these 2 factors, it has been suggested that rapid chilling in water associated with an ice slurry is a suitable killing method for small tropical and subtropical finfish species 3.8 cm in length (tip of the snout to the posterior end of the last vertebra) or smaller, having lower lethal temperatures above 4°C.

To ensure optimal hypothermal shock (ie, rapid killing), transfer of finfish into ice water must be completed as quickly as possible. This means rapid transitions from acclimatization temperature to 2° to 4°C must be achieved. This can be accomplished by using minimal water volume to transfer finfish (ie, using a net to place finfish in chilled water). In addition, finfish should not be in direct contact with the ice in the water; rather a depression should be formed in the ice posure.[560] As an example, immersion in a buffered MS 222 solution having a concentration > 1 g/L is not a reliable method for killing some finfish in younger life stages.[461,462,560] For some species and in some situations, adjunctive methods to guarantee death may need to be applied for these animals after anesthesia with buffered MS 222. Rapid chilling followed by an adjunctive method such as immersion in a dilute sodium hypochlorite or calcium hypochlorite solution is acceptable for zebrafish embryos and larvae as a 2-step method and is also acceptable with conditions as a 2-step method for destruction of other (nonzebrafish) species' embryos and larvae.[327,462]

S6.2.6 Finfish in Particular Environments

S6.2.6.1 Veterinary Private Practice—Companion and Ornamental (Display) Finfish

Clients with pet or display finfish of any species often value them as companion animals and share a human-animal bond similar to that seen between clients and other pets, such as dogs and cats. Therefore, it is important to consider the perception of the client when euthanasia methods are chosen. Clients should be offered the opportunity to be present during euthanasia whenever feasible; however, clients also should be educated as to what method will be used and what they may observe during euthanasia. For example, clients may believe the excitement phase of anesthesia, which can result in increased motor activity or the appearance of agitation,[559] is unduly painful or stressful for the finfish even when it is not.

The following methods are acceptable for use in this environment:

(1) Immersion in solutions of buffered tricaine methanesulfonate (MS 222), buffered benzocaine, isoflurane and sevoflurane, quinaldine sulfate, and 2-phenoxyethanol.

(2) Injections of pentobarbital, ketamine followed by pentobarbital, a combination of ketamine and medetomidine followed by pentobarbital, and propofol followed by pentobarbital. Owners should be advised about the possibility of ketamine-induced muscle spasms during induction when using that agent.

The following methods are acceptable with conditions for use in this environment:

(1) Immersion in eugenol, isoeugenol, or clove oil. Finfish should be left in the solution for a minimum of 10 minutes after cessation of opercular movement.[63,325,559]

The following methods are not recommended for use in this environment:

(1) Immersion in CO_2-saturated water is not recommended because some finfish exposed to this method may become hyperactive, which can be disconcerting for staff and owners.

(2) Manually applied blunt force trauma to the head, decapitation, and pithing are not recommended because their application can be distressing for owners and staff.

Early stages in the lives of finfish, including embryos and larvae, may require higher concentrations of immersion anesthetics or a longer duration of exposure.[560] As an example, immersion in a buffered MS 222 solution having a concentration > 1 g/L is not a reliable method for killing some finfish in early life stages.[461,462,560] For some species and in some situations, adjunctive methods to guarantee death may need to be applied for these animals after anesthesia with buffered MS 222.

Rapid chilling followed by immersion in a dilute sodium hypochlorite or calcium hypochlorite solution is acceptable for zebrafish embryos and larvae as a 2-step method and is also acceptable with conditions as a 2-step method for destruction of other (nonzebrafish) species'embryos and larvae.[327,462]

S6.2.6.2 Aquarium Finfish Wholesale and Retail Facilities

Freshwater and marine aquarium finfish are commercially collected from the wild, and are also bred in captivity. Tropical aquarium finfish are sold at retail pet shops and finfish stores from systems housing one or more species of finfish per tank. Individual finfish or populations of finfish may become injured or diseased and require euthanasia. Methods of euthanasia used in this environment need to be applicable to individual finfish, to all finfish in an aquarium, to finfish held in multiple aquariums on a central filtration system, or for finfish kept in ponds. In certain situations euthanasia may not be feasible and depopulation methods may be required.

The following methods are acceptable for use in this environment:

Immersion in solutions of buffered tricaine methanesulfonate (MS 222), buffered benzocaine, and quinaldine sulfate. Finfish should be left in the anesthetic solution for a minimum of 10 minutes after cessation of opercular movement.[63,325,559]

The following methods are acceptable with conditions for use in this environment:

(1) Immersion in CO_2-saturated water; eugenol, isoeugenol, or clove oil; and ethanol.

(2) Decapitation, cervical transection, or manually applied blunt force trauma as step 1 of a 2-step method, followed by pithing.

(3) Freezing may be used as an adjunctive method following anesthesia.

(4) Rapid chilling (hypothermic shock) for small-bodied (3.8-cm-long or smaller) tropical and subtropical stenothermic finfish, for which the lower lethal temperature range is above 4°C.[316,461,462]

The following methods are not recommended for use in this environment:

Use of injectable anesthetic drugs including barbiturates, requires the oversight of a veterinarian and DEA permitting for controlled substances. Therefore, unless a veterinarian is available on-site to oversee use of these drugs, this method is not recommended in this environment.

Early stages in the lives of finfish, including embryos and larvae, may require higher concentrations of immersion anesthetics or a longer duration of exposure.[560] As an example, immersion in a buffered MS 222 solution having a concentration > 1 g/L is not a reliable method for killing some finfish in early life stages.[461,462,560] For some species and in some situations, adjunctive methods to guarantee death may need to be applied for these animals after anesthesia with buffered MS 222.

Rapid chilling followed by immersion in a dilute sodium hypochlorite or calcium hypochlorite solution is acceptable for zebrafish embryos and larvae as a 2-step method and is also acceptable with conditions as a 2-step method for destruction of other (nonzebrafish) species'embryos and larvae.[327,462]

S6.2.6.3 Research Facilities

Researchers working in laboratories should have materials readily available to provide appropriate euthanasia for their research subjects when required, and should be trained and monitored for proficiency in the use of chosen techniques. Many facilities using finfish as research subjects are engaged in biomedical research. Zebrafish are the most common species used for research and are usually kept in small-scale tank systems; however, some research facilities may also have large-scale housing and production systems and/or keep other larger species of finfish, and consequently, need to consider additional options for euthanasia.[320] The expertise of those knowledgeable about these settings and species should be sought as necessary.

The following methods are acceptable for use in this environment:

(1) Immersion in solutions of buffered tricaine methanesulfonate (MS 222), buffered benzocaine, quinaldine sulfate, and 2-phenoxyethanol. Finfish euthanized with these methods must not enter the food supply.

(2) Rapid chilling (hypothermic shock) is acceptable for zebrafish (*D rerio*) and Australian river gizzard shad (*N erebi*) as long as transfer from acclimatized temperatures to water associated with a 2° to 4°C ice slurry occurs rapidly with as little transfer of warmer water as possible.

The following methods are acceptable with conditions for use in this environment:

(1) Immersion in CO_2-saturated water (as long as observers are advised and can accept that some finfish exposed to this method may exhibit hyperactivity and appears to be in distress), eugenol, isoeugenol, or clove oil.

(2) Rapid chilling (hypothermic shock) to 2° to 4°C is acceptable with conditions for small-bodied (3.8-cm-long or smaller) tropical and subtropical stenothermic finfish, for which the lower lethal temperature range is above 4°C. Because of surface-to-volume consider-
[illegible]

head followed by pithing.

(3) Decapitation followed by pithing. Decapitation alone is not considered a humane form of euthanasia, especially for species that may be particularly tolerant of low O_2 concentrations. Pithing helps ensure rapid death for those species.

(4) Cervical transection followed by pithing. The rationale for this approach is similar to that for decapitation and pithing, except that the head is still physically attached by musculature to the body.

(5) Captive bolt. This method is usually applied to large finfish species.

(6) Rapid chilling (hypothermic shock) in water of 2° to 4°C for small-bodied (3.8-cm-long or smaller) tropical and subtropical stenothermic species (as previously described for zebrafish). Because of surface-to-volume considerations, use of this method is not appropriate in medium to large-bodied finfish until pertinent data for those species becomes available.

Early stages in the lives of finfish, including embryos and larvae, may require higher concentrations of immersion anesthetics or a longer duration of exposure.[560] As an example, immersion in a buffered MS 222 solution having a concentration > 1 g/L is not a reliable method for killing some finfish in early life stages.[461,462,560] For some species and in some situations, adjunctive methods to guarantee death may need to be applied for these animals after anesthesia with buffered MS 222. Rapid chilling followed by immersion in a dilute sodium hypochlorite or calcium hypochlorite solution is acceptable for zebrafish embryos and larvae as a 2-step method and is also acceptable with conditions as a 2-step method for destruction of other (nonzebrafish) species' embryos and larvae.[327,462]

S6.3 AQUATIC INVERTEBRATES

Overdose of a general anesthetic is as appropriate a euthanasia strategy for aquatic invertebrates as it is for finfish. And, immersion is an effective route of administration of anesthetic and euthanasia agents.[133,330]

Because confirming the death of many invertebrates is difficult, 2-step euthanasia procedures are often recommended in which chemical induction of anesthesia, nonresponsiveness, or presumptive death is followed by an adjunctive method that destroys the brain or major ganglia physically (eg, pithing, freezing, boiling) or chemically (eg, alcohol, formalin). Application of the latter methods by themselves is generally not considered to meet the criteria established for euthanasia.[133,330]

S6.3.1 Acceptable First Steps of 2-Step Methods

S6.3.1.1 Noninhaled Agents for Immersion

Magnesium salts—Magnesium salts are a near-universal anesthetic agent, relaxing agent, and euthanasia agent for aquatic invertebrates, although they are ineffective for crustaceans. A range of concentrations has been recommended for various phyla. Research suggests the magnesium ion acts centrally in suppressing neural activity of cephalopods.[134]

Clove oil or eugenol—Clove oil or eugenol has been used effectively as an immersion agent for the euthanasia of crustaceans (0.125 mL/L).[133,573] Isoeugenol is a potential carcinogen[318] so human safety in the application of that agent is of concern.

Ethanol—Ethanol has been used for euthanasia

potential for pain or distress. Most commonly used methods involve terminal anesthesia, followed by physical destruction of the nervous system, to assure lack of sensory perception and death of the animal. The diversity of invertebrate taxa may require equally diverse approaches to euthanasia.

S7.2.1 Acceptable Methods

S7.2.1.1 Noninhaled Agents

Injectable agents—While there is little dosing or outcome data in the peer-reviewed literature, an overdose of pentobarbital or similar agent, at a dose equivalent to that used for other poikilotherm vertebrates (piscine, amphibian, or reptilian) on a weight-to-weight basis will generally suffice. Ideally these agents will be injected directly into the circulating hemolymph. However, because many invertebrates have an open circulatory system, true intravascular application can be difficult if not impossible. In such cases an intracoelomic injection would be warranted unless otherwise contraindicated. Premedication with an injectable or inhaled agent may facilitate administration of barbiturate overdoses.

S7.2.2 Acceptable With Conditions Methods

S7.2.2.1 Inhaled Agents

Inhaled anesthetics—Overdose of an inhaled anesthetic is acceptable with conditions for terrestrial invertebrates where injectable agents are not available. Because confirming death of many species of invertebrates can be difficult, subsequent use of an adjunctive method of euthanasia is recommended.

Carbon dioxide—Carbon dioxide may be useful for euthanasia of some terrestrial invertebrates, but additional information is needed to confirm its efficacy.

S7.2.2.2 Physical and Chemical Methods

P2.2 Physv Tm [((egis 0 0 10 0 gy)1(phys)]TJ 0 Tw83VWpr)2w83ht TJ /c1_1 w 0 -1.057 TD [(ver25ome)1(data)1(in)1(t

tion of euthanasia agents can be challenging for some species. Intracoelomic, subcutaneous lymph spaces, and lymph sacs are acceptable routes of administration. Direct injection into the brain through the parietal eye, while under anesthesia, has been described for some lizard species.[592]

Sodium pentobarbital (60 to 100 mg/kg of body weight) can be administered IV, intracoelomically, in the subcutaneous lymph spaces, or in the lymph sacs, although doses vary by species.[593] Doses as high as 1,100 mg/kg (500 mg/lb) of sodium pentobarbital with sodium phenytoin administered intracoelomically may be required for euthanasia of some species such as *X laevis*.[312] Time to effect may vary, with death occurring instantaneously or up to 30 minutes later.[77,552,589–591,594] Barbiturates are best administered intravascularly to minimize the discomfort upon injection.[595] However, where intravascular administration is not possible or its benefits are outweighed by distress imposed by additional restraint, pain from alternate methods, risk to personnel, or other similar reasons, intracoelomic administration is an acceptable route for administration of barbiturates.

Dissociative agents such as ketamine hydrochloride or combinations such as tiletamine and zolazepam; inhaled agents; and IV administered anesthetics, such as propofol, or other ultra–short-acting barbiturates, may be used for poikilotherms to induce rapid general anesthesia and subsequent euthanasia, although application of an adjunctive method to ensure death is recommended.

External or topical agents—Buffered tricaine methanesulfonate (MS 222) may be administered via water baths (amphibians), or injected directly into the lymph sacs (amphibians) or the coelomic cavity (amphibians and reptiles).[596–599] Prolonged immersion (as long as 1 hour) may be required for 5 to 10 g/L water baths.[312,593] Tricaine methanosulfonate does not create histopathologic artifacts.[596] See the Noninhaled Agents section of the Guidelines for additional information.

Benzocaine hydrochloride, a compound similar to MS 222, may be used as a bath or in a recirculation system at concentrations ≥ 250 mg/L or applied topically to the ventrum as a 7.5% or 20% gel for euthanasia of amphibians.[600] A dose of 182 mg/kg of benzocaine gel (20% concentration, 2.0-cm X 1.0-mm application) has been reported as effective for euthanasia of adult *X laevis*.[312] Pure benzocaine is not water soluble and should be avoided for anesthesia or euthanasia because it requires the use of acetone or ethanol solvents, which may be irritating to tissues.[310]

In general, these noninhaled agents are highly effective, their onset of action is rapid, and they are ap-

of firearms. Refer to ballistics details in the section on Physical Methods and experts for more information on selection and use of firearms.

S7.4.5 Adjunctive Methods

Potassium chloride—Potassium chloride can be administered IV or intracardially to stop the heart of animals that are deeply anesthetized or unconscious. Potassium chloride does not create artifacts that can interfere with histopathologic examination and, therefore, its application may be appropriate when accurate postmortem diagnostic or research results are important. Potassium chloride may also be used adjunctively for large animals that are first anesthetized with barbiturates, particularly where volume of administration is a limitation. In many cases significant agonal reflex activity can be avoided where barbiturates are administered prior to administration of potassium chloride.

Exsanguination—Exsanguination may be useful as a secondary or tertiary method to ensure death. The aesthetics of this procedure and its acceptance by personnel must be considered in its application.

Cervical dislocation or decapitation—Applied to small mammals and birds, this method may be useful as an adjunct or as a first-step method of euthanasia. A paucity of data for wildlife and the potential for interspecies variation creates challenges for establishing specific size recommendations. However, based on domestic animals, manual cervical dislocation may be appropriate for birds < 3 kg (6.6 lb), rodents < 200 g, and rabbits < 1 kg (2.2 lb).[599] A secondary method such as decapitation or exsanguination should be employed to ensure death when feasible.

Thoracic compression—Thoracic compression may be useful in rare circumstances in animals that are deeply anesthetized or otherwise unconscious, or as a final, confirmatory step when the animal's status is uncertain.

S7.4.6 Unacceptable Methods

Methods that are classified as being unacceptable for use in comparable domestic species are unacceptable for use in wild mammals that are not deeply anesthetized.

S7.4.7 Embryos, Fetuses, and Neonates

Euthanasia of embryos, fetuses, and neonates should be conducted using guidelines appropriate for taxonomically similar domestic mammals.

S7.5 CAPTIVE MARINE MAMMALS

Due to their unique anatomic and physiologic adaptations for aquatic environments, the large size of some species, and the challenges associated with performing euthanasia under typical circumstances, marine mammals are considered separately from other mammals. To facilitate making appropriate recommendations regarding euthanasia, marine mammals have been divided into physiologically and anatomically distinct groups. These groups follow taxonomic lines to some extent, though it is appropriate to consider the sea otter (a large mustelid) with small pinnipeds: (1) pinnipeds, (2) odontocetes, (3) mysticetes, and (4) sirinids. Methods addressed under methods of euthanasia for captive mammals (nonmarine species) are applicable to polar bears, and will not be addressed in this section. Sizes of the animals vary dramatically among and within these groups and each group should minimally be divided into subgroups by size (large and small). Recommendations for euthanasia of marine mammals in managed care facilities differ from those used for free-ranging marine mammals, because of differences in environment and facilities, restraint capabilities, and personnel and observers.

S7.5.1 Acceptable Methods

S7.5.1.1 Noninhaled Agents

Intravenous administration of barbiturates and their derivatives can be a rapid and reliable method of euthanasia for small pinnipeds, small odontocetes, and sirinids. Intraperitoneal administration is also acceptable where intravascular administration is not possible or is outweighed by distress from the requirement of additional restraint, pain from alternate methods, risk to personnel, or other similar reasons, although tissue irritation and variable absorption rates must be considered. Safe and effective IV administration of these agents may also be possible in anesthetized, moribund, or unconscious large pinnipeds and in large odontocetes. For the largest odontocetes, drug dilution in large volumes may limit the effectiveness of euthanasia agents administered IV. Intracardiac administration is acceptable only in anesthetized, moribund, or unconscious animals.

The advantage of using barbiturates is that death is usually rapid. Unfortunately, voluntary peripheral vasoconstriction by cetaceans or hypovolemic shock may limit access to peripheral veins. There is also a risk of injury for personnel attempting venipuncture if animals are not restrained. Furthermore tissue residues monly used to euthanize marine mammals because these animals' ability to breath- hold means that ex-

and may be inappropriate. Refer to ballistics details in the Physical Methods section and experts for more information on selection and use of firearms.

S7.6.3.3 Adjunctive Methods

Potassium chloride—Potassium chloride may be administered IV or intracardially to stop the heart of animals that are deeply anesthetized or unconscious. Administration of potassium chloride can also be preferred for large animals when administered with barbiturates, where volume of administration is a limitation.

Exsanguination—Bleeding may be used as an adjunctive method to ensure the death of animals that are anesthetized or otherwise unconscious. The aesthetics of this procedure and its acceptance by personnel and observers should be considered.

Cervical dislocation or decapitation—Applied to small mammals and birds, this method may be useful as an adjunct or as a first-step method of euthanasia. A paucity of data for wildlife and the potential for interspecies variation create challenges for establishing specific size recommendations. However, based on domestic animals, manual cervical dislocation may be appropriate for birds < 3 kg, rodents < 200 g, and rabbits < 1 kg.[599] A secondary method such as decapitation or exsanguination should be employed to ensure death when feasible.

Thoracic compression—Thoracic compression may be useful in rare circumstances in animals that are deeply anesthetized or otherwise unconscious, or as a final, confirmatory method to ensure death when the animal's status is uncertain.

S7.6.3.4 Unacceptable Methods

Approaches to euthanasia that ignore recent advances in technology, and that do not minimize risks to animal welfare, personnel safety, and the environment for a particular set of circumstances, are unacceptable.

S7.6.4 Embryos, Fetuses, and Neonates

Methods that are acceptable for euthanasia of domestic or captive wildlife species in developmental or neonatal stages are generally acceptable for euthanasia of similar stages of free-ranging wildlife.

S7.7 FREE-RANGING MARINE MAMMALS

Selecting a method of euthanasia for free-ranging marine mammals can be a substantial challenge because of large body size, environmental constraints, and concerns for the safety of personnel. It can also be difficult to determine when stranded marine mammals are unconscious or dead.[623] Currently available euthanasia methods generally have significant limitations that fail to meet aesthetic or other conventional standards for euthanasia of marine mammals under field conditions, particularly for large animals. Nevertheless, the options available must be evaluated to identify the best option under a given set of circumstances. Further research is warranted to identify improved methods of euthanasia.

S7.7.1 Acceptable Methods

S7.7.1.1 Noninhaled agents

Overdoses of injectable anesthetics can be used to euthanize marine mammals under field conditions. Anesthetics that can be used alone or in combination include tiletamine-zolazepam, ketamine, xylazine, meperidine, fentanyl, midazolam, diazepam, butorphanol, acepromazine, barbiturates, and etorphine.[613,624,625] Intramuscular administration of anesthetics may be required to achieve restraint of conscious animals before personnel can safely perform euthanasia using injectable agents by an intravascular route. A clear understanding of species anatomy and use of sufficiently long needles are required to ensure that muscle, rather than fat, is the site of injection.

Injectable anesthetics may be administered by multiple routes. Mucocutaneous administration, via the blowhole, can be an effective method that maximizes personnel safety.[625] Intravenous administration can be rapid and reliable for small pinnipeds, small odontocetes, and sirinids. For larger animals, safe IV administration is generally limited to animals that are anesthetized or unconscious. In addition, drug dilution in large blood volumes of large odontocetes and mysticetes may limit the effectiveness of IV administered agents. Intraperitoneal administration can be effective for small marine mammals if sufficiently long needles are available to access the peritoneal cavity. However, delayed absorption may limit the efficacy of drugs administered via this route. Intracardiac administration is acceptable only in anesthetized, moribund, or unconscious animals. This approach requires special, strong, and long needles to ensure that the heart can be accessed.

Advantages of injectable anesthetics are that they act rapidly and personnel experienced with these methods are readily available. Their administration is logistically simple and aesthetically acceptable, and public safety is relatively easy to secure. However, voluntary peripheral vasoconstriction by cetaceans or hypovolemic shock may limit access to peripheral veins and fat layers must be bypassed for effective administration. Large quantities of drug may be required to effectively euthanize large animals, and administration of single types of agents, such as α_2 adrenergic receptor agonists, can result in animals passing through aesthetically displeasing and potentially unsafe excitation phases of anesthesia. There is a risk of injury for personnel attempting to access veins if animals are not appropriately restrained, and personnel may also face self-administration risks (especially for ultrapotent opioids). Environmental contamination and scavenger exposure are possible due to residues in the animal's remains.

S7.7.2 Acceptable With Conditions Methods

S7.7.2.1 Physical Methods

Gunshot—Gunshot is acceptable with conditions for euthanizing small marine mammals when injectsist in identifying appropriate anatomic landmarks and caliber of ballistics.[348,626–630]

Advantages of gunshot include a rapid death and equipment that is generally readily available. Gunshot also poses minimal risk for other animals that may scavenge the animal's remains. However, its efficacy is highly dependent on the knowledge, technical expertise, and experience of the operator. Associated noise can distress other animals (especially in the case of mass strandings) and ricochet poses a risk to bystanders. Euthanasia by gunshot may also be aesthetically displeasing and emotionally distressing for personnel and bystanders. Compliance with firearm regulations is also required. Refer to details for ballistics in the Physical Methods section and experts for more information on selection and use of firearms.

Manually applied blunt force trauma—In situations where other options are not available, a concussive blow to the head may be an effective method of euthanasia for small juvenile marine mammals.[631] The advantages of properly applied manual blunt force trauma are that it results in rapid death, no special equipment is required, and there is limited potential for secondary toxicity for scavengers. However, the efficacy of manually applied blunt force trauma is highly dependent on knowledge and experience of the operator and it is aesthetically displeasing for personnel and observers.

Implosive decerebration—Decerebration of large mysticetes and odontocetes can be effectively accomplished through the detonation of properly placed, shaped, and dimensioned explosive charges.[632,633] Advantages of this technique include a rapid death, limited potential for exposure of scavengers to toxic residues, and protection of personnel from injury by tail flukes. Its efficacy, however, is highly dependent on the knowledge, skills, and experience of the operator; it is aesthetically displeasing; and personnel and bystanders must be sufficiently distant from the resulting explosion to avoid injury. If these conditions can be met, implosive decerebration is an acceptable method of euthanasia.

S7.7.3 Adjunctive Methods

Potassium chloride or succinylcholine—While unacceptable as sole agents of euthanasia in awake animals, potassium chloride or succinylcholine may be used to ensure the death of animals that are anesthetized or unconscious. Saturated potassium chloride solutions can be mixed inexpensively in large volumes and can be administered IV or intracardially, with a low risk of secondary toxicity for scavengers when preferred methods of disposal of the remains (eg, deep burial, rendering) are not available.[613,634]

S7.7.4 Unacceptable Methods

Inhaled agents—While acceptable with conditions from an animal welfare standpoint, practical and human and environmental safety constraints generally prevent use of inhaled agents for euthanasia of marine mammals under field conditions.

Exsanguination—Exsanguination is inappropriate as a sole method of euthanasia because it requires an excessively long time to death, is believed to produce anxiety associated with extreme hypovolemia, and is aesthetically displeasing to bystanders. It can, however, be used as an adjunctive method to ensure the death of unconscious animals.[630]

a. Anthony R, University of Alaska Anchorage, Anchorage, Alaska: Personal communication, 2011.
b. T

20. Morton DB. A hypothetical strategy for the objective evaluation of animal well-being and quality of life using a dog model. *Anim Welf* 2007;16(suppl):75–81.
21. Rollin BE. Animal euthanasia and moral stress. In: Kay WJ, Cohen SP, Fudin CE, et al, eds. *Euthanasia of the companion animal*. Philadelphia: Charles Press, 1988;31–41.
22. Niel L, Stewart, SA, Weary, DM. Effect of flow rate on aversion to gradual-fill carbon dioxide euthanasia in rats. *Appl Anim Behav Sci* 2008;109:77–84.
23. Brown M, Carbone L, Conlee KM, et al. Report of the working group on animal distress in the laboratory. *Lab Anim (NY)* 2006;35:26–30.
24. Demers G, Griffin G, DeVroey G, et al. Animal research. Harmonization of animal care and use guidelines. *Science* 2006;312:700–701.
25. Hawkins P, Playle L, Golledge H, et al. Newcastle consensus meeting on carbon dioxide euthanasia of laboratory animals. London: National Centre for the Replacement, Refinement and Reduction of Animals in Science, 2006. Available at: www.nc3rs.org.uk/downloaddoc.asp?id=416&page=292&skin=0. Accessed Jan 20, 2011.
26. AVMA. Principles of veterinary medical ethics of the AVMA. Available at: www.avma.org/KB/Policies/Pages/Principles-of-Veterinary-Medical-Ethics-of-the-AVMA.aspx. Accessed May 13, 2011.
27. Rollin BE. *An introduction to veterinary medical ethics*. 2nd ed. Ames, Iowa: Blackwell, 2006.
28. Croney CC, Anthony R. Engaging science in a climate of values: tools for animal scientists tasked with addressing ethical problems. *J Anim Sci* 2010;88(suppl 13):E75–E81.
29. Sandoe P, Christiansen SB, Appleby MC. Farm animal welfare: the interaction of ethical questions and animal welfare science. *Anim Welf* 2003;12:469–478.
30. Fraser D. Animal ethics and animal welfare science: bridging the two cultures. *Appl Anim Behav Sci* 1999;65:171–189.
31. Thompson PB. From a philosopher's perspective, how should animal scientists meet the challenge of contentious issues? *J Anim Sci* 1999;77:372–377.
32. Webster J. *Animal welfare: a cool eye towards Eden*. Oxford, England: Blackwell, 1994.
33. Anderson E. Animal rights and the values of nonhuman life. In: Sunstein C, Nussbaum M, eds. *Animal rights: current debates and new directions*. Oxford, England: Oxford University Press, 2004;277–298.
34. Regan T. *Animal rights, human wrongs: an introduction to moral philosophy*. Lanham, Md: Rowman and Littlefield, 2003.
35. Pluhar E. *Beyond prejudice*. Durham, NC: Duke University Press, 1995.
36. Regan T. *The case for animal rights*. Berkeley, Calif: University of California Press, 1983.
37. Anthony R. Ethical implications of the human-animal bond on the farm. *Anim Welf* 2003;12:505–512.
38. Burgess-Jackson K. Doing right by our animal companions. *J Ethics* 1998;2:159–185.
39. Frey R. *Rights, killing and suffering*. Oxford, England: Blackwell, 1983.
40. Singer P. *Practical ethics*. Cambridge: Cambridge University Press, 1978.
41. Rollin BE. The use and abuse of Aesculapian authority in veterinary medicine. *J Am Vet Med Assoc* 2002;220:1144–1149.
42. Hendrickx JF, Eger EI II, Sonner JM, et al. Is synergy the rule? A review of anesthetic interactions producing hypnosis and immobility. *Anesth Analg* 2008;107:494–506.
43. Antognini JF, Barter L, Carstens E. Overview: movement as an index of anesthetic depth in humans and experimental animals. *Comp Med* 2005;55:413–418.
44. Alkire MT, Hudetz AG, Tononi G. Consciousness and anesthesia. *Science* 2008;322:876–880.
45. Gregory NG. Animal welfare at markets and during transport and slaughter. *Meat Sci* 2008;80:2–11.
46. Finnie JW. Neuropathologic changes produced by non-penetrating percussive captive bolt stunning of cattle. *N Z Vet J* 1995;43:183–185.
47. Blackmore DK, Newhook JC. The assessment of insensibility in sheep, calves and pigs during slaughter. In: Eikelenboom G, ed. *Stunning of animals for slaughter*. Boston: Martinus Nijhoff Publishers, 1983;13–25.
48. Gregory NG, Lee CJ, Widdicombe JP. Depth of concussion in cattle shot by penetrating captive bolt. *Meat Sci* 2007;77:499–503.
49. Vogel KD, Badtram G, Claus JR, et al. Head-only followed by cardiac arrest electrical stunning is an effective alternative to head-only electrical stunning in pigs. *J Anim Sci* 2011;89:1412–1418.
50. Blackmore DK, Newhook JC. Electroencephalographic studies of stunning and slaughter of sheep and calves. 3. The duration of insensibility induced by electrical stunning in sheep and calves. *Meat Sci* 1982;7:19–28.
51. Cartner SC, Barlow SC, Ness TJ. Loss of cortical function in mice after decapitation, cervical dislocation, potassium chloride injection, and CO_2 inhalation. *Comp Med* 2007;57:570–573.
52. Close B, Banister K, Baumans V, et al. Recommendations for euthanasia of experimental animals: part 2. DGXT of the European Commission. *Lab Anim* 1997;31:1–32.
53. Close B, Banister K, Baumans V, et al. Recommendations for euthanasia of experimental animals: part 1. DGXI of the European Commission.. *Lab Anim* 1996;30:293–316.
54. Gregory NG, Wotton SB. Effect of slaughter on the spontaneous and evoked activity of the brain. *Br Poult Sci* 1986;27:195–205.
55. Bates G. Humane issues surrounding decapitation reconsidered. *J Am Vet Med Assoc* 2010;237:1024–1026.
56. Holson RR. Euthanasia by decapitation: evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. *Neurotoxicol Teratol* 1992;14:253–257.
57. Derr RF. Pain perception in decapitated rat brain. *Life Sci* 1991;49:1399–1402.
58. Vanderwolf CH, Buzak DP, Cain RK, et al. Neocortical and hippocampal electrical activity following decapitation in the rat. *Brain Res* 1988;451:340–344.
59. Mikeska JA, Klemm WR. EEG evaluation of humaneness of asphyxia and decapitation euthanasia of the laboratory rat. *Lab Anim Sci* 1975;25:175–179.
60. Muir WW. Considerations for general anesthesia. In: Tranquilli WJ, Thurmon JC, Grimm KA, eds. *Lumb and Jones' veterinary anesthesia and analgesia*. 4th ed. Ames, Iowa: Blackwell, 2007;7–30.
61. Erhardt W, Ring C, Kraft H, et al. CO_2 stunning of swine for slaughter from the anesthesiological viewpoint. *Dtsch Tierarztl Wochenschr* 1989;96:92–99.
62. International Association for the Study of Pain. Pain terms. Available at: www.iasp-pain.org/AM/Template.cfm?Section=Pain_Definitions&Template=/CM/HTMLDisplay.cfm&ContentID=1728#Pain. Accessed Feb 7, 2011.
63. AVMA. AVMA guidelines on euthanasia. June 2007. Available from AVMA Animal Welfare Division (animalwelfare@avma.org).
64. Tarsitano MS, Jackson RR. Araneophagic jumping spiders discriminate between detour routes that do and do not lead to prey. *Anim Behav* 1997;53:257–266.
65. Jackson RR, Carter CM, Tarsitano MS. Trial-and-error solving of a confinement problem by a jumping spider, *Portia fibriata*. *Behaviour* 2001;138:1215–1234.
66. Dyakonova VE. Role of opiod peptides in behavior of invertebrates. *J Evol Biochem Physiol* 2001;37:335–347.
67. Sladky KK, Kinney ME, Johnson SM. Analgesic efficacy of butorphanol and morphine in bearded dragons and corn snakes. *J Am Vet Med Assoc* 2008;233:267–273.
68. Baker BB, Sladky KK, Johnson SM. Evaluation of the analgesic effects of oral and subcutaneous tramadol administration in red-eared slider turtles.

72. Nordgreen J, Horsberg TE, Ranheim B, et al. Somatosensory evoked potentials in the telencephalon of Atlantic salmon (*Salmo salar*) following galvanic stimulation of the tail. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2007;193:1235–1242.
73. Dunlop R, Laming P. Mechanoreceptive and nociceptive responses in the central nervous system of goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*). *J Pain* 2005;6:561–568.
74. Braithwaite VA. Cognition in fish. *Behav Physiol Fish* 2006;24:1–37.
75. Kitchen N, Aronson AL, Bittle JL, et al. Panel report on the Colloquium on Recognition and Alleviation of Animal Pain and Distress. *J Am Vet Med Assoc* 1987;191:1186–1191.
76. Wack R, Morris P, Sikarskie J, et al. Criteria for humane euthanasia and associated concerns. In: American Association of Zoo Veterinarians (AAZV). *Guidelines for euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;3–5.
77. National Research Committee on Pain and Distress in Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. *Recognition and alleviation of pain and distress in laboratory animals*. Washington, DC: National Academy Press, 1992.
78. Breazile JE. Physiologic basis and consequences of distress in animals. *J Am Vet Med Assoc* 1987;191:1212–1215.
79. McMillan FD. Comfort as the primary goal in veterinary medical practice. *J Am Vet Med Assoc* 1998;212:1370–1374.
80. Coppola CL, Grandin T, Enns MR. Human interaction and cortisol: can human contact reduce stress in shelter dogs? *Physiol Behav* 2006;87:537–541.
81. Van Reenen CG, O'Connell NE, Van der Werf JT, et al. Response of calves to acute stress: individual consistency and relations between behavioral and physiological measures. *Physiol Behav* 2005;85:557–570.
82. Dantzer R, Mormède P. Stress in farm animals: a need for reevaluation. *J Anim Sci* 1983;57:6–18.
83. Moberg GP, Wood VA. Effect of differential rearing on the behavioral and adrenocortical response of lambs to a novel environment. *Appl Anim Ethol* 1982;8:269–279.
84. Baran BE, Allen JA, Rogelberg SG, et al. Euthanasia-related strain and coping strategies in animal shelter employees. *J Am Vet Med Assoc* 2009;235:83–88.
85. Collette JC, Millam JR, Klasing KC, et al. Neonatal handling of Amazon parrots alters stress response and immune function. *Appl Anim Behav Sci* 2000;66:335–349.
86. Grandin T. Assessment of stress during handling and transport. *J Anim Sci* 1997;75:249–257.
87. Boandl KE, Wohlt JE, Carsia RV. Effect of handling, administration of a local anesthetic and electrical dehorning on plasma cortisol in Holstein calves. *J Dairy Sci* 1989;72:2193.
88. Lay DC, Friend TH, Bowers CL, et al. A comparative physiological and behavioral study of freeze and hot-iron branding using dairy cows. *J Anim Sci* 1992;70:1121–1125.
89. Lay DC, Friend TH, Randel RD, et al. Behavioral and physiological effects of freeze and hot-iron branding on crossbred cattle. *J Anim Sci* 1992;70:330–336.
90. Duke JL, Zammit TG, Lawson DM. The effects of routine cage-changing in cardiovascular and behavioral parameters in male Sprague-Dawley rats. *Contemp Top Lab Anim Sci* 2001;40:17–20.
91. Ramsay EC, Wetzel RW. Comparison of five regimens for oral administration of medication to induce sedation in dogs prior to euthanasia. *J Am Vet Med Assoc* 1998;213:240–242.
92. Wetzel RW, Ramsay EC. Comparison of four regimens for intraoral administration of medication to induce sedation in cats prior to euthanasia. *J Am Vet Med Assoc* 1998;213:243–245.
93. Houpt KA. Domestic animal behavior for veterinarians and animal scientists. 3rd ed. Ames, Iowa: Iowa State University Press, 1998.
94. Beaver BV. *Canine behavior: a guide for veterinarians*. Philadelphia: WB Saunders Co, 1998;.
95. Beaver BV. *The veterinarian's encyclopedia of animal behavior*. Ames, Iowa: Iowa State University Press, 1994;.
96. Schafer M. *The language of the horse: habits and forms of expression*. New York: Arco Publishing Co, 1975.
97. White RG, DeShazer JA, Tressler CJ, et al. Vocalization and physiological response of pigs during castration with and without a local anesthetic. *J Anim Sci* 1995;73:381–386.
98. Warriss PD, Brown SN, Adams M. Relationships between subjective and objective assessments of stress at slaughter and meat quality in pigs. *Meat Sci* 1994;38:329–340.
99. Dunn CS. Stress reactions of cattle undergoing ritual slaughter using two methods of restraint. *Vet Rec* 1990;126:522–525.
100. Grandin T. Cattle vocalizations are associated with handling and equipment problems in slaughter plants. *Appl Anim Behav Sci* 2001;71:191–201.
101. Grandin T. Objective scoring of animal handling and stunning practices at slaughter plants. 1g 45 (quality in pigGallar)2o4.2295ude for veter 101.

workers to euthanasia. *J Am Vet Med Assoc* 1996;208:846–849.
125. Wolfle T. Laboratory animal technicians: their role in stress reduction and human-companion animal bonding. *Vet Clin North 125.*

cidence for an old disease. *Undersea Hyperb Med* 2007;34:163–168.
175. Lowe-Ponsford FL, Henry JA. Clinical aspects of carbon monoxide poisoning. *Adverse Drug React Acute Poisoning Rev* 1989;8:217–240.
176. Bloom JD. Some considerations in establishing divers' breathing gas purity standards for carbon monoxide. *Aerosp Med* 1972;43:633–636.
177. Norman CA, Halton DM. Is carbon monoxide a workplace teratogen? A review and evaluation of the literature. *Ann Occup Hyg* 1990;34:335–347.
178. Wojtczak-Jaroszowa J, Kbow S. Carbon monoxide, carbon disulfide, lead and cadmium—four examples of occupational toxic agents linked to cardiovascular disease. *Med Hypotheses* 1989;30:141–150.

226. Yavari P, McCulloch PF, Panneton WM. Trigeminally-mediated alteration of cardiorespiratory rhythms during nasal application of carbon dioxide in the rat. *J Auton Nerv Syst* 1996;61:195–200.
227. Moosavi SH, Golestanian E, Binks AP, et al. Hypoxic and hypercapnic drives to breathe generate equivalent levels of air hunger in humans. *J Appl Physiol* 2003;94:141–154.
228. Millar RA. Plasma adrenaline and noradrenaline during diffusion respiration. *J Physiol* 1960;150:79–90.
229. Nahas GG, Ligou JC, Mehlman B. Effects of pH changes on O_2 uptake and plasma catecholamine levels in the dog. *Am J Physiol* 1960;198:60–66.
230. Liotti M, Brannan S, Egan G, et al. Brain responses associated with consciousness of breathlessness (air hunger). *Proc Natl Acad Sci* 2001;98:2035–2040.
231. Dripps RD, Comroe JH. The respiratory and circulatory response of normal man to inhalation of 7.6 percent CO_2 and 10.4 percent CO_2 with a comparison of the maximal ventilation produced by severe muscular exercise, inhalation of CO_2 and maximal voluntary hyperventilation. *Am J Physiol* 1947;149:43–51.
232. Hill L, Flack M. The effect of excess of carbon dioxide and of want of oxygen upon the respiration and the circulation. *J Physiol* 1908;37:77–111.
233. Banzett RB, Lansing RW, Evans KC, et al. Stimulus-response characteristics of CO_2-induced air hunger in normal subjects. *Respir Physiol* 1996;103:19–31.
234. Shea SA, Harty HR, Banzett RB. Self-control of level of mechanical ventilation to minimize CO_2-induced air hunger. *Respir Physiol* 1996;103:113–125.
235. Fowler WS. Breaking point of breath-holding. *J Appl Physiol* 1954;6:539–545.
236. Kirkden RD, Niel L, Stewart SA, et al. Gas killing of rats: the effect of supplemental oxygen on aversion to carbon dioxide. *Anim Welf* 2008;17:79–87.
237. Coenen AM, Drinkenburg WH, Hoenderken R, et al. Carbon dioxide euthanasia in rats: oxygen supplementation minimizes signs of agitation and asphyxia. *Lab Anim* 1995;29:262–268.
238. Hewett TA, Kovacs MS, Artwohl JE, et al. A comparison of euthanasia methods in rats, using carbon dioxide in prefilled and fixed flow-rate filled chambers. *Lab Anim Sci* 1993;43:579–582.
239. Borovsky V, Herman M, Dunphy G, et al. CO_2 asphyxia increases plasma norepinephrine in rats via sympathetic nerves. *Am J Physiol* 1998;274:R19–R22.
240. Reed B, Varon J, Chait BT, et al. Carbon dioxide-induced anesthesia result in a rapid increase in plasma levels of vasopressin. *Endocrinology* 2009;150:2934–2939.
241. Raff H, Roarty TP. Renin, ACTH, and aldosterone during acute hypercapnia and hypoxia in conscious rats. *Am J Physiol* 1988;254:R431–R435.
242. Marotta SF, Sithichoke N, Garcy AM, et al. Adrenocortical responses of rats to acute hypoxic and hypercapnic stresses after treatment with aminergic agents. *Neuroendocrinology* 1976;20:182–192.
243. Raff H, Shinsako J, Keil LC, et al. Vasopressin, ACTH, and corticosteroids during hypercapnia and graded hypoxia in dogs. *Am J Physiol* 1983;244:E453–E458.
244. Argyropoulos SV, Bailey JE, Hood SD, et al. Inhalation of 35% CO_2 results in activation of the HPA axis in healthy volunteers. *Psychoneuroendocrinology* 2002;27:715–729.
245. Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci* 1997;20:78–84.
246. Kc P, Haxhiu MA, Trouth CO, et al. CO_2-induced c-Fos expression in hypothalamic vasopressin containing neurons. *Respir Physiol* 2002;129:289–296.
247. Hackbarth H, Kuppers N, Bohnet W. Euthanasia of rats with carbon dioxide—animal welfare aspects. *Lab Anim* 2000;34:91–96.
248. Blackshaw JK, Fenwick DC, Beattie AW, et al. The behavior of chickens, mice and rats during euthanasia with chloroform, carbon dioxide and ether. *Lab Anim* 1988;22:67–75.
249. Britt DP. The humaneness of carbon dioxide as an agent of euthanasia for laboratory rodents. In: *Euthanasia of unwanted, injured or diseased animals or for educational or scientific purposes*. Potters Bar Hertfordshire, England: Universities Federation for Animal Welfare, 1987;19–31.
250. Raj ABM, Gregory NG. Welfare implications of the gas stunning of pigs: 2. Stress of induction of anaesthesia. *Anim Welf* 1996;5:71–78.
251. Jongman EC, Barnett JL, Hemsworth PH. The aversiveness of carbon dioxide stunning in pigs and a comparison of the CO_2 stunner crate vs the V-restrainer. *Appl Anim Behav Sci* 2000;67:67–76.
252. Troeger K, Woltersdorf W. Gas anesthesia of slaughter pigs. 1. Stunning experiments under laboratory conditions with fat pigs of known halothane reaction type—meat quality, animal protection. *Fleischwirtschaft* 1991;72:1063–1068.
253. Dodman NH. Observations on use of Wernberg dip-lift carbon dioxide apparatus for pre-slaughter anesthesia of pigs. *Br Vet J* 1977;133:71–80.
254. Gerritzen MA, Lambooij E, Reimert HG, et al. Susceptibility of duck and turkey to severe hypercapnic hypoxia. *Poult Sci* 2006;85:1055–1061.
255. Gerritzen M, Lambooij B, Reimert H, et al. A note on behaviour of poultry exposed to increasing carbon dioxide concentrations. *Appl Anim Behav Sci* 2007;108:179–185.
256. McKeegan DEF, McIntyre JA, Demmers TGM, et al. Physiological and behavioural responses of broilers to controlled atmosphere stunning: implications for welfare. *Anim Welf* 2007;16:409–426.
257. Abeyesinghe SM, McKeegan DEF, McLeman MA, et al. Controlled atmosphere stunning of broiler chickens. I. Effects on behaviour, physiology and meat quality in a pilot scale system at a processing plant. *Br Poult Sci* 2007;48:406–423.
258. Cooper J, Mason G, Raj M. Determination of the aversion of farmed mink (*Mustela vison*) to carbon dioxide. *Vet Rec* 1998;143:359–361.
259. Battaglia M, Ogliari A, Harris J, et al. A genetic study of the acute anxious response to carbon dioxide stimulation in man. *J Psychiatr Res* 2007;41:906–917.
260. Nardi AE, Freire RC, Zin WA. Panic disorder and control of breathing. *Respir Physiol Neurobiol* 2009;167:133–143.
261. Grandin T. Effect of genetics on handling and CO_2 stunning of pigs (updated July 2008). *Meat Focus Int* 1992;July:124–126. Available at: www.grandin.com/humane/meatfocus7-92.html. Accessed Dec 13, 2010.
262. Ziemann AE, Allen JE, Dahdaleh NS, et al. The amygdala is a chemosensor that detects carbon dioxide and acidosis to elicit fear behavior. *Cell* 2009;139:1012–1021.
263. Kohler I, Moens Y, Busato A, et al. Inhalation anaesthesia for the castration of piglets: CO_2 compared to halothane. *Zentralbl Veterinarmed A* 1998;45:625–633.
264. Glen JB, Scott WN. Carbon dioxide euthanasia of cats. *Br Vet J* 1973;129:471–479.
265. Franson JC. Euthanasia. In: Friend M, Franson JC, eds. *Field manual of wildlife diseases. General field procedures and diseases of birds*. Biological Resources Division information and technology report 1999–001. Washington, DC: US Department of the Interior and US Geological Survey, 1999;49–53.
266. Mohan Raj AB, Gregory NG. Effect of rate of induction of carbon dioxide anaesthesia on the time of onset of unconsciousness and convulsions. *Res Vet Sci* 1990;49:360–363.
267. Mohan Raj AB, Wotton SB, Gregory NG. Changes in the somatosensory evoked potentials and spontaneous electoencephalogram of hens during stunning with a carbon dioxide and argon mixture. *Br Vet J* 1992;148:147–156.
268. Raj M, Gregory NG. An evaluation of humane gas stunning methods for turkeys. *Vet Rec* 1994;135:222–223.
269. Poole GH, Fletcher DL. A comparison of argon, carbon dioxide, and nitrogen in a broiler killing system. *Poult Sci* 1995;74:1218–1223.
270. Latimer KS, Rakich PM. Necropsy examination. In: Ritchie BW, Harrison GJ, Harrison LR, eds. *Avian medicine: principles and application*. Lake Worth, Fla: Wingers Publishing Inc, 1994;355–379.
271. Jaksch W. Euthanasia of day-old male chicks in the poultry industry. *Int J Study Anim Probl* 1981;2:203–213.
272. Pritchett-Corning KR. Euthanasia of neonatal rats with carbon dioxide. *J Am Assoc Lab Anim Sci* 2009;48:23–27.
273. Pritchett K, Corrow D, Stockwell J, et al. Euthanasia of neonatal

mice with carbon dioxide. *Comp Med* 2005;55:275–281.
274. Hayward JS, Lisson PA. Carbon dioxide tolerance of rabbits and its relation to burrow fumigation. *Aust Wildl Res* 1978;5:253–261.
275. Iwarsson K, Rehbinder C. A study of different euthanasia techniques in guinea pigs, rats, and mice. Animal response and postmortem findings. *Scan J Lab Anim Sci* 1993;20:191–205.
276. US Department of Health and Human Services. *Compounding of drugs for use in animals*. Compliance policy guide section 608.400. Rockville, Md: US FDS, 2003;1–7. Available at: www.fda.gov/downloads/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/UCM200461.pdf. Accessed Mar 26, 2010.
277. Campbell VL, Butler AL, Lunn KF. Use of a point-of-care urine drug test in a dog to assist in diagnosing barbiturate toxicosis secondary to ingestion of a euthanized carcass. *J Vet Emerg Crit Care (San Antonio)* 2009;19:286–291.
278. Jurczynski K, Zittlau E. Pentobarbital poisoning in Sumatran tigers (*Panthera tigris sumatrae*). *J Zoo Wildl Med* 2007;38:583–584.
279. US FDA. 21 CFR Part 522. Injectable or implantable dosage form new animal drugs; euthanasia solution; technical amendment. *Fed Regist* 2003;68:42968–42969.
280. Wilkins JR III, Bowman ME. Needlestick injuries among female veterinarians: frequency, syringe contents and side-effects. *Occup Med (Lond)* 1997;47:451–457.
281. Lewbart GA, ed. *Invertebrate medicine*. Oxford, England: Blackwell, 2006.
282. Schwartz JA, Warren RJ, Henderson DW, et al. Captive and field tests of a method for immobilization and euthanasia of urban deer. *Wildl Soc Bull* 1997;25:532–541.
283. Bucher K, Bucher KE, Waltz D. Irritant actions of unphysiological pH values. A controlled procedure to test for topical irritancy. *Agents Actions* 1979;9:124–132.
284. Grier RL, Schaffer CB. Evaluation of intraperitoneal and intrahepatic administration of a euthanasia agent in animal shelter cats. *J Am Vet Med Assoc* 1990;197:1611–1615.
285. Schoell AR, Heyde BR, Weir DE, et al. Euthanasia method for mice in rapid time-course pulmonary pharmacokinetic studies. *J Am Assoc Lab Anim Sci* 2009;48:506–511.
286. Philbeck TE, Miller LJ, Montez D, et al. Hurts so good. Easing IO pain and pressure. *JEMS* 2010;35(9):58–62, 65–66, 68, 69.
287. Montez D, Miller LJ, Puga T, et al. Pain management with the use of IO: easing IO pain and pressure. Available at: www.jems.com/article/intraosseous/pain-management-use-io. Accessed Jun 13, 2011.
288. US FDA. *Tributame Euthanasia Solution: embutramide/chloroquine phosphate/lidocaine*. Freedom of Information summary. NADA 141-245 Silver Spring, Md: FDA, 2005..
289. US FDA. 21 CFR Part 522. Implantation or injectable dosage form new animal drugs; embutramide, chloroquine, and lidocaine solution. *Fed Regist* 2005;70:36336–36337.
290. US FDA. 21 CFR Part 1308. Schedules of controlled substances: placement of embutramide into schedule III. *Fed Regist* 2006;71:51115–51117.
291. Sodfola OA. The cardiovascular effect of chloroquine in anesthetized dogs. *Can J Physiol Pharmacol* 1980;58:836–841.
292. Don Michael TA, Alwassadeh S. The effects of acute chloroquine poisoning with special references to the heart. *Am Heart J* 1970;79:831–842.
293. Webb AI. Euthanizing agents. In: Reviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Ames, Iowa: Wiley Blackwell, 2009;401–408.
294. Webb AI, Pablo LS. Local anesthetics. In: Reviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Ames, Iowa: Wiley Blackwell, 2009;381–400.
295. Hellebrekers LJ, Baumans V, Bertens APMG, et al. On the use of T61 for euthanasia of domestic and laboratory animals; an ethical evaluation. *Lab Anim* 1990;24:200–204.
296. Park CK, Kim K, Jung SJ, et al. Molecular mechanism for local anesthetic action of eugenol in the rat trigeminal system. *Pain* 2009;144:84–94.
297. Kearns KS, Swenson B, Ramsay EC. Dosage trials with transmucosal carfentanil citrate in non-human primates. *Zoo Biol* 1999;18:397–402.
298. Flecknell PA. *Laboratory animal anaesthesia*. 2nd ed. San Diego: Elsevier Academic Press, 1996;168–171.
299. Saxena K. Death from potassium chloride overdose. *Postgrad Med* 1988;84:97–98, 101–102.
300. Lumb WV. Euthanasia by noninhalant pharmacologic agents. *J Am Vet Med Assoc* 1974;165:851–852.
301. Ciganovich E. Barbiturates. In: *Field manual of wildlife diseases. General field procedures and diseases of birds*. Biological Resources Division information and technology report 1999–001. Washington, DC: US Department of the Interior and US Geological Survey, 1999;349–351.
302. Raghav R, Taylor M, Guincho M, et al. Potassium chloride as a euthanasia agent in psittacine birds: clinical aspects and consequences for histopathologic assessment. *Can Vet J* 2011;52:303–306.
303. Luckl J, Keating J, Greenberg JH. Alpha-chloralose is a suitable anesthetic for chronic focal cerebral ischemia studies in the rat: a comparative study. *Brain Res* 2008;1191:157–167.
304. Belant JL, Tyson LA, Seamans TW. Use of alpha-chloralose by the Wildlife Services program to capture nuisance birds. *Wildl Soc Bull* 1999;27:938–942.
305. Cobaugh DJ. Ethanol. In: Brent J, Phillips SD,Wallace KL, et al, eds. *Critical care toxicology*. Philadelphia: Mosby, 2005;1553–1558.
306. Harms C. Anesthesia in fish. In: Fowler ME, Miller RE, eds. *Zoo and wild animal medicine: current therapy 4*. Philadelphia: WB Saunders Co, 1999;158–163.
307. Lord R. Use of ethanol for euthanasia of mice. *Aust Vet J* 1989;66:268.
308. US Food and Drug Administration. ANADA 200-226 Tricaine-

2004;1:46–53.
320. Borski RJ, Hodson RG. Fish research and the institutional animal care and use committee. *ILAR J* 2003;44:286–294.
321. Sladky KK, Swanson CR, Stoskopf MK, et al. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (2003-

135.
373. Blackmore DK, Newhook JC. Insensibility during slaughter of pigs in comparison to other domestic stock. *N Z Vet J* 1981;29:219–222.
374. Grandin T. Solving return-to-sensibility problems after electrical stunning in commercial pork slaughter plants. *J Am Vet Med Assoc* 2001;219:608–611.
375. Anil MH. Studies on the return of physical reflexes in pigs following electrical stunning. *Meat Sci* 1991;30:13–21.
376. Hoenderken R. Electrical and carbon dioxide stunning of pigs for slaughter. In: Eikelenboom G, ed. *Stunning of animals for slaughter*. Boston: Martinus Nijhoff Publishers, 1983;59–63
377. Denicourt M, Klopfenstein C, DuFour V, et al. *Developing a safe and acceptable method for on-farm euthanasia of pigs by electrocution. Final report*. Montreal: Faculty of Veterinary Medicine, University of Montreal, 2009.
378. Vogel KD, Badtram G, Claus JR, et al. Head-only followed by cardiac arrest electrical stunning is an effective alternative to head-only electrical stunning in pigs. *J Anim Sci* 2011;89:1412–1418.
379. Weaver AL, Wotton SB. The Jarvis Beef Stunner: effect of a prototype chest electrode. *Meat Sci* 2009;81:51–56.
380. Meerburg BGH, Brom FWA, Kijlstra A. The ethics of rodent control. *Pest Manag Sci* 2008;64:1205–1211.
381. Federal Provincial Committee for Humane Trapping. *Final report: Committee of the Federal Provincial Wildlife Conference*. Ottawa: Canadian Wildlife Service, 1981.
382. Department of Foreign Affairs and International Trade. *Agreement on international humane trapping standards between the European Community, Canada, and the Russian Federation*. Ottawa: Department of Foreign Affairs and International Trade, 1997;1–32.
383. Canadian General Standards Board. *Animal (mammal) traps—mechanically powered, trigger-activated killing traps for use on land*. No. CAN/CGSB-144.1–96. Ottawa: Canadian General Standards Board, 1996;1–36.
384. Nolan JW, Barrett MW. *Description and operation of the humane trapping research facility at the Alberta Environmental Centre*. AECV90–R3. Vegreville, AB, Canada: Alberta Environmental Centre, 1990.
385. International Organization for Standardization. *Animal (mammal) traps-part 4: methods for testing killing trap systems used on land or underwater*. TC 191, ISO/DIS 19009–4E. Geneva: International Organization for Standardization, 2000;1–15.
386. Gilbert FF. Assessment of furbearer response to trapping devices, in *Proceedings*. Worldw Furbearer Conf 1981;1599–1611.
387. Proulx G, Barrett MW. Evaluation of the Bionic trap to quickly kill fisher (*Martes pennanti*) in simulated natural environments. *J Wildl Dis* 1993;29:310–316.
388. Proulx G, Barrett MW, Cook SR. The C120 Magnum with pan trigger: a humane trap for mink (*Mustela vison*). *J Wildl Dis* 1990;26:511–517.
389. Hiltz M, Roy LD. Rating of killing traps against humane trapping standards using computer simulations, in *Proceedings*. 19th Vertebrate Pest Conf 2000;197–201.
390. Association of Fish and Wildlife Agencies. Best management practices. Available at: jjcdev.com/~fishwild/?section=best_management_practices Accessed July 22, 2012.
391. International Association of Fish and Wildlife Agencies. *Summary of progress. 1999–2000 field season: testing restraining and body-gripping traps for development of best management practices for trapping in the United States*. Washington, DC: International Association of Fish and Wildlife Agencies, 2003.
392. Warburton B, Gregory NG, Morriss G. Effect of jaw shape in kill-traps on time to loss of palpebral reflexes in brushtail possums. *J Wildl Dis* 2000;36:92–96.
393. King CM. The effects of two types of steel traps upon captured stoats (*Mustela erminea*). *J Zool (Lond)* 1981;195:553–554.
394. Proulx G, Kolenosky AJ, Cole PJ, et al. A humane killing trap for lynx (*Felis lynx*): the Conibear 330 with clamping bars. *J Wildl Dis* 1995;31:57–61.
395. Warburton B, Hall JV. Impact momentum and clamping force thresholds for developing standards for possum kill traps. *N Z J Zool* 1995;22:39–44.
396. Naylor BJ, Novak M. Catch efficiency and selectivity of various traps and sets used for capturing American martens. *Wildl Soc Bull* 1994;22:489–496.
397. Proulx G, Barrett MW. Field testing of the C120 magnum trap for mink. *Wildl Soc Bull* 1993;21:421–426.
398. Proulx G, Kolenosky AJ, Badry MJ, et al. Assessment of the Savageau 2001-8 trap to effectively kill artic fox. *Wildl Soc Bull* 1993;21:132–135.
399. Proulx G, Kolenosky AJ, Cole PJ. Assessment of the Kania trap to humanely kill red squirrels (*Tamiasciurus hudsonicus*) in enclosures. *J Wildl Dis* 1993;29:324–329.
400. Proulx G, Pawlina IM, Wong RK. Re-evaluation of the C120 magnum and Bionic traps to humanely kill mink. *J Wildl Dis* 1993;29:184.
401. Cooper JE, Ewbank R, Platt C, et al. *Euthanasia of amphibians and reptiles*. London: Universities Federation for Animal Welfare and World Society for the Protection of Animals, 1989.
402. Proulx G, Cook SR, Barrett MW. Assessment and preliminary development of the rotating jaw Conibear 120 trap to effectively kill marten (*Martes americana*). *Can J Zool* 1989;67:1074–1079.
403. Hill EP. *Evaluation of improved traps and trapping techniques*. Project report W-44-6, Job IV-B. Montgomery, Ala: Alabama Department of Conservation and Natural Resources, 1981;1–19.
404. Guidelines of the American Society of Mammalogists for the use of mammals in research. *J Mammal* 2011;92:235–253.
405. *Improving animal welfare in US trapping programs*. Washington, DC: International Association of Fish and Wildlife Agencies, 1997.
406. American Association of Avian Pathologists (AAAP) Animal

Assoc 2005;226:370–373.
468. Daly CC, Whittington PE. Concussive methods of pre-slaughter stunning in sheep: effects of captive bolt stunning in the poll position on brain function. *Res Vet Sci* 1986;41:353–355.
469. Gregory NG, Spence JY, Mason CW, et al. Effectiveness of poll stunning water buffalo with captive bolt guns. *Meat Sci* 2009;81:178–182.
470. Schulze W, Schultze-Petzold H, Hazem AS, et al. Experiments on the objective assessment of pain and consciousness in slaughtering sheep and calves by the conventional method (humane killer stunning) and by ritual slaughtering laws (shechita). *Dtsch Tierarztl Wochenschr* 1978;85:62–66.
471. Bager F, Devine CE, Gilbert KV. Jugular blood flow in calves after head-only electrical stunning and throat-cutting. *Meat Sci* 1988;22:237–243.
472. Daly CC, Kallweit E, Ellendorf F. Cortical function in cattle during slaughter: conventional captive bolt stunning followed by exsanguination compared with shechita slaughter. *Vet Rec* 1988;122:325–329.
473. Newhook JC, Blackmore DK. Electroencephalographic studies of stunning and slaughter of sheep and calves: part 1— the onset of permanent insensibility in sheep during slaughter. *Meat Sci* 1982;6:221–233.
474. Gregory NG, Fielding HR, von Wenzlawowicz M, et al. Time to collapse following slaughter without stunning in cattle. *Meat Sci* 2010;85:66–69.
475. Rosen SD. Physiological insights into shechita. *Vet Rec* 2004;154:759–765.
476. Gregory NG. Physiology of stress, distress, stunning and slaughter. In: Animal welfare and meat science. Wallingford, Oxfordshire, England: CABI Publishing, 1998;64–92.
477. Gibson TJ, Johnson CB, Murrell JC, et al. Components of electroencelphalographic responses to slaughter in halothane-anesthetized calves: effects of cutting neck tissues compared with major blood vessels. *N Z Vet J* 2009;57:84–89.
478. Mellor DJ, Gibson TJ, Johnson CB. A re-evaluation of the need to stun calves prior to slaughter by ventral-neck incision : an introductory review. *N Z Vet J* 2009;57:74–76.
479. Evers AS, Crowder CM, Balser JR. General anesthetics. In: Brunton LL, Lazo JS, Parker KL, eds. *Goodman and Gillman's the pharmacological basis of therapeutics*. 11th ed. New York: McGraw-Hill Medical Publishing Division, 2006;362.
480. Finnie JW, Blumbergs PC, Manavis J, et al. Evaluation of brain damage resulting from penetrating and non-penetrating captive bolt stunning using lambs. *Aust Vet J* 2000;78:775–778.
481. Finnie JW, Manavis J, Blumberg PC, et al. Brain damage in sheep from penetrating captive bolt stunning. *Aust Vet J* 2002;80:67–69.
482. OIE. Chapter 7.5.5: management of fetuses during slaughter of pregnant animals. In: *Terrestrial animal health code*. 17th ed. Paris: OIE, 2008;284.
483. Jochems CE, van der Valk JB, Stafleu FR, et al. The use of fetal bovine serum: ethical or scientific problem? *Altern Lab Anim* 2002;30:219–227.
484. Yan EB, Barburamani AA, Walker AM, et al. Changes in cerebral blood flow, cerebral metabolites, and breathing movements in the sheep fetus following asphyxia produced by occlusion of the umbilical cord. *Am J Physiol Regul Integr Comp Physiol* 2009;297:R60–R69.
485. Mellor DJ. Integration of perinatal events, pathophysiological changes and consequences for the newborn lamb. *Br Vet J* 1988;144:552–569.
486. Peisker N, Preissel AK, Rechenbach HD, et al. Foetal stress responses to euthanasia of pregnant sheep. *Berl Munch Tierarztl Wochenschr* 2010;123:2–10.
487. Klaunberg BA, O'Malley J, Clark T, et al. Euthanasia of mouse fetuses and neonates. *Contemp Top Lab Anim Sci* 2004;43:29–34.
488. Küchenmeister U, Kuhn G, Ender K. Preslaughter handling of pigs and the effect on heart rate, meat quality, including tenderness, and sarcoplasmic reticulum Ca2+ transport. *Meat Sci* 2005;71:690–695.
489. Küchenmeister U, Kuhn G, Stabenow B, et al. The effect of experimental stress on sarcoplasmic reticulum Ca2+ transport and meat quality in pig muscle. *Meat Sci* 2002;61:375–380.
490. Geverink NA, Schouten WGP, Gort G, et al. Individual differences in behavioral and physiological responses to restraint stress in pigs. *Physiol Behav* 2002;77:451–457.
491. Magnusson U, Wattrang E, Tsuma V, et al. Effects of stress resulting from short-term restraint on in vitro functional capacity of leukocytes obtained from pigs. *Am J Vet Res* 1998;59:421–425.
492. Neubert E, Gurtler H, Vallentin G. Effect of restraining growth pigs with snare restraints on plasma levels of catecholamines, cortisol, insulin and metabolic parameters. *Berl Munch Tierarztl Wochenschr* 1996;109:409–413.
493. Roozen AW, Magnusson U. Effects of short-term restraint stress on leukocyte counts, lymphocyte proliferation and lysis of erythrocytes in gilts. *Zentralbl Veterinarmed B* 1996;43:505–511.
494. Roozen AWM, Tsuma VT, Magnusson U. Effects of short-term restraint stress on plasma concentrations of catecholamines, β-endorphin, and cortisol in gilts. *Am J Vet Res* 1995;56:1225–1227.
495. Farmer C, Dubreuil P, Couture Y, et al. Hormonal changes following an acute stress in control and somatostatin-immunized pigs. *Domest Anim Endocrinol* 1991;8:527–536.
496. Muir W. *Handbook of veterinary anesthesia*. 3rd ed. St Louis: Mosby, 2000.
497. Maisch A, Ritzmann M, Heinritzi K. The humane euthanasia of pigs with pentobarbital. *Tierarztl Umsch* 2005;60:679–683.
498.

563. Peng J, Wngle M, Mueller T, et al. Ethanol-modulated camouflage response screen in zebrafish uncovers a novel role for camp and extracellular signal-regulated kinase signaling in behavioral sensitivity to ethanol. *J Neurosci* 2009;29:8408–8418.
564. Dlugos CA, Rabin RA. Ethanol effects on three strains of zebrafish: model system for genetic investigations. *Pharmacol Biochem Behav* 2003;74:471–480.
565. Gerlai R, Lahav M, Guo S, et al. Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacol Biochem Behav* 2000;67:773–782.
566. Gladden JN, Brainard BM, Shelton JL, et al. Evaluation of isoeugenol for anesthesia in koi carp (*Cyprinus carpio*). *Am J Vet Res* 2010;71:859–866.
567. Holloway A, Keene JL, Noakes DG, et al. Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Aquaculture Res* 2004;35:1025–1030.
568. Lewbart GA. Fish. In: Carpenter JW, ed. *Exotic animal formulary*. 3rd ed. St Louis: Elsevier Saunders, 2005;5–29.
569. FDA. *Concerns related to the use of clove oil as an anesthetic for fish*. Guidance for industry 150. Washington, DC: Department of Health and Human Services, 2007. Available at: www(raa)Tj t5.urosci

2006;19–22.
615. Hyman J. Euthanasia in marine mammals. In: Dierauf LA, ed. *Handbook of marine mammal medicine: health, disease and rehabilitation*. Boca Raton, Fla: CRC Press Inc, 1990;265–266.
616. Needham DJ. Cetacean strandings. In: Fowler ME, ed. Zoo and wild animal medicine: current therapy 3. 3rd ed. Philadelphia: WB Saunders Co, 1993;415–425.
617. Gullett PA. Euthanasia. In: Friend M, ed. *Field guide to wildlife diseases. Volume 1: general field procedures and diseases of migratory birds*. Resource publication #167. Washington, DC: US Department of the Interior, Fish and Wildlife Service, 1987;59–63.
618. Fair JM. *Guidelines for the use of wild birds in research*. 3rd ed. Washington, DC: The Ornithological Council,52,ca Raton, Fla6Tw -2.25103ce, 1987;59–63.

Glossary

Acceptable: A method considered to reliably meet the requirements of euthanasia. See EUTHANASIA.

Acceptable With Conditions: A method considered to reliably meet the requirements of euthanasia when specified conditions are met. See EUTHANASIA.

Adjunctive Method: A method of assuring death that may be used after an animal has been made unconscious.

Affect: The external expression of emotion.

Altricial: Immobile, blind, naked young animals (including but not limited to birds and some rodents) requiring parental care and feeding.

Anesthesia, General: A method used to produce unconsciousness. See UNCONCIOUSNESS.

Animal: Any nonhuman animal (Kingdom: Animalia).

Aversion: A desire to avoid or retreat from a stimulus.

Avian: Relating to birds.

Captive Bolt: A device used to kill or stun animals where a tethered metal rod is discharged into the brain of the animal.

Chick: A young bird.

Cremation: To incinerate a dead body. See INCINERATION.

Depopulation: The killing of animals in large numbers in response to an animal health emergency (eg, catastrophic infectious disease, mass intoxication, natural disaster) where all due consideration is given to the terminal experience of the animal, but the circumstances surrounding the event are understood to be exigent and extenuating. Depopulation may not meet the requirements of euthanasia due to situational constraints.

Distress: The effect of stimuli that initiate adaptive responses that are not beneficial to the animal—thus, the animal's response to stimuli interferes with its welfare and comfort.

Ectotherm: An organism that is dependent on environmental heat sources for regulating its body temperature.

Eustress: The effect of stimuli that initiate adaptive responses that are beneficial to the animal.

Euthanasia: A method of killing that minimizes pain, distress, and anxiety experienced by the animal prior to loss of consciousness, and causes rapid loss of consciousness followed by cardiac or respiratory arrest and death (see sections I3, I5, I6).

Exsanguination: The action of draining an animal of blood.

Fear: An unpleasant emotional experience caused by an awareness of a threat of danger.

Feral: A free-roaming, unowned animal of a domestic species that has reverted to wild behavior.

Field Conditions: Any situation outside of a controlled or clinical environment.

Fin sh: a term used to describe true (vertebrate) fish as opposed to other non-fish aquatic animals such as the invertebrates "starfish" and "cuttlefish"

Good Death: see EUTHANASIA.

Harvest: The act or process of killing an animal for food or other products.

Humane Killing: Killing performed in a manner that minimizes animal distress, but may not meet the requirements of euthanasia due to situational constraints.

Incineration: To burn completely, to ashes.

Insensible: See UNCONSCIOUS.

Livestock: Domestic animals raised for use, consumption, or profit, typically on a farm.

Mass euthanasia: see DEPOPULATION.

Nociception: Neuronal impulses generated by noxious stimuli, which threaten to, or actually do, destroy tissue. Nociception can occur without consequential pain perception.

Pain: A sensation (perception) that results from nociceptive nerve impulses reaching areas of the brain capable of conscious perception via ascending neural pathways.

Pithing: Physical destruction of the brain with a wire, air jet, or rod.

Poikilotherm: An animal with a variable internal temperature. These animals are generally ectothermic.

Poult: A young fowl.

Poultry: Domestic fowl raised for meat or eggs, such as chickens, turkeys, ducks, or geese.

Precocious: Capable of a high degree of independent activity (ie, mobility, feeding) from birth.

Secondary Method: A euthanasia method employed subsequent to a primary method to ensure death of an unconscious animal before it can recover consciousness. See ADJUNCTIVE METHOD.

Sedation: A state of CNS depression in which the animal is awake but calm, and with sufficient stimuli may be aroused.

Slaughter: Killing animals for the purposes of harvesting commodities such as meat or hides.

Stress: The effect of physical, physiologic, or emotional factors (stressors) that induce an alteration in an animal's homeostasis or adaptive state.

Stunning: Rendering an animal unconscious by use of a physical, gas, or electrical method.

Suffocate: To kill by preventing access to air or oxygen.

Unacceptable: A method that does not meet the requirements of euthanasia. See EUTHANASIA.

Unconsciousness: Unconsciousness, defined as lnUn

Appendix 2

Some acceptable* agents and methods of euthanasia.

Agen	Cla i ca ion	Mode of ac ion	Rapidi	Ea e of performance	Safe for per onnel	Specie i abili	Ef cac and commen	Condi ion
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible] 200 [illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] > 5 [illegible]		[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Agen	Cla i ca ion	Mode of ac ion	Rapidi	Ea e of performance	Safe for per onnel	Specie i abili	Ef cac and commen	Condi ion
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] 222)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2- [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Appendix 3

Some agents and methods that are unacceptable as primary methods of euthanasia.

Agent or method	Comment
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]