

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*

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*The AVMA Panel on Euthanasia develops the content of the guidelines, with support from its working groups. The panel is required to do a comprehensive review and update of the report at least every 10 years, although more frequent major revisions are possible based on substantive information gleaned from new research and experience with practical implementation. To ensure the guidelines remain as up-to-date as possible, interim revisions (reflecting substantive updates, but of a less extensive nature than a major revision) are also accommodated.

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as a compassionate treatment option when the alternative is prolonged and unrelenting suffering.⁴⁴ However, accommodating a pluralism of values, interests, and duties in animal ethics is challenging. This underscores the need for veterinarians to consider the broader context in thinking about what animal care she or he will prescribe. There are no easy reductionist formulas to which to appeal. In many cases, advice will need to be responsive to the needs at hand. Attention must be given to how the welfare and suffering of the animal are understood within the context of its whole life and in light of socially acceptable ways in which humans and animals interact in different environments.

Because veterinarians are committed to improving animal and human health and welfare, and because they work tirelessly to discover causes and cures for animal diseases and promote good animal management, some may feel a sense of disquiet or defeat when euthanasia becomes the better course of action. The POE hopes that these Guidelines and other AVMA policies will assist veterinarians who may be struggling with what may seem to be gratuitous euthanasia, the acceptability of certain procedures, and the sometimes routine nature of performing euthanasia. Toward that end, the decision aids in **Figures 1 and 2^a** are offered as a resource.

15 Evaluating Euthanasia Methods

In evaluating methods of euthanasia, the POE considered the following criteria: (1) ability to induce loss of consciousness and death with a minimum of pain and distress; (2) time required to induce loss of consciousness; (3) reliability; (4) safety of personnel; (5) irreversibility; (6) compatibility with intended animal use and purpose; (7) documented emotional effect on observers or operators; (8) compatibility with subsequent evaluation, examination, or use of tissue; (9) drug availability and human abuse potential; (10) compatibility with species, age, and health status; (11) ability to maintain equipment in proper working order; (12) safety for predators or scavengers should the animal's remains be consumed; (13) legal requirements; and (14) environmental impacts of the method or disposition of the animal's remains.

Euthanasia methods are classified in the Guidelines as acceptable, acceptable with conditions, and unacceptable. Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia. Methods acceptable with conditions are those techniques that may require certain conditions to be met to consistently produce humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure death. Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method can be met. Unacceptable techniques are those methods deemed inhumane under any conditions or that

the POE found posed a substantial risk to the human applying the technique. The Guidelines also include information about adjunctive methods, which are those that should not be used as a sole method of euthanasia, but that can be used in conjunction with other methods to bring about euthanasia.

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

As with many other procedures involving animals, some methods of euthanasia require physical handling of the animal. The amount of control and kind of restraint required will be determined by the species, breed, and size of animal involved; the degree of domestication, tolerance to humans, level of excitement, and prior handling experience of the animal; the presence of painful injury or disease; the animal's social environment; and the method of euthanasia and competence of the person(s) performing the euthanasia. Proper handling is vital to minimize pain and distress in animals, to ensure the safety of the person performing euthanasia, and, often, to protect other people and animals. Handling animals that are not accustomed to humans or that are severely injured or otherwise compromised may not be possible without inducing stress, so some latitude in the means of euthanasia is needed in some situations. The POE discussed the criteria for euthanasia used in the Guidelines as they apply to circumstances when the degree of control over the animal makes it difficult to ensure death without pain and distress. Pre-medication with the intent of providing anxiolysis, analgesia, somnolence for easier and safer IV access, and 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. Information in the scientific literature and available from practical experience focuses primarily on domesticated animals, but the same general considerations should be applied to all species.

Euthanasia must be performed in accord with applicable federal, state, and local laws governing drug acquisition and storage, occupational safety, and methods used for euthanasia and disposal of animals, with special attention to species requirements where possible. The AVMA encourages those responsible for performing euthanasia of nonhuman animals to review current federal, state, and local regulations. If drugs have been used, careful consideration must be given to appropriate disposal of the animal's remains and steps should be taken to avoid environmental contamination or harm to other animals.

Circumstances may arise that are not clearly covered by the Guidelines. Whenever such situations arise, a veterinarian experienced with the species should apply professional judgment, knowledge of clinically acceptable techniques, professional ethos, and social conscience in selecting an appropriate technique for ending an animal's life.

It is imperative that death be verified after euthanasia and before disposal of the animal. An animal in deep narcosis following administration of an injectable or inhalant agent may appear to be dead, but might eventually recover. Death must be confirmed by examining the animal for cessation of vital signs. Consideration should be given to the animal species and method of euthanasia when determining appropriate criteria for confirming death.

Safe handling and disposal of the resulting animal remains are also critically important when the presence of zoonotic disease, foreign animal diseases, or other diseases of concern to population health is suspected. Appropriate diagnostic samples should be collected for testing, pertinent regulatory authorities should be notified, and the animal's body should be incinerated, if possible. Use of 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.

15.1 CONSCIOUSNESS AND UNCONSCIOUSNESS

Consciousness refers to the subjective or inner qualitative experience of an animal in question. In humans, consciousness is common during both sleep and anesthesia, as evidenced by dreaming.⁴⁵ One defining feature of dreaming is that, even while conscious, we do not experience our environment—we are disconnected from it. Ideally, general anesthesia prevents the experience of surgery and pain (connected consciousness), as well as producing behav-

ioral unresponsiveness, either by inducing unconsciousness or by disconnecting consciousness from the environment.⁴⁵

Unconsciousness, defined as loss of individual awareness, occurs when the brain's ability to integrate information is blocked or disrupted. In humans, onset of anesthetic-induced unconsciousness has been functionally defined by loss of appropriate response to verbal command; in animals, by loss of the righting reflex.^{46,47} This definition, introduced with the discovery of general anesthesia more than 160 years ago, is still useful because it is an easily observable, integrated whole-animal response.

Anesthetics produce unconsciousness either by preventing integration (blocking interactions among specialized brain regions) or by reducing information (shrinking the number of activity patterns available to cortical networks) received by the cerebral cortex or equivalent structure(s). Further, the abrupt loss of consciousness that occurs at a critical concentration of anesthetic implies that the integrated repertoire of neural states underlying consciousness may collapse nonlinearly.⁴⁸ Cross-species data suggest that memory and awareness are abolished with less than half the concentration required to abolish movement. Thus, an anesthetic state (unconsciousness and amnesia) can be produced at concentrations of anesthetic that do not prevent physical movements.⁴⁷

Measurements of brain electrical function have been used to objectively quantify the unconscious state. At some level between behavioral unresponsiveness and the induction of a flat EEG (indicating the cessation of the brain's electrical activity and brain death), consciousness must vanish. However, EEG data cannot provide definitive answers as to onset of unconsciousness. Brain function monitors based on EEG are limited in their ability to directly indicate presence or absence of unconsciousness, especially around the transition point⁴⁸; also, it is not always clear which EEG patterns are indicators of activation by stress or pain.²⁸

Physical methods that destroy or render nonfunctional the brain regions responsible for cortical integration (eg, gunshot, captive bolt, cerebral electrocution, blunt force trauma, maceration) produce instantaneous unconsciousness. When physical methods directly destroy the brain, signs of unconsciousness include immediate collapse and a several-second period of tetanic spasm, followed by slow hind limb movements of increasing frequency⁴⁹⁻⁵¹ in cattle; however, there is species variability in this response. The corneal reflex will be absent.⁵² Signs of effective electrocution are loss of righting reflex, loss of eye-blink and moving object tracking, extension of the limbs, opisthotonos, downward rotation of the eyeballs, and tonic spasm changing to clonic spasm, with eventual muscle flaccidity.^{53,54}

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,⁵⁵⁻⁵⁸ has been controversial.⁵⁹ As indicated previously, EEG methods cannot provide definitive answers as to onset of unconsciousness. Other studies⁶⁰⁻⁶³ indicate such activity does not imply the ability to perceive pain and conclude that loss of consciousness develops rapidly.

Once loss of consciousness occurs (ie, there is no longer an inner qualitative experience) subsequently observed activities, such as convulsions, vocalization, reflex struggling, breath holding, and tachypnea, can be attributed to the second stage of anesthesia, which by definition lasts from loss of consciousness to the onset of a regular breathing pattern.^{64,65} Thus, events observed following loss of the righting reflex are likely not consciously perceived. Some agents may induce convulsions, but these generally follow loss of consciousness. Agents inducing convulsions prior to loss of consciousness are unacceptable for euthanasia.

15.1.1 A REVIEW

Sedatives and immobilizing agents should not be confused with anesthetics, since animals are not necessarily rendered unconscious by the former 2 agents. Sedated and immobilized animals may still be aware of their environment. During anesthesia, consciousness is not necessarily associated with connectedness, responsiveness, or even recall. The concept of a transition zone between consciousness and unconsciousness has been discussed by Terlouw et al.^{66,67} This is especially true as it pertains to animals in slaughter plants. When animals are exsanguinated without stunning,⁶⁸ EEG studies^{69,70} show that a corneal reflex in response to touch can occur in unconscious animals. To clarify assessment of unconsciousness and consciousness, it is recommended to separate signs of definite consciousness from signs of unconsciousness or death. Following this paragraph is a list of 6 signs that an animal is definitely conscious⁶⁷; the subsequent paragraph is followed by a list of 3 signs that an animal is unconscious or (brain) dead. Consciousness likely depends on integrity of the corticothalamic networks. Spontaneous responsiveness may depend on subcortical and spinal cord networks and connectedness (namely, an awareness of one's environment) and may depend on continued information integration in corticothalamic circuits and unperturbed norepinephrinergic signaling.^{45,71} According to Terlouw et al,⁶⁷ terrestrial animals are definitely conscious when they exhibit any 1 of these 6 indicators: standing posture, head or body righting reflex, voluntary vocalization, spontaneous blinking (no touching), eye pursuit, and response to threat or menace test (no touching). Some modification of these indicators may be required on the basis of factors such as species and developmental stage. A terrestrial animal that is unconscious and brain-dead will not have corneal reflex, eyelash reflex (in response to touch), or rhythmic breathing.⁶⁷ Determin-

ing similar indicators for other species of animals is desired, and research into them is highly encouraged to help practitioners distinguish between animals that are brain-dead, unconscious (by anesthesia), immobilized, or sedated. Following are the 6 indicators of definite consciousness, in list form:

- Standing posture.
- Head or body righting reflex.
- Voluntary vocalization.
- Spontaneous blinking (no touching).
- Eye pursuit.
- Response to threat or menace test (no touching).

Before carcass disposal or invasive dressing procedures occur at a slaughter plant, it should be confirmed that an animal is unconscious or brain-dead. Ensuring that an animal is unconscious or brain-dead requires all 3 of the following indicators:

- Absence of corneal reflex.
- Absence of eyelash reflex (response to touch).
- Absence of rhythmic breathing.⁶⁷

15.2 PAIN AND ITS PERCEPTION

Criteria for painless death can be established only after the mechanisms of pain are understood. The perception of pain is defined as a conscious experience.⁴⁷ The International Association for the Study of Pain (IASP) describes pain as “[a]n unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state, even though we may well appreciate that pain most often has a proximate physical cause.”⁷²

The perception of pain based on mammalian models requires nerve impulses from peripheral nociceptors to reach a functioning conscious cerebral cortex and the associated subcortical brain structures. Noxious stimulation that threatens to damage or destroy tissue produces activity in primary nociceptors and other sensory nerve endings. In addition to mechanical and thermal stimulation, a variety of endogenous substances can generate nociceptive impulses, including prostaglandins, hydrogen ions, potassium ions, substance P, purines, histamine, bradykinin, and leukotrienes, as can electrical currents.

Nociceptive impulses are conducted by nociceptor primary afferent fibers to either the spinal cord or the brainstem and 2 general sets of neural networks. Reflex withdrawal and flexion in response to nociceptive input are mediated at the spinal level while ascending nociceptive pathways carry impulses to the reticular formation, hypothalamus, thalamus, and cerebral cortex (somatosensory cortex and limbic system) for sensory processing and spatial localization. Thus, movement observed in response to nociception can be due to spinally mediated reflex activity, cerebral cortical and subcortical processing, or a combination of the two. For example, it is well recognized clinically that spinally mediated nociceptive

reflexes may remain intact distal to a compressive spinal lesion or complete spinal transection that blocks the ascending nociceptive pathways. In contrast, administration of a local anesthetic into the epidural space suppresses both spinally mediated nociceptive reflexes and ascending nociceptive pathways; in either case, noxious stimuli are not perceived as pain in conscious human or nonhuman animals because activity in the ascending pathways, and thus access to the higher cortical centers, is suppressed or blocked. It is therefore incorrect to substitute the term *pain* for stimuli, receptors, reflexes, or pathways because the term implies higher sensory processing associated with conscious perception. Consequently, the choice of a euthanasia agent or method is less critical if it is to be used on an animal that is anesthetized or unconscious, provided that the animal does not regain consciousness prior to death.

Pain is subjective in the sense that individuals can differ in their perceptions of pain intensity as well as in their physical and behavioral responses to it. Pain can be broadly categorized as sensory-discriminative, where the origin and the stimulus causing pain are determined, or as motivational-affective, where the severity of the stimulus is perceived and a response to it determined.⁷³ Sensory-discriminative nociceptive processing occurs within cortical and subcortical structures using mechanisms similar to those used to process other sensory-discriminatory input and provides information on stimulus intensity, duration, location, and quality. Motivational-affective processing involves the ascending reticular formation for behavioral and cortical arousal, as well as thalamic input to the forebrain and limbic system for perception of discomfort, fear, anxiety, and depression. Motivational-affective neural networks also provide strong inputs to the limbic system, hypothalamus, and autonomic nervous system for reflex activation of the cardiovascular, pulmonary, and pituitary-adrenal systems.

Although the perception of pain requires a conscious experience, defining consciousness, and therefore the ability to perceive pain, across many species is quite difficult. Previously it was thought that fish, amphibians, reptiles, and invertebrates lacked the anatomic structures necessary to perceive pain as we understand it in birds and mammals. For example, the invertebrate taxa include animals with no nervous system (eg, sponges) and nervous systems with no ganglionation or minimal ganglionation (eg, starfish). However, there are also invertebrate taxa with well-developed brains and/or complex behaviors that include the ability to analyze and respond to complex environmental cues (eg, octopus, cuttlefish, spiders,^{74,75} honeybees, butterflies, ants). Most invertebrates do respond to noxious stimuli and many have endogenous opioids.⁷⁶

Amphibians and reptiles also represent taxa with a diverse range of anatomic and physiologic characteristics such that it is often difficult to ascertain that an amphibian or reptile is, in fact, dead. Although

amphibians and reptiles respond to noxious stimuli and are presumed to feel pain, our understanding of their nociception and response to stimuli is incomplete. Nevertheless, there is increasing taxa-specific evidence of the efficacy of analgesics to minimize the impact of noxious stimuli on these species.^{77,78} Consequently, euthanasia techniques that result in “rapid loss of consciousness” and “minimize pain and distress” should be strived for, even where it is difficult to determine that these criteria have been met.

Compelling recent evidence indicates finfish possess the components of nociceptive processing systems similar to those found in terrestrial vertebrates,^{59-65,72-80} 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 fish responses to pain merely represent simple reflexes⁸¹ have been refuted by studies^{82,83} 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 fish cognition and sentience forward⁸⁴ 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 fish), 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 fishes', 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.

15.3 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.⁸⁵ The response of an animal to stress represents the adaptive process that is necessary to restore the baseline mental and physiologic state. These responses may involve changes in an animal's neuroendocrinologic

system, autonomic nervous system, and mental status that may result in overt behavioral changes. An animal's response varies according to its experience, age, species, breed, and current physiologic and psychological state, as well as handling, social environment, and other factors.^{86,87}

Stress and the resulting responses have been divided into 3 phases.⁸⁸ Eustress results when harmless stimuli initiate adaptive responses that are beneficial to the animal. Neutral stress results when the animal's response to stimuli causes neither harmful nor beneficial effects to the animal. Distress results when an animal's response to stimuli interferes with its well-being and comfort.⁸⁹ To avoid distress, veterinarians should strive to euthanize animals within the animals' physical and behavioral comfort zones (eg, preferred temperatures, natural habitat, home) and, when possible, prepare a calming environment.

15.4 ANIMAL BEHAVIOR

Although evaluations of euthanasia methods in the veterinary context are driven by science, clinical considerations and expectations from the public that high ethical standards will be observed may, in some cases, also play a role. When addressing euthanasia, veterinarians may disagree about what constitutes humane measures and a compassionate outcome for an animal or group of animals. This is reflective of the complexity or messiness of real-world situations veterinarians can sometimes find themselves in, where difficult decisions must be made involving euthanasia, and the multifaceted nature of animal welfare. In the latter case, conceptions of animal welfare are linked to varying normative approaches to how an animal is doing as described by different human assessors.^b Here, this disagreement may not necessarily involve disagreements about empirical information or clinical measures but instead may be due to a values-based disagreement about what constitutes good animal welfare⁹⁰ or how an animal may be harmed or distressed by a particular clinical option. So, while the core issue concerning euthanasia is how to bring about a good death for an animal, a disagreement may persist among veterinarians about how to weigh or weight various social and clinical trade-offs. For example, there may be disagreement over whether a quick death with some short-lived but acute distress, aversion, or suffering is preferable to one where the animal becomes unconscious over a longer period of time but does not demonstrate much behavioral aversion. More specifically, veterinarians in the laboratory context may debate which type of inhalant to use or its optimal flow rate to get rodents quicker to death or which can be anxiety producing and may not create a desired anesthetic state in the animal. Furthermore, depending on which conception of welfare is emphasized, behavioral aversion as an indicator of poor animal welfare may be viewed as problematic by some but not others if, for example, more weight is given to the intensity of negative states

experienced by an animal instead of the duration of exposure to a noxious agent. Measures designed to minimize pain or distress before animals become unconscious will likely achieve widespread support only if veterinarians are sensitive to the variety of conceptions of animal welfare and are willing to engage openly about how animals may be impacted by various alternatives. In the context of laboratory animals, for example, resolution of a disagreement in emphasis or interpretation regarding affective states, basic functioning, and evidence of frustration, anxiety, or fear will likely be influenced by programmatic policies and practices that have been identified by the institution's IACUC as ensuring high animal welfare standards.

The need to minimize animal distress, including negative affective or experientially based states like fear, aversion, anxiety, and apprehension, must be considered in determining the method of euthanasia. Ethologists and animal welfare scientists are getting better at discerning the nature and content of these states. Veterinarians and other personnel involved in performing euthanasia should familiarize themselves with pre-euthanasia protocols and be attentive to species and individual variability. For virtually all animals, being placed in a novel environment is stressful⁹¹⁻⁹⁴; therefore, a euthanasia approach that can be applied in familiar surroundings may help reduce stress.

For animals accustomed to human contact, gentle restraint (preferably in a familiar and safe environment), careful handling, and talking during euthanasia often have a calming effect and may also be effective coping strategies for personnel.⁹⁵ Sedation and/or anesthesia may assist in achieving the best conditions for euthanasia. It must be recognized that sedatives or anesthetics given at this stage that change circulation may delay the onset of the euthanasia agent.

Animals that are in social groups of conspecifics or that are wild, feral, injured, or already distressed from disease pose another challenge. For example, mammals and birds that are not used to being handled have higher corticosteroid levels during handling and restraint compared with animals accustomed to frequent handling by people.⁹⁶⁻⁹⁸ For example, beef cattle that are extensively raised on pasture or range have higher corticosteroid levels when restrained in a squeeze chute compared with intensively raised dairy cattle that are always in close association with people,^{99,100} and being placed in a new cage has been shown to be stressful for rodents.¹⁰¹ Because handling may be a stressor for animals less accustomed to human contact (eg, wildlife, feral species, zoo animals, and some laboratory animals), the methods of handling and degree of restraint (including none, such as for gunshot) required to perform euthanasia should be considered when evaluating various methods.⁸⁶ When handling such animals, calming may be accomplished by retaining them (as much as possible) in familiar environments, and by minimizing visual,

ness of their environment, and the same is likely true for animals. Indeed, humans experienced connected awareness of their environment during sedation with dexmedetomidine sufficient to lose responsiveness,¹⁴⁴ and a state of surgical anesthesia could not be produced even when xylazine was administered at 55 to 88 times the usual dose (0.1 mg/kg [0.05 mg/lb]) required to produce recumbency in cattle.^c Immobilizing, tranquilizing, or sedative agents should not be relied on to produce a truly unresponsive, disconnected unconscious state, regardless of the dose administered. Instead, an effective dose of a general anesthetic should be used when performing euthanasia with methods causing distress or noxious stimulation prior to loss of consciousness.

16 Mechanisms of Euthanasia

Euthanizing agents cause death by 3 basic mechanisms: (1) direct depression of neurons necessary for life function, (2) hypoxia, and (3) physical disruption of brain activity. The euthanasia process should minimize or eliminate pain, anxiety, and distress prior to loss of consciousness. As loss of consciousness resulting from these mechanisms can occur at different rates, the suitability of a particular agent or method will depend on whether an animal experiences distress prior to loss of consciousness.

Unconsciousness, defined as loss of individual awareness, occurs when the brain's ability to integrate information is blocked or disrupted (see comments on unconsciousness for additional information). Ideally, euthanasia methods should result in rapid loss of consciousness, followed by cardiac or respiratory arrest and the subsequent loss of brain function. Loss of consciousness should precede loss of muscle movement. Agents and methods that prevent movement through muscle paralysis, but that do not block or disrupt the cerebral cortex or equivalent structures (eg, succinylcholine, strychnine, curare, nicotine, potassium, or magnesium salts), are not acceptable as sole agents for euthanasia of vertebrates because they result in distress and conscious perception of pain prior to death. In contrast, magnesium salts are acceptable as the sole agent for euthanasia in many invertebrates due to the absence of evidence for cerebral activity in some members of these taxa,^{145,146} and there is evidence that the magnesium ion acts centrally in suppressing neural activity of cephalopods.¹⁴⁷

Depression of the cortical neural system causes loss of consciousness followed by death. Depending on the speed of onset of the particular agent or method used, release of inhibition of motor activity may be observed accompanied by vocalization and muscle contraction similar to that seen in the initial stages of anesthesia. Although distressing to observers, these responses do not appear to be purposeful. Once ataxia and loss of righting reflex occur, subsequent observed motor activity, such as convulsions, vocalization, and reflex struggling, can be attributed

to the second stage of anesthesia, which by definition lasts from the loss of consciousness to the onset of a regular breathing pattern.^{64,65}

Hypoxia is commonly achieved by exposing animals to high concentrations of gases that displace oxygen (O₂), such as carbon dioxide (CO₂), nitrogen (N₂), or argon (Ar), or by exposure to carbon monoxide (CO) to block uptake of O₂ by RBCs. Exsanguination, an adjunctive method, is another method of inducing hypoxia, albeit indirectly, and can be a way to ensure death in an already unconscious or moribund animal. As with other euthanasia methods, some animals may exhibit motor activity or convulsions following loss of consciousness due to hypoxia; however, this is reflex activity and is not consciously perceived by the animal. In addition, methods based on hypoxia will not be appropriate for species that are tolerant of prolonged periods of hypoxemia.

Physical disruption of brain activity can be produced through a blow to the skull resulting in concussive stunning; through direct destruction of the brain with a captive bolt, bullet, or pithing rod; or through depolarization of brain neurons following electrocution. Death quickly follows when the midbrain centers controlling respiration and cardiac activity fail. Convulsions and exaggerated muscle activity can follow loss of consciousness. Physical disruption methods are often followed by exsanguination. These methods are inexpensive, humane, and painless if performed properly, and leave no drug residues in the animal's remains. Furthermore, animals presumably experience less fear and anxiety with methods that require little preparatory handling. However, physical methods usually require a more direct association of the operator with the animals to be euthanized, which can be offensive to, and upsetting for, the operator. Physical methods must be skillfully executed to ensure a quick and humane death, because failure to do so can cause substantial suffering.

In summary, the cerebral cortex or equivalent structure(s) and associated subcortical structures must be functional for pain to be perceived. If the cerebral cortex is nonfunctional because of neuronal depression, hypoxia, or physical disruption, pain is not experienced. Reflex motor activity that may occur following loss of consciousness, although distressing to observers, is not perceived by the animal as pain or distress. Given that we are limited to applying euthanasia methods based on these 3 basic mechanisms, efforts should be directed toward educating individuals involved in the euthanasia process, achieving technical proficiency, and refining the application of existing methods.¹⁴⁸

17 Confirmation of Death

Death must be confirmed before disposal of any animal remains. A combination of criteria is most reliable in confirming death, including lack of pulse, breathing, corneal reflex, and response to firm toe pinch; inability to hear respiratory sounds and heart-

M2 Noninhaled Agents

M2.1 COMMON CONSIDERATIONS

Noninhaled agents of euthanasia include chemical agents that are introduced into the body by means other than through direct delivery to the respiratory tract. 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 recover. Therefore, as for any euthanasia method, death must be confirmed prior to final disposition of the animal's remains.

M2.1.1 COMPOUNDING

Products approved by the Center for Veterinary Medicine at the FDA should be used whenever feasible. When not feasible, euthanasia agents compounded in compliance with applicable guidance document(s) and compliance policy guide(s) in effect at the time of euthanasia should be used whenever feasible.¹⁵² 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 accord with local, state, and federal laws. Disposal of euthanized animals has become increasingly problematic because most rendering facilities will no longer take animals euthanized with agents that pose residue hazards (eg, barbiturates). The potential for ingestion of euthanasia agents is an important consideration in the euthanasia of animals that are disposed of in outdoor settings where scavenging by other animals is possible¹⁵³ or when euthanized animals are fed to zoo and exotic animals.¹⁵⁴ Veterinarians and laypersons have been fined for causing accidental deaths of endangered birds that ingested animal remains that were poorly buried.¹⁵⁵ Environmental warnings must now be included on animal euthanasia drugs approved by the FDA Center for Veterinary Medicine.¹⁵⁶

M2.2 ROUTES OF ADMINISTRATION

M2.2.1 PARENTERAL INJECTION

The use of injectable euthanasia agents is one of the most rapid and reliable methods of performing

euthanasia. It is usually the most desirable method when it can be performed without causing fear or distress in the animal. When appropriately administered, acceptable injectable euthanasia agents result in smooth loss of consciousness prior to cessation of cardiac and/or respiratory function, minimizing pain and distress to the animal. However, heightened awareness for personnel safety is imperative when using injectable euthanasia agents because needlestick injuries involving these drugs have been shown to result in adverse effects (41.6% of the time); 17% of these adverse effects were systemic and severe.¹⁵⁷

Intravenous injections deliver euthanasia agents directly into the vascular system, allowing for rapid distribution of the agent to the brain or neural centers, resulting in rapid loss of consciousness (for some invertebrates with closed circulatory systems, intrahemolymph injection is considered analogous to IV injection).¹⁵⁸ When the restraint necessary for giving an animal an IV injection is likely to impart added distress to the animal or pose undue risk to the operator, sedation, anesthesia, or an acceptable alternate route or method of administration should be used. Aggressive or fearful animals should be sedated prior to restraint for IV administration of the euthanasia agent. Paralytic immobilizing agents (eg, neuromuscular blocking agents) are unacceptable as a sole means of euthanasia, because animals under their influence remain awake and able to feel pain. Having said this, there may be select circumstances (eg, for wild or feral animals) where the administration of paralytic agents (eg, neuromuscular blocking agents) may be the most rapid and humane means of restraint prior to euthanasia due to their more rapid onset compared with other immobilizing agents.¹⁵⁹ In such situations, paralytic immobilizing agents may only be used if the chosen method of euthanasia (eg, captive bolt, IV injection of euthanasia solution) can be applied immediately following immobilization. Paralytic immobilizing agents must never be used as a sole means of euthanasia, nor should they be used if delay is expected between immobilization and euthanasia.

When intravascular administration is considered impractical or impossible, IP or intracoelomic administration of a nonirritating¹⁶⁰ barbiturate or other approved solution is acceptable. In laboratory rats, addition of lidocaine or bupivacaine to pentobarbital reduced abdominal writhing following intraperitoneal injection.¹⁶¹ Intracoelomic administration of buffered MS 222^a is acceptable for some poikilotherms. When injectable euthanasia agents are administered into the peritoneal or coelomic cavities, vertebrates may be slow to pass through stages I and II of anesthesia.¹⁶² Accordingly, they should be placed in small enclosures in quiet areas to minimize excitement and trauma. Intra-abdominal administration of euthanasia agents is an acceptable means of delivery in invertebrates with open circulatory systems.

In anesthetized mice, retrobulbar injection of no

tion. (2) Barbiturates induce euthanasia smoothly, with minimal discomfort for the animal. (3) Barbiturates are less expensive than many other euthanasia agents. (4) Food and Drug Administration–approved barbiturate-based euthanasia solutions are readily available.

Disadvantages—(1) Intravenous injection is necessary for best results and this requires trained personnel. (2) Each animal must be appropriately restrained. (3) Current federal drug regulations require strict accounting for barbiturates, and these must be used under the supervision of personnel registered with the US DEA. (4) An aesthetically objectionable terminal gasp may occur in unconscious animals. (5) Some animals may go through an excitatory phase that may be distressing to observers. (6) These drugs persist in the animal's remains and may cause sedation or even death of animals that consume the body. (7) Tissue artifacts (eg, splenomegaly) may occur in some species euthanized with barbiturates.

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), IM, 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 combine sodium pentobarbital with agents such as lidocaine or phenytoin are interchangeable with those of pure barbituric acid derivatives.

Mixing of pentobarbital with a neuromuscular blocking agent in the same injection apparatus is not an acceptable approach to euthanasia because of the potential for the neuromuscular blocking agent to induce paralysis prior to onset of unconsciousness.

M2.5 TRIBUTAME

Tributame euthanasia solution is an injectable, nonbarbiturate euthanasia agent with each milliliter containing 135 mg of embutramide, 45 mg of chloroquine phosphate USP, and 1.9 mg lidocaine USP dissolved in water and ethyl alcohol. The final formulation has a teal blue color with the bittering agent, denatonium benzoate, added to minimize the risk of the solution being ingested accidentally. Tributame was approved by the FDA in 2005 as an IV agent for euthanasia of dogs, and embutramide was classified as a schedule III controlled substance in 2006, making Tributame a C-III controlled agent.^{166–168}

Embutramide is a derivative of γ -hydroxybutyrate that was investigated as a general anesthetic in the late 1950s, but was never used as a pharmaceutical agent due to a poor margin of safety, with severe cardiovascular effects including hypotension, myocardial depression, and ventricular dysrhythmias.¹⁶⁹ Embutramide can be injected alone to cause death, but the time until death can exceed 5 minutes. Subsequently, chloroquine phosphate, an antimalarial drug with profound cardiovascular depressant effects, was added to embutramide, and studies verified a significantly shorter time until death.^{170,171} Studies on dogs showed this combination of 2 drugs to be effective, but when tested for euthanasia of cats, a substantial response to IV injection via peripheral vein was evident. This effect was almost completely eliminated by addition of lidocaine. The addition of chloroquine and lidocaine also lowers the dosage of embutramide required for euthanasia.¹⁷⁰ Death from Tributame results from severe CNS depression, hypoxia, and circulatory collapse.

Tributame produces unconsciousness in dogs in fewer than 30 seconds, with death occurring within 2 minutes; agonal breathing may occur in 60% to 70% of patients.¹⁷² Injection is to be given IV over a period of 10 to 15 seconds through a preplaced catheter or hypodermic needle at a dosage of 1 mL for each 5 lb (0.45 mL/kg [0.2 mL/lb]).

Advantages—(1) Tributame has a rapid onset of action. This effect depends on the dose, concentration, route, and rate of the injection. (2) Tributame induces euthanasia smoothly, with minimal discomfort to the animal. (3) Schedule III status makes Tributame somewhat simpler to obtain, store, and administer than Schedule II drugs such as sodium pentobarbital.

Disadvantages—(1) At the time of compilation of this report, while Tributame is FDA approved for use in dogs, it is not currently being manufactured. (2) Intravenous injection by trained personnel is necessary. (3) Each animal must be individually restrained. (4) Aesthetically objectionable agonal breathing may occur in unconscious animals. (5) The component drugs tend to persist in the animal's remains and may cause sedation or even death of animals that consume the body.

General recommendations—If it becomes available, Tributame is an acceptable euthanasia drug for

embryonic form and capable of experiencing distress or pain.²⁰⁹ Hypochlorite has also been used to terminate embryos in various research settings.

Advantages—(1) Sodium hypochlorite and calcium hypochlorite are inexpensive, are readily available, and, at the concentrations used for embryonic and larval stage destruction (1% to 10%), pose minimal hazards to personnel. (2) These products are not controlled substances.

Disadvantages—(1) Concentrated hypochlorite solutions are corrosive and pose risk of dermal, ocular, and respiratory injury to personnel if mishandled.

General recommendations—When used on early embryonic and larval stages prior to development of nociceptive abilities, application of hypochlorites is an acceptable means of euthanasia. Hypochlorites are unacceptable as the sole means of euthanasia of organisms beyond these embryonic and larval stages.

M2.19 FORMALDEHYDE

Formaldehyde causes cellular damage through oxidative injury as well as through formation of cross-linkages with DNA, RNA, and proteins.²¹⁰ Formaldehyde can be used to euthanize and preserve Porifera (sponges) as these invertebrates lack nervous tissue.

Advantages—(1) Formaldehyde is inexpensive, easily obtainable, and not a controlled substance. (2) Formaldehyde rapidly fixes tissues, preserving structure for later study.

Disadvantages—(1) Formaldehyde poses substantial health risks for personnel, including respiratory, dermal, and ocular irritation and hypersensitivity. Formaldehyde is also a known human carcinogen.²¹¹

General recommendations—Formaldehyde is an acceptable method of euthanasia for Porifera species. Formaldehyde is acceptable as an adjunctive method of euthanasia for Coelenterates (comb jellies, corals, anemones) and Gastropod molluscs (snails, slugs) only after these animals have been rendered nonresponsive by other methods (eg, magnesium chloride²¹²). Formaldehyde is unacceptable as a first step or adjunctive method of euthanasia for other animal species.

M2.20 LIDOCAINE HYDROCHLORIDE

Lidocaine hydrochloride is a local anesthetic that acts on ion channels of nerves, blocking the movement of sodium into the cell and resulting in failure of nerve conduction due to inability to generate action potentials.²¹³ Additional alteration of nerve transmission occurs due to lidocaine-induced inhibition of G-protein-coupled receptors and *N*-methyl-D-aspartate receptors.²¹⁴ Local anesthetics have occasionally been incorporated into IV barbiturate- or embutramide-based euthanasia solutions, primarily for their cardiodepressant effects.

Advantages—(1) Lidocaine is inexpensive, widely available, and not a controlled substance. (2) Lidocaine causes relatively rapid loss of cerebrocortical

function (brain death) when administered intrathecally to anesthetized animals. (3) Lidocaine leaves relatively low tissue residues and is not expected to pose hazards to scavenging animals that might feed on the carcass.²¹⁴

Disadvantages—(1) Anesthesia and intrathecal administration require technical expertise to perform. (2) Risk to scavenging animals from anesthetic drug residues must be considered. (3) Reflexive (agonal) breathing occurred occasionally after loss of brain electrical activity. (4) Exposure of personnel to encephalitic diseases (eg, rabies) from CSF removed from animals with unknown illness is possible.

Recommendations—Intrathecal 2% lidocaine hydrochloride is an acceptable secondary method of euthanasia in animals under anesthesia in situations where other euthanasia methods are unavailable or cost prohibitive or where proper carcass disposal cannot be assured.

M2.21 UNACCEPTABLE AGENTS

Strychnine, nicotine, insulin, caffeine, cleaning agents, solvents, pesticides, disinfectants, and other toxicants not specifically designed for therapeutic or euthanasia use are unacceptable for use as euthanasia agents under any circumstances.

Magnesium sulfate, potassium chloride, and neuromuscular blocking agents are unacceptable for use as euthanasia agents in conscious vertebrate animals. These agents may be used for euthanasia of anesthetized or unconscious animals as previously described.

M3 Physical Methods

M3.1 COMMON CONSIDERATIONS

Physical methods of euthanasia include captive bolt, gunshot, cervical dislocation, decapitation, electrocution, focused beam microwave irradiation, exsanguination, maceration, stunning, and pithing. When properly used by skilled personnel with well-maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane, and practical than other forms of euthanasia. Exsanguination, stunning, and pithing are not recommended as a sole means of euthanasia, but may be considered as adjuncts to other agents or methods.

Some consider physical methods of euthanasia aesthetically displeasing. There are occasions, however, when what is perceived as aesthetic and what is most humane are in conflict. Despite their aesthetic challenges, in certain situations physical methods may be the most appropriate choice for euthanasia and rapid relief of pain and suffering. Personnel using physical methods of euthanasia must be well trained and monitored for each type of physical method performed to ensure euthanasia is conducted appropriately. They must also be sensitive to the aesthetic implications of the method and convey to onlookers what they should expect to observe when at all possible.

Part III—Methods of Euthanasia by Species and Environment

SI Companion Animals

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

SI.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.¹ Factors leading to the decision to euthanize should be discussed openly,² 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.^{1,3} 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.⁴

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.⁵ 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.⁶ 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 2 people to move around freely in different types of animal-handling situations.^{6,7} 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.

SI.2 ACCEPTABLE METHODS

SI.2.1 NONINHALED AGENTS

Barbiturates and barbituric acid derivatives—Intravenous injection of a barbituric acid derivative (eg, pentobarbital, pentobarbital combination product) is the preferred method for euthanasia of dogs, cats, and other small companion animals. Barbiturates administered IV may be given alone as the sole agent of euthanasia or as the second step after sedation or general anesthesia. Refer to the product label or appropriate species references⁸ for recommended doses. Current federal drug regulations require strict accounting for barbiturates, and these must be used under the supervision of personnel registered with the US DEA.

When IV access would be distressful, dangerous, or impractical (eg, small patient size such as puppies, kittens, small dogs and cats, rodents, and some other nondomestic species or behavioral considerations for some small exotic mammals and feral domestic animals), barbiturates and barbituric acid derivatives may be administered IP (eg, sodium pentobarbital, secobarbital; not pentobarbital combination products as these have only been approved for IV and intracardiac administration). Because of the potential for peritoneal irritation and pain (observed in rats),⁹ lidocaine has been used with some success in rats to ameliorate discomfort.^{10,11} Lidocaine was also used in combination with sodium pentobarbital in a laboratory comparison of IP and intrahepatic injection routes in cats from animal shelters.¹² Additional studies are necessary to determine applicability to and dosing for other species.

Nonbarbiturate anesthetic overdose—Injectable anesthetic overdose (eg, combination of ketamine and xylazine given IV, IP, or IM or propofol given IV) is acceptable for euthanasia when animal size, restraint requirements, or other circumstances indicate these drugs are the best option for euthanasia. Assurance of death is paramount and may require a second step, such as a barbiturate, or additional doses of the anesthetic. For additional information see Section M2, Noninhaled Agents, and Section S2, Laboratory Animals.

Tributame—While it is not currently being manufactured, Tributame is an acceptable euthanasia drug for dogs provided it is administered IV by an appropriately trained individual at recommended dosages and at proper injection rates. If barbiturates are not available, its extralabel use in cats is also acceptable. Routes of administration other than IV injection are not acceptable. Aesthetically objectionable agonal breathing may occur in unconscious animals and, consequently, the use of Tributame for owner-attended euthanasia is not recommended. While disconcerting for observers, because the animal is unconscious, agonal breathing has limited impact on its welfare.

T-61—T-61 is acceptable as an agent of euthanasia, provided it is administered appropriately by trained

individuals. Slow IV injection is necessary to avoid muscular paralysis prior to unconsciousness.¹³ Routes other than IV are unacceptable. T-61 is also not currently being manufactured in the United States but is obtainable from Canada.

Should sodium pentobarbital become unavailable and manufacturing resume in the United States for Tributame and T-61, the latter 2 agents may become important for euthanasia of dogs and cats.

SI.3 ACCEPTABLE WITH CONDITIONS METHODS

SI.3.1 NONINHALED AGENTS

Barbiturates and barbituric acid derivatives (alternate routes of administration)—The IP route is not practical for medium or large dogs due to the volume of agent that must be administered and a prolonged time to death. A better choice for these animals when IV access is unachievable using manual restraint is general anesthesia followed by intraorgan injection. In unconscious or anesthetized animals, intraorgan injections (eg, intraosseous [Figure 4], intracardiac [Figure 5], intrahepatic [Figure 6], and intrarenal [Figure 7]^{14,15,a}) may be used as an alternative to IV or IP injection of barbiturates when IV access is difficult.¹⁵ Intraorgan 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.¹⁶ The intrahepatic injection of a combination of sodium pentobarbital and lidocaine in awake cats from animal shelters caused rapid unconsciousness and was more accurately placed than IP injections.¹² Therefore, intrahepatic injection in awake cats may have limited application in controlled environments when conducted by trained personnel. However, positioning of awake cats for intrahepatic injection is in an upright position with the forequarters elevated rather than in lateral recumbency.

SI.3.2 INHALED AGENTS

Inhaled anesthetics—Overdoses of inhaled anesthetics administered via chamber (eg, isoflurane, sevoflurane) are acceptable with conditions for euthanasia of small mammals and some other species < 7 kg because most vertebrates display aversion behavior to inhaled anesthetics (see Inhaled Agents section for details). Because of the potential for recovery, care must be taken to ensure death has occurred prior to disposing of animal remains. Inhaled anesthetics may also be used to anesthetize small fractious animals prior to administration of an injectable euthanasia agent.

Carbon monoxide—Carbon monoxide can be used effectively for euthanasia when required conditions for administration (see detailed discussion in Inhaled Agents section of the Guidelines) can be met. These conditions can be challenging and costly to meet on a practical basis, and there is substantial risk to personnel (hypoxia) if safety precautions are

not observed. Consequently, CO is acceptable with conditions for use in institutional situations where appropriately designed and maintained equipment and trained and monitored personnel are available to administer it, but it is not recommended for routine euthanasia of cats and dogs. It may be considered in unusual or rare circumstances, such as natural disasters and large-scale disease outbreaks. Alternate methods with fewer conditions and disadvantages are recommended for companion animals where feasible.

Carbon dioxide—Carbon dioxide can be used effectively for euthanasia when required conditions for administration (see detailed discussion in Inhaled Agents section of the Guidelines) can be met. However, just as for use of CO, this can be challenging and costly to do on a practical basis. Narcosis is a human safety risk associated with the use of CO₂. Carbon dioxide is acceptable with conditions for use in institutional situations where appropriately designed and maintained equipment and trained and monitored personnel are available to administer it, but it is not recommended for routine euthanasia of cats and dogs. It may be considered in unusual or rare circumstances, including but not limited to, natural disasters and large-scale disease outbreaks. Alternate methods with fewer conditions and disadvantages are recommended for companion animals where feasible.

SI.3.3 PHYSICAL METHODS

Gunshot—Gunshot should only be performed by highly skilled personnel trained in the use of firearms (eg, animal control and law enforcement officers, properly trained veterinarians) and only in jurisdictions that allow for legal firearm use. A method acceptable with conditions, use of gunshot may be appropriate in remote areas or emergency situations in which withholding death by gunshot will result in prolonged, unrelieved pain and suffering of the animal or imminent danger to human life. Protocols for ensuring a humane death by gunshot have been described^{17,18} and preferred anatomic sites for use of gunshot for dogs and cats are provided in **Figures 8 and 9**, respectively. Pre-euthanasia sedation (eg, medication added to food) is recommended, whenever possible, for cats since they may be difficult to shoot humanely.¹⁷ Gunshot 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.

PCB—Use of a PCB by trained personnel in a controlled laboratory setting has been described as an effective and humane method of euthanasia for rabbits and dogs.¹⁹ 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.

SI.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 N₂ 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 N₂ or Ar (< 2% O₂) 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.

SI.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.

SI.6 SPECIAL CONSIDERATIONS

SI.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,²⁰ liquid formulations squirted into mouths²¹) 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.²²

SI.6.2 DISPOSAL OF ANIMAL REMAINS

Residues of injectable agents commonly used for euthanasia of companion animals (eg, sodium pento-

barbital) 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.

SI.7 FETUSES AND NEONATES

Scientific data²³ 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 pregnancy and remain unconscious until after birth due to chemical inhibitors (eg, adenosine, allopregnanolone, pregnanolone, prostaglandin D₂, placental peptide neuroinhibitor) and hypoxic inhibition of cerebrocortical activity.²³ 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.”²³ Information about developing nonmammalian eggs is available in the S5, Avians; S6, Finfish and Aquatic Invertebrates; and S7, Zoologic 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 Zoologic 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 ovariectomy 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,²⁴ 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).

SI.8 EUTHANASIA IN SPECIFIC ENVIRONMENTS

SI.8.1 INDIVIDUAL ANIMALS IN PRESENCE OF OWNERS

Pre-euthanasia sedation or anesthesia should be provided whenever practicable, either before or after the owner(s) has had the opportunity to spend some final moments with their pet. Once the animal is calm, either direct venipuncture or use of an IV catheter is acceptable for IV injection of the euthanizing agent. Use of an IV catheter prevents repeat injections and minimizes the need for restraint while pet owners are present. When circulation is compromised by the animal's condition and sedation or anesthesia may reduce the likelihood of successful injection, it may be necessary to proceed with IV injection in the awake animal, or another route of administration of euthanizing agent might be considered. Alternatively, general anesthesia may be induced, followed by administration of a euthanasia agent.

SI.8.2 BREEDING FACILITIES

Euthanasia protocols in large breeding facilities may differ from those utilized in a clinical practice setting. Indications for euthanasia in breeding facilities include neonates with congenital defects, acquired abnormalities or diseases within any segment of the population, or other conditions that render animals unsuitable for breeding or sale. Euthanasia may be performed on an individual-animal basis, or in groups. Euthanasia method is determined by animal species, size, age, and number of animals to be euthanized. Barbiturates are commonly administered IV or IP for individual euthanasia of any species, and for all ages of dogs and cats. Carbon dioxide euthanasia is commonly utilized for individual or group euthanasia of small animals, including ferrets, rodents, and rabbits. Regardless of method and number of animals being euthanized, procedures must be performed in a professional, compassionate manner by trained individuals under veterinary oversight. Appropriate techniques for assuring death must be applied individually, regardless of the number of animals being euthanized.

SI.8.3 ANIMAL CONTROL, SHELTERING, AND RESCUE FACILITIES

The preferred method of euthanasia in these facilities is injection of a barbiturate or barbituric acid

raised to meet niche market demands for fiber and food, are also included.

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 is monitored.¹¹⁷⁻¹²¹

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 heartbeat) or the presence of rigor mortis.

S3.2 BOVIDS AND SMALL RUMINANTS

S3.2.1 BOVIDS

S3.2.1.1 Acceptable methods

S3.2.1.1.1 Noninhaled agents

Barbiturates and barbituric acid derivatives—

Barbiturates act rapidly and normally induce a smooth transition from consciousness to unconsciousness and death—a desirable outcome for the operator and observers. Although cost is a deterrent to the use of barbiturates for euthanasia of large numbers of animals, these agents tend to be less expensive than other injectable pharmaceuticals. Drawbacks to the use of barbiturates are that their administration requires adequate restraint of the animal, personnel who are registered with the US DEA (and other appropriate state authority where required), strict control over the drug with accounting of the amount used,¹²² and fewer options for disposal of animal remains because of potential residues.

S3.2.1.2 Acceptable with conditions methods

S3.2.1.2.1 Physical methods

In emergency situations, such as euthanasia of a bovid that is not restrained, it may be difficult to restrain a dangerous animal for IV injection. While administration of a sedative might be desirable, in some situations it is possible the animal 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 bovid IM or IV, but the bovid must be euthanized via an appropriate method as soon as the bovid can be controlled. Succinylcholine alone or without sufficient anesthetic is not acceptable for euthanasia.

Gunshot—Gunshot is the most common method used for on-farm euthanasia of cattle.¹²³ Death is caused by destruction of brain tissue and the degree of brain damage inflicted by the bullet is dependent on the firearm, type of bullet (or shotshell for shotguns), and accuracy of aim.

Handguns—Handguns or pistols are short-barreled firearms that may be fired with 1 hand. For euthanasia, use of handguns is limited to close-range

shooting (within 1 to 2 feet or 30 to 60 cm) of the intended target. Calibers ranging from .32 to .45 are recommended for euthanasia of cattle.¹²⁴ Bullets should be selected to have adequate penetration ability. Older types of hollow-point bullets are designed to expand and fragment on impact with their targets, which reduces the depth of penetration. Under ideal conditions and good penetration of the skull, hollow-point bullets are able to cause extensive damage to neural tissues; however, because penetration of the skull is the first criterion in euthanasia, a solid lead bullet is preferred. Since the publication of the previous edition, many new types of bullets and firearms are now available. These must be of sufficient muzzle velocity to ensure penetration. The muzzle velocity specifications are on most ammunition packaging. The .22 caliber handgun is generally not recommended for routine euthanasia of adult cattle regardless of bullet used, because of the inability to consistently achieve desirable muzzle energies with standard commercial loads.¹²⁴

Rifles—A rifle is a long-barreled firearm that is usually fired from the shoulder. Unlike the barrel of a shotgun, which has a smooth bore for shot shells, the bores of handgun and rifles contain a series of helical grooves (called rifling) that cause the bullet to spin as it travels through the barrel. Rifling imparts stability to the bullet and improves accuracy. For this reason, rifles are the preferred firearm for euthanasia when it is necessary to shoot from a distance. Another reason a rifle is preferred is that a longer barrel may improve bullet performance.

Rifles are capable of delivering bullets at much higher muzzle velocities and energies and thus are not the ideal choice for euthanasia of animals in indoor or short-range conditions. General recommendations on rifle selection for use in euthanasia of cattle include .22, .223, .243, .270, .308, and others.¹²⁴⁻¹²⁶ Results of at least 1 study¹²⁶ suggest that the .22 LR may not be the best selection of a firearm for euthanasia of adult cattle because of poor penetration, deflection, and fragmentation of the bullet. Standard- and high-velocity bullets fired from a .22 caliber rifle at a range of 25 m failed to penetrate skulls of steers and heifers studied. On the other hand, the .223 and .30-06 performed satisfactorily (eg, traversed the skull and caused sufficient brain damage to cause death) when fired from a distance of 25 m.¹²⁶ This is in agreement with similar information indicating that .22 Magnum or larger-caliber firearms provide higher muzzle energies and more consistent results when delivered to the proper anatomic site.¹²⁵

When the most appropriate firearm is being chosen for the purpose of euthanasia, there are several factors to be considered, including caliber of the firearm, type of bullet or shotshell, distance from the target, age of the animal (aged animals have harder skulls), sex of the animal (bull or cow), and accuracy of aim. Based upon available information, if a .22 LR is to be used the following conditions apply: 1) the fire-

sia, it is acceptable to exsanguinate birds that are fully anesthetized or otherwise unconscious as a means to ensure death. Biosecurity precautions during and following exsanguination should be observed as part of appropriate disease response.

S3.4.4 EMBRYOS AND NEONATES

In addition to methods involving inhaled agents mentioned previously, the following methods are acceptable with conditions for euthanasia of embryos or neonates.

Embryonated eggs may be destroyed by prolonged exposure (20 minutes) to CO₂ or before 80% of incubation, cooling (4 hours at 40°F), or freezing.⁹⁵ In some cases inhaled anesthetics can be administered through the air cell at the large end of the egg. Egg addling can also be used.²⁴⁴ Embryos that have been exposed can be decapitated.

Maceration, via use of a specially designed mechanical apparatus having rotating blades or projections, causes immediate fragmentation and death of newly hatched poultry and embryonated eggs.²²¹ A review by the American Association of Avian Pathologists²⁴⁵ of the use of commercially available macerators for euthanasia of chicks, poults, and pipped eggs indicates that death by maceration in poultry up to 72 hours old occurs immediately with minimal pain and distress. Maceration is an alternative to the use of CO₂ for euthanasia of poultry up to 72 hours old. Maceration is believed to be equivalent to cervical dislocation and cranial compression as to time element, and is considered to be an acceptable means of euthanasia for newly hatched poultry by the Federation of Animal Science Societies,²⁴⁶ Agriculture Canada,²⁴⁷ World Organization for Animal Health,¹³⁶ and European Council.²⁴⁸

Maceration requires special equipment that must be kept in excellent working order. Newly hatched poultry must be delivered to the macerator in a way and at a rate that prevents a backlog at the point of entry into the macerator and without causing injury, suffocation, or avoidable distress before maceration.

S4 Equids

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

S4.1 GENERAL CONSIDERATIONS

S4.1.1 HUMAN SAFETY

When equids are euthanized, consideration should be given to the unpredictability of a falling or thrashing equid. Most methods of euthanasia will result in some degree of exaggerated muscular activity after the equid falls even if the equid is not experiencing pain or distress. Whatever euthanasia method is used should not put personnel at unnecessary risk.

S4.1.2 DISPOSAL OF REMAINS

For equids euthanized with pentobarbital, disposal of remains must be carried out promptly through on-farm burial, incineration or cremation, direct haul to a solid waste landfill, or biodigestion. This will help prevent exposure of wildlife and domestic animals to potentially toxic barbiturate residues. Disposal of remains must be conducted in accord with all federal, state, and local regulations.

S4.2 METHODS

S4.2.1 ACCEPTABLE METHODS

S4.2.1.1 Noninhaled agents

Barbiturates or barbituric acid derivatives—

Pentobarbital or a pentobarbital combination is the principal choice for equine euthanasia by chemical means. Because a large volume of solution must be injected, use of an IV catheter placed in the jugular vein will facilitate the procedure. To facilitate catheterization of an excitable or fractious equid, a tranquilizer, such as acepromazine, or an α_2 -adrenergic receptor agonist can be administered, but these drugs may prolong time to loss of consciousness because of their effect on circulation and may result in varying degrees of muscular activity and agonal gasping. Opioid agonists or agonist-antagonists in conjunction with α_2 -adrenergic receptor agonists may further facilitate restraint.

S4.2.2 ACCEPTABLE WITH CONDITIONS METHODS

S4.2.2.1 Physical methods

PCB and gunshot—

Penetrating captive bolt and gunshot are considered acceptable with conditions for euthanasia of equids. Both should only be used by well-trained personnel who are regularly monitored to ensure proficiency, and firearms must be well maintained. Appropriate restraint is required for application of the PCB and special care should be taken to ensure that personnel are not injured by ricochet from free bullets.

The correct anatomic site for application of gunshot and PCB is illustrated in **Figure 23**.²⁴⁹ The site for entry of the projectile is described as being on the intersection of 2 diagonal lines each running from the outer corner of the eye to the base of the opposite ear.

S4.2.3 ADJUNCTIVE METHODS

Recently, rendering plants and landfills have refused equine carcasses euthanized with pentobarbital. For this reason, adjunctive methods should be considered. Anesthetizing the equid with xylazine-ketamine should be followed by one of the following: (1) saturated solution of potassium chloride injected IV or intracardially; (2) saturated solution of magnesium sulfate injected IV; or (3) 60 mL of 2% lidocaine injected intrathetically.²⁵⁰ Each of these performed in an equid in a deep surgical plane of anesthesia is an acceptable method to invoke cardiac arrest and death.

Intrathecal administration of 2% lidocaine hydrochloride to anesthetized horses resulted in sequential loss of respiration, loss of cerebrocortical activity, loss of brainstem function, and loss of cardiovascular activity, with loss of cerebrocortical activity occurring within 3.38 minutes after intrathecal lidocaine administration.²¹⁴ Heart sounds persisted for up to 10 minutes, and ECG activity lasted up to 21 minutes, long after all brain activity had ceased. Tissues from horses euthanized via intrathecal lidocaine administration contained drug residues considered well below concentrations expected to pose hazards to scavenging animals.²¹³

S4.2.4 UNACCEPTABLE METHODS

Chloral hydrate—Chloral hydrate has an almost immediate sedative action, but unless it is combined with other anesthetics, onset of anesthesia is delayed. Associated adverse effects can be severe and aesthetically objectionable, and chloral hydrate also has limited 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 Reptiles, Zoo Animals, and Wildlife 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 discussions and editorials.^{224,251–256,c} There are scientific studies^{233,235,238,257–259} comparing various

methods for depopulation of commercial poultry, but these methods may or may not meet the criteria for euthanasia, and may or may not be applicable to individual birds or small groups of birds.

Because this taxon comprises more than 8,000 species, the choice of euthanasia method for a particular bird will depend greatly on its species, size, anatomic and physiologic characteristics, environment, degree of domestication, clinical state, and anticipated and actual response to restraint. Personnel performing euthanasia should be familiar with the species being euthanized, be able to interpret avian behavior indicative of stress, and use their knowledge and experience to choose restraint and euthanasia options that alleviate or minimize distress and result in rapid death. Legal requirements may apply in cases involving endangered or migratory species.

S5.1.1 ANATOMY AND PHYSIOLOGY

Birds differ anatomically and physiologically from mammals and these differences will affect whether and how particular euthanasia methods may be acceptably applied. Because birds lack a diaphragm, they have a single coelomic cavity, rather than separate thoracic and abdominal cavities. When giving intracoelomic injections care must be taken that material is not injected into the air sacs, which could potentially drown the bird or expose its respiratory system to irritating substances. Air sacs act as a bellows to ventilate birds' small, nonexpanding lungs.²⁶⁰ Because there is no diaphragm, birds need to be able to move their sternum ventrally and cranially to breathe.²⁶¹ Birds also have hollow, pneumatic bones, such as the humerus and femur, which communicate directly with the respiratory system. Pre-euthanasia and euthanasia drugs should not be administered via the intraosseous route into the humerus or femur because drowning or irritation to the respiratory system may occur. Intraosseous catheters can, however, be safely placed in birds, preferably in the distal ulna or proximal tibiotarsus.

A bird's respiratory system has greater capacity to process air than a mammal's due to a unique unidirectional flow of air through the lungs (which prevents mixing of inspired and expired air), more efficient gas exchange, and a greater surface area over which O₂ can be exchanged (more and smaller air capillaries [3 µm] than the smallest mammalian alveoli [35 µm]).²⁶¹ Because of their greater capacity to process air, birds are more sensitive than mammals to inspired toxicants (eg, the proverbial canary in the coal mine collapsing before humans detect the methane in the air).²⁶²

S5.1.2 RESTRAINT

Manual restraint for administering pre-euthanasia or euthanasia drugs is possible for many bird species. Nets or other equipment may be required or may improve conditions for both birds and people when handling birds less acclimated to human con-

thanasia procedure. Pithing requires detailed anatomic knowledge of the species in question. These methods are not acceptable, however, as a single-step procedure, nor as the first step of a 2-step procedure.

S6.3.3 LIFE STAGE CONSIDERATIONS

The effectiveness of euthanasia methods described in the Guidelines may vary depending on life stage and species. As for fish, this should be considered when euthanizing aquatic invertebrates. Methods used for different life stages of the same species may require modification to maximize their effectiveness. Recommendations regarding use of adjunctive methods (as described previously) may also be necessary to guarantee death.

S6.3.4 UNACCEPTABLE METHODS

Methods of killing that do not cause rapid death or that cause trauma prior to loss of consciousness are not considered humane methods of death, or euthanasia.

These can include removing a fish or aquatic invertebrate from the water and allowing it to die by hypoxia secondary to desiccation of gill tissue; leaving fish or aquatic invertebrates in a container of water without adequate aeration, causing death by anoxia; or any death due to exposure to caustic chemicals or traumatic injury without first inducing unconsciousness in the fish or aquatic invertebrate.

S7 Zoologic and Free-Ranging Nondomestic Animals

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

S7.1 GENERAL CONSIDERATIONS

The nondomestic captive and free-ranging animals discussed in the following sections vary substantially in their anatomic and physiologic characteristics, native environment, behavior, social structure, responses to humans, and other traits. These variations challenge the application and effectiveness of euthanasia methods for the many different species. The efficacy of these methods can be further limited by the circumstances under which euthanasia is performed. Consequently, the best means of terminating an animal's life might not strictly conform to the definition of euthanasia. For nondomestic captive or free-ranging animals, the methods selected will often be situation specific, as a means of minimizing potential risks to the animal's welfare and personnel safety. In addition, challenges associated with disposal of the remains of animals with drug residues that have been addressed in the section of the document on Disposal of Remains (eg, secondary toxicosis, environmental contamination, and other topics) are relevant to disposal of the remains of nondomestic animals, particularly under field conditions. Given the

complexity of issues that euthanasia of nondomestic animals presents, personnel are encouraged to consult references on anatomy, physiology, natural history, husbandry, and other disciplines that will aid in understanding how various methods may impact an animal's euthanasia experience.^{95,271,336-338} Consultation with experienced colleagues is recommended, particularly when novel circumstances and/or species are encountered.

Animals may become distressed due to physical discomfort, anxiety in atypical social settings and physical surroundings, pheromones or odors from nearby or previously euthanized animals, and the presence of humans. In addition, human safety, observers' perceptions, availability of trained personnel, potential infectious disease concerns, conservation and other population objectives, regulatory oversight that may be species specific, available equipment and facilities, options for disposal, potential secondary toxicity, research objectives, and other factors must be considered. Human safety is of utmost importance for all euthanasia procedures, and appropriate protocols and equipment (including supplies for addressing human injury due to animal handling or exposure to immobilizing drugs) must be available prior to handling animals.³³⁹ Laws and regulations pertaining to the species being euthanized, the euthanasia methods employed, and disposal of the remains must be followed.

Euthanasia of captive wild animals requires consideration of basic stewardship, physiologic and behavioral variation, and relief from pain and anxiety. Management can be guided by the physical and social setting the animal is in (eg, small enclosures, seminatural conditions), the animal's temperament, seasonal factors (eg, reproductive stage, physical condition, age and size), and differences from similar domestic species. Appropriate handling and modifying the animal's physical and social environment to minimize distress, as well as administration of anxiolytics, are recommended. Provision of preferred bedding, temperature, humidity, and security in the period leading up to euthanasia will allow the animal to be as comfortable as possible. Most small animals will find security in a dimly lighted, appropriately bedded and ventilated crate, box, tube, or similar container as this simulates a natural tendency to hide from perceived threats. Some species respond well to being left within typical social groups or familiar surroundings as long as possible prior to euthanasia to minimize anxiety.

Best practice for many captive wild animal species includes a multistep approach, beginning with administration of a sedative or anesthetic to relieve anxiety and pain. For wild animals in captivity, physical and/or chemical restraint is usually required before euthanasia can be performed. Physical restraint is appropriate when skilled staff, facilities, suitable equipment, and the animal's characteristics allow rapid immobilization with minimal distress.³³⁹ Refer-

debilitated, sedated, or anesthetized. Use of inhaled agents may be appropriate for small pinnipeds after administration of an injectable sedative or anesthetic under circumstances where acceptable methods are not practical or appropriate for other reasons.

Inhaled agents present some advantages in that they do not require phlebotomy skills and may present minimal concern for tissue residues.³⁸³ Disadvantages include that they are expensive, require an extended delivery time with associated risks of distress and injury for animals and personnel, and may be noxious to the animal.

S7.5.2.2 Physical methods

Physical methods, although used to euthanize free-ranging marine mammals, will generally not be used on captive mammals due to limited efficacy for these species, risk for personnel, and aesthetics.

S7.6 FREE-RANGING WILDLIFE

S7.6.1 GENERAL CONSIDERATIONS

Free-ranging wildlife are present in all habitats across North America including fresh and salt water. Wildlife includes representatives of all known animal taxa, but for the purpose of the Guidelines, will be restricted to amphibians, reptiles, birds, and mammals, including some feral and exotic species. Wildlife are enjoyed and used by people in a number of ways including nonconsumptive uses (wildlife viewing, bird watching, bird feeding) and legal harvest (hunting, fishing, commercial take). Varied interests and perspectives can influence what methods are used to terminate the lives of free-ranging wildlife.³⁸⁴ This section of the Guidelines updates and expands upon previous editions by recognizing an inherent lack of control over free-ranging wildlife, accepting that firearms may be the most appropriate approach to their euthanasia, and acknowledging that the quickest and most humane means of terminating the life of free-ranging wildlife in a given situation may not always meet all criteria established for euthanasia (ie, distinguishes between euthanasia and methods that are more accurately characterized as humane killing).

Because of the variety of situations that may be encountered, it is difficult to strictly classify methods for termination of free-ranging wildlife as acceptable, acceptable with conditions, or unacceptable. Furthermore, classification of a given method as a means of euthanasia or humane killing may vary by circumstances. These acknowledgments are not intended to condone a lower standard for the humane termination of wildlife. The best methods possible under the circumstances must be applied, and new technology and methods demonstrated to be superior to previously used methods must be embraced.

Multiple federal, state, and local regulations apply to the euthanasia of wildlife. In the United States, management of wildlife is primarily under state jurisdiction. However, some species (eg, migratory birds, endangered species, marine mammals) are protected and managed by federal agencies or through collabo-

ration between state and federal agencies. Within the context of wildlife management, personnel associated with state and federal agencies and Native American tribes may handle or capture individual animals or groups of animals for various purposes, including research. During the course of these management actions, individual animals may become injured or debilitated and may require euthanasia; in other cases, research or collection protocols dictate that some of them be killed. Sometimes population management requires the lethal control of wildlife species. And, the public may identify and/or present individual animals to state or federal personnel because they are orphaned, sick, injured, diseased (eg, rabid), or becoming a nuisance. Another aspect of wildlife management is rehabilitation of orphaned or injured wildlife. For the most part, wildlife rehabilitation is done by private citizens and requirements for handling these animals vary by state and species.

S7.6.2 SPECIAL CONSIDERATIONS

The primary factor influencing methods selected for euthanasia of free-ranging wildlife is lack of control over the animal. In addition, some species may be too large to effectively euthanize by conventional means. Marine mammals are of particular concern due to their large size and the lack of standardized equipment and techniques (see Free-Ranging Marine Mammals for more information). Other species, such as reptiles, may be refractory to conventional euthanasia agents. The potential for secondary toxicity and environmental hazards associated with the remains of animals euthanized by chemical means are of substantial concern, as is disposal of large or numerous animal remains. Therefore, while some methods described in the taxonomically based sections for non-domestic animals may be useful for euthanizing free-ranging wildlife, their applicability will vary.

Given that close human contact is stressful and difficult to achieve for most free-ranging animals, these animals may have to be euthanized or immobilized from a distance. In some cases (eg, suburban areas), discharge of a firearm is illegal, is considered a serious threat to human safety, or may be inappropriate for other reasons. Consequently, free-ranging animals may need to be killed quickly and efficiently in ways that may not fulfill the criteria for euthanasia established by the POE.

Remotely delivered chemical immobilization may be required when wildlife cannot be captured. If a free-ranging animal is within an acceptable range, trained individuals may use species and situation-specific anesthetic agents and remote injection equipment to anesthetize that animal to allow handling. Once anesthetized, many wildlife species can be euthanized via methods similar to those applied to domestic or captive wild animals of similar species and size. Other techniques used in wildlife management for trapping or capturing animals may also be applied to allow some degree of control over the animal.