# 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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**ABBREVIATIONS**

| | |
|---|---|
| ASIC | Acid-sensing ion channel |
| CAS | Controlled atmospheric stunning |
| DEA | Drug Enforcement Agency |
| EEG | Electroencephalogram *or* electroencephalographic |
| EPA | Environmental Protection Agency |
| HPA | Hypothalamic-pituitary axis |
| IACUC | Institutional animal care and use committee |
| MS 222 | Tricaine methanesulfonate |
| NPCB | Nonpenetrating captive bolt |
| PCB | Penetrating captive bolt |
| POE | Panel on Euthanasia |
| SNS | Sympathetic nervous system |

# Part I—Introduction and General Comments

## I1 Preface

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

In creating the 2020 and 2013 edition of the AVMA Guidelines for the Euthanasia of Animals (Guidelines), the POE made every effort to identify and apply the best research and empirical information available. As new research is conducted and more practical experience gained, recommended methods of euthanasia may change. As such, the AVMA and its POE have made a commitment to ensure the Guidelines reflect an expectation and paradigm of continuous improvement that is consistent with the obligations of the Veterinarian's Oath.[10] As for other editions of the document, modifications of previous recommendations are also informed by continued professional and public sensitivity to the ethical care of animals.

While some euthanasia methods may be utilized in slaughter (which refers to humane killing of animals destined for human consumption) or harvest and depopulation, recommendations related to humane slaughter and depopulation fall outside the purview of the Guidelines and are addressed by separate documents.

The Guidelines set criteria for euthanasia, specify appropriate euthanasia methods and agents, and are intended to assist veterinarians in their exercise of professional judgment. The Guidelines acknowledge that euthanasia is a process involving more than just what happens to an animal at the time of its death. Apart from delineating appropriate methods and agents, these Guidelines also recognize the importance of considering and applying appropriate pre-euthanasia (eg, sedation) and animal handling practices, as well as attention to disposal of animals' remains.

## I2 Historical Context and Current Edition

### I2.1 HISTORY OF THE PANEL ON EUTHANASIA

Since 1963 the AVMA has convened a POE to evaluate methods and potential methods of euthanasia for the purpose of creating guidelines for veterinarians who carry out or oversee the euthanasia of animals. The scope of the 1963 edition was limited to methods and recommendations applicable to dogs, cats, and other small mammals. Subsequent editions published in 1972 and 1978 encompassed more methods and species (laboratory animals and food animals, respectively), and included additional information about animals' physiologic and behavioral responses to euthanasia (specifically, pain, stress, and distress), euthanasia's effects on observers, and the economic feasibility and environmental impacts of various approaches. In 1986 information on poikilothermic, aquatic, and fur-bearing wildlife was introduced; in 1993 recommendations for horses and wildlife were

added; and in 2000 an update acknowledged a need for more research on approaches suitable for depopulation. An interim revision by the AVMA Animal Welfare Committee in 2007 incorporated information derived from an existing, but separate, AVMA policy on the use of maceration to euthanize day-old chicks, poults, and pipped eggs, and the name of the report was changed to the AVMA Guidelines on Euthanasia.

In 2013 the process for compiling the POE's report was substantially changed to include more breadth and depth of expertise in the affected species and environments in which euthanasia is performed. More than 3 years of deliberation by more than 60 individuals, including veterinarians, animal scientists, behaviorists, psychologists, and an animal ethicist, resulted in robust commentary and recommendations. A comment period allowed AVMA members an opportunity to provide input and share their experiences directly with POE members.

The 2020 iteration of the Guidelines constitutes the ninth edition of the POE's report. The process for compiling this edition was similar to that of the 2013 edition. Two years of review, discussion, and revision by the POE culminated in this edition. A comment period was held and the input from AVMA members helps ensure the resulting document is not only scientifically robust, but practically sound.

## I2.2 SUBSTANTIVE CHANGES SINCE THE LAST EDITION

In this interim update of the Guidelines, methods, techniques, and agents of euthanasia have been updated and detailed descriptions have been included to assist veterinarians in applying their professional judgment. Species-specific sections have been expanded or added to include more guidance for terrestrial and aquatic species kept for a variety of purposes and under different conditions. Where possible, appropriate flowcharts, illustrations, tables, and appendices have been used to clarify recommendations. **Appendices 1 and 2** may be useful as a quick reference guide, but should never be used in lieu of the full text of the document by those performing euthanasia. All illustrations and figures have been moved to **Appendix 3** of the document.

Some of the more significant changes are as follows:

- Language was added to clarify the distinction between sedation and anesthesia. Specifically, animals under sedation may be aroused to a conscious state with sufficient stimulation. Recognizing this is critical when categorizing the effects of agents and distinguishing even deep states of sedation from unconsciousness.
- The conditions for the use of $CO_2$ with rodents in the laboratory have changed from a recommended 10% to 30% of the chamber or cage volume/min to a recommended 30% to 70% of the chamber or cage volume/min. The extensive literature used to make this recommendation is cited and the AVMA appreciates the proactive efforts made by the international research community to provide the evidence needed to make this determination.
- Euthanasia techniques appropriate for use with rabbits raised for meat are categorized and described. This material is located in the Laboratory Animals section to place them with other techniques used with these species.
- The Animals Farmed for Food and Fiber section has been expanded to include American bison, water buffalo, camelids, and cervids. Updates to the application of captive bolt in several species have been made and new illustrations are available to assist veterinarians in proper usage.
- In the Avians section the recommendation for when avian embryos achieve the potential for perception has been amended from 50% to 80% of incubation for all avian eggs. This recommendation should be applied across avians with consideration for species-specific differences in development and using the best available data.

## I2.3 STATEMENT OF USE

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

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

Veterinarians performing or overseeing euthanasia must assess the potential for animal distress due to physical discomfort, abnormal social settings, novel physical surroundings, pheromones or odors from nearby or previously euthanized animals, the presence of humans, or other factors (including impact on the environment and other animals). In addition, human safety and perceptions, availability of trained personnel, potential infectious disease concerns, conservation or other animal population objectives, regulatory oversight that may be species specific, available equipment and facilities, options for disposal, potential secondary toxicity, and other factors must be considered. Human safety is of utmost importance, and appropriate safety equipment, protocols, and knowledge must be available before animals are handled. Advance preparation includes protocols and supplies for addressing personnel injury due to animal handling or exposure to drugs and equipment used during the process. Once eutha-

nasia has been carried out, death must be carefully verified. All laws and regulations pertaining to the species being euthanized, the methods employed, and the disposal of the animal's remains and/or any water containing pharmaceuticals used for euthanasia must be followed.

The POE's objective in creating the Guidelines is to provide guidance for veterinarians about how to prevent and/or relieve the pain and suffering of animals that are to be euthanized. While every effort has been made to identify and recommend appropriate approaches for common species encountered under common conditions, the POE recognized there will be less than perfect situations in which a recommended method of euthanasia may not be possible and a method or agent that is best under the circumstances will need to be applied. For this reason, although the Guidelines may be interpreted and understood by a broad segment of the general population, a veterinarian should be consulted in their application.

## 13 What Is Euthanasia?

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

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

Debate exists about whether *euthanasia* appropriately describes the killing of some animals at the end of biological experiments[11] and of unwanted shelter animals. The Panel believes that evaluating the social acceptability of various uses of animals and/or the rationale for inducing death in these cases is beyond its purview; however, current AVMA policy supports the use of animals for various human purposes,[12] and also recognizes the need to euthanize animals that are unwanted or unfit for adoption.[13] Whenever animals are used by humans, good animal care practices should be implemented and adherence to those good practices should be enforced. When evaluating our responsibilities toward animals, it is important to be sensitive to the context and the practical realities of the various types of human-animal relationships. Impacts on animals may not always be the center of the valuation process, and there is disagreement on how to account for conflicting interspecific interests. The Panel recognizes these are complex issues since how to bring about a "good death" for animals is regarded as "essentially contested" (morally and conceptually),[14] raising concerns across a large number of domains, including scientific, ethical, economic, environmental, political, and social.

### 13.1 A GOOD DEATH AS A MATTER OF HUMANE DISPOSITION

Humane disposition reflects the veterinarian's desire to do what is best for the animal and serves to bring about the best possible outcome for the animal. Thus, euthanasia as a matter of humane disposition can be either intent or outcome based.

Euthanasia as a matter of humane disposition occurs when death is a welcome event and continued existence is not an attractive option for the animal as perceived by the owner and veterinarian. When animals are plagued by disease that produces insurmountable suffering, it can be argued that continuing to live is worse for the animal than death or that the animal no longer has an interest in living. The humane disposition is to act for the sake of the animal or its interests, because the animal will not be harmed by the loss of life. Instead, there is consensus that the animal will be relieved of an unbearable burden. As an example, when treating a companion animal that is suffering severely at the end of life due to a debilitating terminal illness, a veterinarian may recommend euthanasia, because the loss of life (and attendant natural decline in physical and psychological faculties) to the animal is not relatively worse compared with a continued existence that is filled with prolonged illness, suffering, and duress. In this case, euthanasia does not deprive the animal of the opportunity to enjoy more goods of life (ie, to have more satisfactions fulfilled or enjoy more pleasurable experiences). And, these opportunities or experiences are much fewer or lesser in intensity than the presence or intensity of negative states or affect. Death, in this case, may be a welcome event and euthanasia helps to bring this about, because the animal's life is not worth living but, rather, is worth avoiding.

Veterinarians may also be motivated to bring about the best outcome for the animal. Often, veterinarians face the difficult question of trying to decide (or helping the animal's owner to decide) when euthanasia would be a good outcome. In making this decision many veterinarians appeal to indices of welfare or quality of life. Scientists have described welfare as having 3 components: that the animal functions well, feels well, and has the capacity to perform behaviors that are innate or species-specific adaptations[15–17] (an alternative view is also available[18]). An animal has good welfare if, overall, its life has positive value for it. When an animal no longer continues to enjoy good welfare (when it no longer has a life worth living because, on balance, its life no longer has positive value for it, or will shortly be overcome by negative states), the humane thing to do is to give it a good death. Euthanasia relieves the animal's suffering, which is the desired outcome.

## I3.2 A GOOD DEATH AS A MATTER OF HUMANE TECHNIQUE

When the decision has been made to euthanize and the goal is to minimize pain, distress, and negative effect to the animal, the humaneness of the technique (ie, how we bring about the death of animals) is also an important ethical issue. As veterinarians and human beings it is our responsibility to ensure that if an animal's life is to be taken, it is done with the highest degree of respect, and with an emphasis on making the death as painless and distress free as possible. When euthanasia is the preferred option, the technique employed should result in rapid loss of consciousness followed by cardiac or respiratory arrest and, ultimately, a loss of brain function. In addition, animal handling and the euthanasia technique should minimize distress experienced by the animal prior to loss of consciousness. The POE recognized that complete absence of pain and distress cannot always be achieved. The Guidelines attempt to balance the ideal of minimal pain and distress with the reality of the many environments in which euthanasia is performed.

While recommendations are made, it is important for those utilizing these recommendations to understand that, in some instances, agents and methods of euthanasia identified as appropriate for a particular species may not be available or may become less than an ideal choice due to differences in circumstances. Conversely, when settings are atypical, methods normally not considered appropriate may become the method of choice. Under such conditions, the humaneness (or perceived lack thereof) of the method used to bring about the death of an animal may be distinguished from the intent or outcome associated with an act of killing. Following this reasoning, it may still be an act of euthanasia to kill an animal in a manner that is not perfectly humane or that would not be considered appropriate in other contexts. For example, due to lack of control over free-ranging wildlife and the stress associated with close human contact, use of a firearm may be the most appropriate means of euthanasia. Also, shooting a suffering animal that is in extremis, instead of catching and transporting it to a clinic to euthanize it using a method normally considered to be appropriate (eg, barbiturates), is consistent with one interpretation of a good death. The former method promotes the animal's overall interests by ending its misery quickly, even though the latter technique may be considered to be more acceptable under normal conditions.[19] Neither of these examples, however, absolves the individual from their responsibility to ensure that recommended methods and agents of euthanasia are preferentially used.

# I4 Euthanasia and Veterinary Medical Ethics

The AVMA has worked to ensure that veterinarians remain educated about public discourse around animal ethics and animal welfare issues and that they are able to participate in meaningful ways. While an essential ingredient in public discourses about animals, sound science is by itself inadequate to address questions of ethics and values that surround the appropriate treatment of animals, especially as they relate to end-of-life issues. Since the 2013 edition, a number of authors[20,21] have probed in greater depth the issue of a good death for animals in both philosophical and ethical terms. To this end, and consistent with its charge, the POE hopes to provide veterinarians, those under their supervision, and the public with well-informed and credible arguments on how to approach the ethically important and sometimes complex issue of the death of an animal. In so doing, it hopes to promote greater understanding regarding the contexts or settings involving euthanasia and the complexity of end-of-life issues involving animals.

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

The AVMA does not take the death of nonhuman animals lightly and attempts to provide guidance for its members on both the morality and practical necessity of the intentional killing of animals. Veterinarians, in carrying out the tenets of their Oath, may be compelled to bring about the intentional death of animals for a variety of reasons. The finality of death is, in part, what makes it an ethically important issue; death forever cuts off future positive states, benefits, or opportunities.[22] In cases where an animal no longer has a good life, however, its death also extinguishes permanently any and all future harms associated with poor welfare or quality of life.[19] What constitutes a good life and what counts as an impoverished life, or one that has limited quality such that the death of the animal is the most humane option, are research areas in need of further study by the veterinary and ethics communities.[23,24] Animal scientists and veterinarians are also investigating the processes by which an animal dies during the antemortem period and euthanasia methods and techniques that mitigate harmful effects.[25–28] Further research is also needed regarding the different contexts within which euthanasia occurs, so that improvements in the performance and outcomes of euthanasia can be made.

The intentional killing of healthy animals, as well as those that are impaired, is a serious concern for the public. When animals must be killed and veterinarians are called upon to assist, the AVMA encourages careful consideration of the decision to euthanize

and the method(s) used. This is also true for euthanasia carried out during the course of disease control or protection of public health, as a means of domestic or wild animal population control, in conjunction with animal use in biomedical research, and in the process of food and fiber production. Killing of healthy animals under such circumstances, while unpleasant and morally challenging, is a practical necessity. The AVMA recognizes such actions as acceptable if those carrying out euthanasia adhere to strict policies, guidelines, and applicable regulations.

In thinking seriously about veterinary medical ethics, veterinarians should familiarize themselves with the plurality of public moral views surrounding animal issues and also be cognizant of personal views and complicating factors that may impact their own ethical decision-making. While the Veterinarian's Oath,[10] Principles of Veterinary Medical Ethics of the AVMA,[29] state veterinary practice acts, and other guidance emanating from veterinary professional organizations and regulatory bodies provide direction for how veterinarians should interact with clients and their animals, different veterinarians may have different personal ethical values[1,30] and this may impact their recommendations.

In their capacity as animal advocate and client advisor, the precision and credibility of advice provided by veterinarians will help to advance client compliance. In many instances when veterinarians are called upon to benefit society through their scientific knowledge, practical experience, and understanding of how animals are benefited and harmed, straightforward answers may not be forthcoming. In such cases, veterinarians and animal welfare scientists may have to facilitate conscientious decision-making by promoting ethical dialogue.[31–34] As advisor and conduit for information (and while respecting the autonomy of their clients to make decisions on behalf of their animals), veterinarians should advance pertinent scientific knowledge and ethical concerns related to practices and procedures so that their clients and/or society can make informed decisions.[1]

Veterinarians who are committed to a broad understanding of the "do no harm" principle may have to determine whether an animal's life is worth living, especially when there is no consensus on when it is appropriate to let that life go. While welfare or quality of life is typically adopted as part of the assessment of an animal's interests, what is in an animal's interest need not be singularly identified with its welfare, especially if welfare is defined narrowly and if the animal is harmed more by its continued life than its death. For example, if welfare is defined solely in terms of an animal's subjective experience, euthanasia may be warranted even if the animal is not showing signs of suffering at the present time and if there is some commitment to avoid harm. Euthanasia may be considered to be the right course to spare the animal from what is to come (in conjunction with a more holistic or objective account of what is in an animal's interest), if medical intervention would only prolong a terminal condition, or if current health conditions cannot be successfully mitigated. In these instances, intentional killing need not be motivated by narrow welfare-based interests[35] but may be connected to the overall value of death to the animal. That some animals are subjects-of-a-life,[36–39] and that human caretakers have moral responsibilities to their animals and do not want to see them endure continued harm,[40,41] may be factors in deciding whether death is in an animal's interest. (A subject-of-a-life is a being that is regarded as having inherent value and should not be treated as a mere means to an end. It is a being that possesses an internal existence and has needs, desires, preferences, and a psychosocial identity that extends through time.[3,6])

In some cases (eg, animals used for research), intentional killing of the animal to minimize harm to it may be trumped by more pressing ends. Here, the decision to kill an animal and how to do so will be complicated by external factors, such as productivity, the greater public and general good, economics, and concern for other animals. In human-animal relationships there usually are other mitigating factors that are relevant besides ones pertaining only to animal welfare or the animal's interest(s). In laboratory situations, for example, where animals are employed as research subjects and death may be a terminal point, animal welfare considerations are balanced against the merits of the experimental design and merits of the research. In such cases, ensuring the respectful and humane treatment of research animals will be largely up to IACUCs. These committees must apply the principles of refinement, replacement, and reduction, and ensure a respectful death for research animals. The decision to induce death may also involve whether replacements can be created for the animals that are killed.[42,43] These other factors might justify killing an animal, despite the fact that the animal might otherwise have had a life worth living. For example, killing may be justified for disease control or public health purposes, population control, biomedical research, or slaughter for food and/or fiber. In other instances, keeping an animal alive that does not have a life worth living can be justified (eg, research circumstances where it would be impractical to kill the animal or when ensuring its survival would promote a greater good[19]).

There may be instances in which the decision to kill an animal is questionable, especially if the animal is predicted to have a life worth living if it is not killed. One example is the healthy companion animal whose owner wants to euthanize it because keeping it in the home is no longer possible or convenient. In this case, the veterinarian, as advisor and animal advocate, should be able to speak frankly about the animal's condition and suggest alternatives to euthanasia.

Prima facie, it is the ethical responsibility of veterinarians to direct animal owners toward euthanasia

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.

## I5 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. Premedication 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.

## I5.1 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 behavioral 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 point[48]; 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 frequency[49–51] 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 eyeblink 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 studies[60–63] 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.

### I5.1.1 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 conscious[67]; 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] Determining 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]

## I5.2 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 transaction 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 reflexes[81] 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 forward[84] 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.

## I5.3 STRESS AND DISTRESS

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

## I5.4 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 welfare[90] 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 stressful[91–94]; 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,

auditory, and tactile stimulation. When struggling during capture or restraint may cause pain, injury, or anxiety to the animal or danger to the operator, the use of tranquilizers, analgesics, and/or anesthetics may be necessary. A method of administration should be chosen that causes the least distress in the animal for which euthanasia must be performed. Various techniques for oral delivery of sedatives to dogs and cats have been described that may be useful under these circumstances.[102,103]

Expressions and body postures that indicate various emotional states of animals have been described for some species.[104–107] Behavioral responses to noxious stimuli in conscious animals include distress vocalization, struggling, attempts to escape, and defensive or redirected aggression. In cattle and pigs, vocalization during handling or painful procedures is associated with physiologic indicators of stress.[108–110] Vocalization is associated with excessive pressure applied by a restraint device.[111,112] Salivation, urination, defecation, evacuation of anal sacs, pupillary dilatation, tachycardia, sweating, and reflex skeletal muscle contractions causing shivering, tremors, or other muscular spasms may occur in unconscious as well as conscious animals. Fear can cause immobility or playing dead in certain species, particularly rabbits and chickens.[113] This immobility response should not be interpreted as loss of consciousness when the animal is, in fact, conscious. Distress vocalizations, fearful behavior, and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals.[114,115] Therefore, for sensitive species, it is desirable that other animals not be present when individual animal euthanasia is performed. Often, simple environmental modifications can help reduce agitation and stress, such as providing a nonslip floor for the animals to stand on, reducing noise, blocking the animal's vision with a blindfold or a barrier, or removing distracting stimuli that cause animals to become agitated.[112,116–119]

## I5.5 HUMAN BEHAVIOR

The depth of the emotional attachment between animals and their owners or caretakers requires an additional layer of professional respect and care beyond the ethical obligation to provide a good death for the animal. Human concerns associated with the euthanasia of healthy and unwanted animals can be particularly challenging, as can situations where the health interests of groups of animals and/or the health interests of people conflict with the welfare of individual animals (eg, animal health emergencies).

The human-animal relationship should be respected by discussing euthanasia openly,[120] providing an appropriate place to conduct the process, offering the opportunity for animal owners and/or caretakers to be present when at all possible (consistent with the best interests of the animal and the owners and caretakers), fully informing those present about what they will see (including possible unpleasant side effects), and giving emotional support and information about grief counseling as needed.[121–123] Regardless of the euthanasia method chosen, it is important to consider the level of understanding and perceptions of those in attendance as they witness euthanasia. When death has been achieved and verified, owners and caretakers should be verbally notified.[122]

Owners and caretakers are not the only people affected by the euthanasia of animals. Veterinarians and their staffs may also become attached to patients and struggle with the ethics of the caring-killing paradox,[124,125] particularly when they must end the lives of animals they have known and treated for many years. Repeating this scenario regularly may lead to emotional burnout, or compassion fatigue. The various ways in which veterinarians cope with euthanasia have been discussed elsewhere.[126]

There are 6 settings in which the Panel was most aware of the potential for substantive psychological impacts of animal euthanasia on people.

The first setting is the veterinary clinical setting (clinics and hospitals or mobile veterinary practices) where owners have to make decisions about whether and when to euthanize. Although many owners rely heavily on their veterinarian's judgment, others may have misgivings about making a decision. This is particularly likely if an owner feels responsible for an animal's medical or behavioral problem. Owners choose euthanasia for their animals for a variety of reasons, including prevention of suffering from a terminal illness, their inability to care for the animal, the impact of the animal's condition on other animals or people, and/or financial considerations. The decision to euthanize often carries strong feelings of emotion such as guilt, sadness, shock, and disbelief.[127] As society continues to pay more attention to questions about the moral status of animals, loss of animal life should be handled with the utmost respect and compassion by all animal care staff. The ability to communicate well is crucial to helping owners make end-of-life decisions for their animals and is a learned skill that requires training.[128]

Almost 80% of clients who recently experienced the death of a pet (87% by euthanasia) reported a positive correlation between support from the veterinarian and staff and their ability to handle the grief associated with their pet's death.[127] Owners should be given the opportunity to be present during euthanasia, when feasible, and they should be prepared for what to expect.[122,127,129] What drugs are being used and how the animal could respond should be discussed. Behaviors such as vocalization, agonal breaths, muscle twitches, failure of the eyelids to close, urination, or defecation can be distressing to owners. Counseling services for owners having difficulty coping with animal death are available in some communities, and veterinarians are encouraged to seek grief support training to assist their clients.[130–132] While good euthanasia practices (ie, client communication and education, compassionate species-appropriate handling

and selection of technique, pre-euthanasia sedatives or anesthetics as needed to minimize anxiety and facilitate safe restraint, and careful confirmation of death) are often applied in the euthanasia of dogs and cats, they should also be followed for other species that are kept as pets, including small mammals, birds, reptiles, farm animals, and aquatic animals.

The second setting is in animal care and control facilities where unwanted, homeless, diseased, and injured animals must be euthanized in large numbers. The person performing euthanasia must be technically proficient (including the use of humane handling methods and familiarity with the method of euthanasia being employed), and must be able to understand and communicate to others the reasons for euthanasia and why a particular approach was selected. This requires organizational commitment to provide ongoing professional training on the latest methods, techniques, and materials available for euthanasia.

Distress may develop among personnel directly involved in performing euthanasia repeatedly,[133] and may include a psychological state characterized by a strong sense of work dissatisfaction or alienation, which may be expressed by absenteeism, belligerence, or careless and callous handling of animals.[134] The impact on personnel may be worse when euthanasia is conducted in frequent, shorter sessions compared with fewer, longer sessions.[135] In addition, animal shelter personnel have been shown to have more difficulty dealing emotionally with the euthanasia of healthy, unwanted animals than those that are old, sick, injured, or wild.[136] Specific coping strategies that can make the task more tolerable include adequate training programs so that euthanasia is performed competently, rotation of duties and shared responsibilities for staff performing euthanasia, peer support in the workplace, professional support as necessary, focusing on animals that are successfully adopted or returned to owners, devoting some work time to educational activities, and providing time off when workers feel distressed. Management should be aware of potential personnel problems related to animal euthanasia and determine whether it is necessary to institute a program to prevent, decrease, or eliminate this problem.

The third setting is the laboratory. Researchers, technicians, and students may become attached to animals that must be euthanized in laboratory settings, even though the animals are often purpose-bred for research.[137] The human–research animal bond positively impacts quality of life for a variety of research animals, but those caring for the animals often experience euthanasia- related stress symptoms comparable to those encountered in veterinary clinics and animal shelters.[138–140] The same considerations afforded pet owners or shelter employees should be provided to those working in laboratories, particularly the provision of training to promote grief coping skills.[141]

The fourth setting is wildlife conservation and management. Wildlife biologists, wildlife managers, and wildlife health professionals are often responsible for euthanizing animals that are injured, diseased, or in excessive number or those that threaten property or human safety. Although relocation of some animals may be appropriate and attempted, relocation is often only a temporary solution and may be insufficient to address a larger problem. People who must deal with these animals, especially under public pressure to save the animals rather than destroy them, can experience extreme distress and anxiety. In addition, the perceptions of not only the wildlife professionals, but of onlookers, need to be considered when selecting a euthanasia method.

The fifth setting is livestock and poultry production. As for shelter and laboratory animal workers, on-farm euthanasia of individual animals by farm workers charged with nurturing and raising production animals can take a heavy toll on employees both physically and emotionally.[142]

The sixth setting is that in which there is broad public exposure. Because euthanasia of zoo animals, animals involved in roadside or racetrack accidents, stranded marine animals, and nuisance or injured wildlife can draw public attention, human attitudes and responses must be considered whenever these animals are euthanized. Natural disasters and foreign animal disease programs also present public challenges. Attention to public perceptions, however, should not outweigh the primary responsibility of doing what is in the animal's best interest under the circumstances (ie, using the most appropriate and painless euthanasia method possible).

In addition to ensuring good care of animals during euthanasia and considering the psychological well-being of human participants, the physical safety of personnel handling the animals and performing euthanasia needs to be protected. The safe use of controlled substances and diversion control to prevent abuse is also part of the responsibility of those using such substances in the performance of euthanasia.[143]

## I5.6 SEDATION VERSUS ANESTHESIA

A distinction must be made between the terms sedation, tranquilization, and anesthesia as utilized in these Guidelines. A common characteristic of both sedatives and tranquilizers is that arousal to a conscious state can occur with sufficient stimulation, such that animals sedated or immobilized with these agents may still be consciously aware of, and connected to, their environment. Unlike properly applied physical euthanasia methods where loss of consciousness is instantaneous and unambiguous (eg, captive bolt, gunshot, electrocution), application of other approved euthanasia methods requires animals be first rendered fully unconscious (eg, intracardiac pentobarbital, IV $MgSO_4$ or KCl, exsanguination). While sedatives, hypnotics, and tranquilizers, when administered in sufficient quantity, can produce a sleep-like state, humans may recall connected aware-

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.

## I6 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 ($O_2$), such as carbon dioxide ($CO_2$), nitrogen ($N_2$), or argon (Ar), or by exposure to carbon monoxide (CO) to block uptake of $O_2$ 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]

## I7 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-

beat by use of a stethoscope; graying of the mucous membranes; and rigor mortis. None of these signs alone, except rigor mortis, confirms death.

In small animals, particularly in animal shelter settings, verification of death may be supplemented by percutaneous cardiac puncture after the animal is unconscious. Failure of the needle and attached syringe to move after insertion into the heart (aspiration of blood provides evidence of correct location) indicates lack of cardiac muscle movement and death.[149]

## I8 Disposal of Animal Remains

Regardless of the euthanasia method chosen, animal remains must be handled appropriately and in accord with state and local law. Regulations apply not only to the disposition of the animal's remains (eg, burial, incineration, rendering), but also to the management of chemical residues (eg, pharmaceuticals [including but not limited to barbiturates, such as pentobarbital] and other residues, such as lead) that may adversely affect scavengers or result in the adulteration of rendered products used for animal feed.

Use of pentobarbital invokes legal responsibilities for veterinarians, animal shelters, and animal owners to properly dispose of animal remains after death. Animal remains containing pentobarbital are potentially poisonous for scavenging wildlife, including birds (eg, bald and golden eagles, vultures, hawk species, gulls, crows, ravens), carnivorous mammals (eg, bears, coyotes, martens, fishers, foxes, lynxes, bobcats, cougars), and domestic dogs.[150] Federal laws protecting many of these species apply to secondary poisoning from animal remains containing pentobarbital. The Migratory Bird Treaty Act, the Endangered Species Act, and the Bald and Golden Eagle Protection Act may carry civil and criminal penalties, with fines in civil cases up to $25,000 and in criminal cases up to $500,000 and incarceration for up to 2 years.[150] Serious repercussions may occur when veterinary health professionals who should be well-informed about the necessity for proper disposal of animal remains fail to provide it, or fail to inform their clients how to provide it, whether there was intent to cause harm or not.[151,152] Cases of suspected wildlife death from animal remains containing pentobarbital are investigated by the regional US Fish and Wildlife Service law enforcement office.

Recommendations by the US Fish and Wildlife Service for prevention of secondary poisoning from pentobarbital are to (1) incinerate or cremate animal remains whenever possible, (2) immediately bury deeply according to local laws and regulations, (3) securely cover or store animal remains if the ground is frozen until such time as deep burial is practical, (4) review and modify local landfill practices to prevent access of scavengers to legally disposed animal remains, (5) educate clients about proper disposal, (6) include a warning regarding disposal of animal remains on the euthanasia consent form, and (7) tag animal remains and outer bags or containers with prominent poison tags.[150]

Rendering is an important means of disposal of dead livestock and horses, and since many horses are euthanized with barbiturates, related residues can be hazardous. Rendered protein is used in animal feed for cattle, swine, poultry, fish, and companion animals, but products rendered from ruminants are prohibited by law for use in ruminant feed. Many pet food manufacturers have lowered their acceptance thresholds for barbiturate concentrations in rendered product. Advances in analytical chemistry have spawned increasingly sensitive assays, and pet food manufacturers are using these techniques to ensure the purity of the rendered protein incorporated in their products. Accordingly, increased analytical sensitivity has led many renderers to reconsider accepting horses euthanized using barbiturates. This places renderers and those wishing to employ rendering as a means of disposal for animals euthanized using pentobarbital in a difficult position, and may result in renderers being reluctant to accept more animal remains than they can reasonably manage without creating residue concerns. Alternatives for disposal of animal remains must be considered in advance, in case the renderer cannot or will not accept animal remains containing barbiturate residues.

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

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

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

Safe handling and disposal of the resulting animal remains are also critically important when zoonotic diseases, foreign animal diseases, or diseases

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

## 19 Footnotes

a. Anthony R, University of Alaska Anchorage, Anchorage, Alaska: Personal communication, 2011.
b. Fraser D. Understanding animal welfare (abstr). *Acta Vet Scand* 2008;50(suppl 1):S1.
c. Dewell RD, Bergamasco LL, Kelly CK, et al. Clinical study to assess the level of unconsciousness in cattle following the administration of high doses of xylazine hydrochloride (abstr), in *Proceedings*. 46th Annu Conf Am Assoc Bovine Pract 2013;183.

## 110 References


1. Sandoe P, Christiansen SB. *Ethics of animal use*. Chichester, England: Wiley-Blackwell, 2008;1–14, 15–32, 49–66.
2. Rollin BE. Animal agriculture and emerging social ethics for animals. *J Anim Sci* 2004;82:955–964.
3. DeGrazia D. Self-awareness in animals. In: Lurz R, ed. *The philosophy of animal minds*. Cambridge, England: Cambridge University Press, 2009;201–217.
4. Thompson PB. Ethics on the frontiers of livestock science. In: Swain DL, Charmley E, Steel JW, et al, eds. *Redesigning animal agriculture: the challenge of the 21st century*. Cambridge, Mass: CABI, 2007;30–45.
5. Thompson PB. Getting pragmatic about farm animal welfare. In: McKenna E, Light A, eds. *Animal pragmatism: rethinking human-nonhuman relationships*. Bloomington, Ind: Indiana University Press, 2004;140–159.
6. DeGrazia D. Animal ethics around the turn of the twenty-first century. *J Agric Environ Ethics* 1999;11:111–129.
7. DeGrazia D. *Taking animals seriously: mental life and moral status*. Cambridge, England: Cambridge University Press, 1996.
8. Thompson PB. *Agricultural ethics: research, teaching, and public policy*. Ames, Iowa: Iowa State University Press, 1998.
9. Varner G. *In nature's interests? Interests, animal rights and environmental ethics*. Oxford, England: Oxford University Press, 1998.
10. AVMA. Veterinarian's oath. Available at www.avma.org/about_avma/whoweare/oath.asp. Accessed May 13, 2011.
11. Pavlovic D, Spassov A, Lehmann C. Euthanasia: in defense of a good, ancient word. *J Clin Res Bioeth* 2011;2:105.
12. AVMA. AVMA animal welfare principles. Available at: www.avma.org/issues/policy/animal_welfare/principles.asp. Accessed May 7, 2011.
13. AVMA. Euthanasia of animals that are unwanted or unfit for adoption. Available at: www.avma.org/issues/policy/animal_welfare/euthanasia.asp. Accessed May 7, 2011.
14. ulAin Q, Whiting TL. Is a "good death" at the time of animal slaughter an essentially contested concept? *Animals (Basel)* 2017;7:99.
15. Haynes R. *Animal welfare: competing conceptions and their ethical implications*. Dort, Netherlands: Springer, 2008.
16. Appleby MC. *What should we do about animal welfare?* Oxford, England: Blackwell, 1999.
17. Fraser D, Weary DM, Pajor EA, et al. A scientific conception of animal welfare that reflects ethical concerns. *Anim Welf* 1997;6:187–205.
18. Duncan IJH. Animal welfare defined in terms of feelings. *Acta Agric Scand. Anim Sci* 1996;(suppl 27):29–35.
19. Yeates J. Death is a welfare issue. *J Agric Environ Ethics* 2010;23:229–241.
20. Višak T, Garner R, Singer P. *The ethics of killing animals*. Oxford, England: Oxford University Press, 2016.
21. Meijboom FLB, Stassen EN. *The end of animal life: a start for ethical debate. Ethical and societal considerations on killing animals*. Wageningen, Netherlands: Wageningen Academic Publishers, 2016.
22. Kamm FM. *Morality, mortality*. Vol 1. Oxford, England: Oxford University Press, 1993.
23. Morton DB. A hypothetical strategy for the objective evaluation of animal well-being and quality of life using a dog model. *Anim Welf* 2007;16(suppl):75–81.
24. Rollin BE. Animal euthanasia and moral stress. In: Kay WJ, Cohen SP, Fudin CE, et al, eds. *Euthanasia of the companion animal*. Philadelphia: Charles Press, 1988;31–41.
25. Niel L, Stewart SA, Weary DM. Effect of flow rate on aversion to gradual-fill carbon dioxide euthanasia in rats. *Appl Anim Behav Sci* 2008;109:77–84.
26. Brown M, Carbone L, Conlee KM, et al. Report of the working group on animal distress in the laboratory. *Lab Anim (NY)* 2006;35:26–30.
27. Demers G, Griffin G, DeVroey G, et al. Animal research. Harmonization of animal care and use guidelines. *Science* 2006;312:700–701.
28. Hawkins P, Playle L, Golledge H, et al. Newcastle consensus meeting on carbon dioxide euthanasia of laboratory animals. London: National Centre for the Replacement, Refinement and Reduction of Animals in Science, 2006. Available at: www.nc3rs.org.uk/downloaddoc.asp?id=416&page=292&skin=0. Accessed Jan 20, 2011.
29. AVMA. Principles of veterinary medical ethics of the AVMA. Available at: www.avma.org/issues/policy/ethics.asp. Accessed May 13, 2011.
30. Rollin BE. *An introduction to veterinary medical ethics*. 2nd ed. Ames, Iowa: Blackwell, 2006.
31. Croney CC, Anthony R. Engaging science in a climate of values: tools for animal scientists tasked with addressing ethical problems. *J Anim Sci* 2010;88(suppl 13):E75–E81.
32. Sandoe P, Christiansen SB, Appleby MC. Farm animal welfare: the interaction of ethical questions and animal welfare science. *Anim Welf* 2003;12:469–478.
33. Fraser D. Animal ethics and animal welfare science: bridging the two cultures. *Appl Anim Behav Sci* 1999;65:171–189.
34. Thompson PB. From a philosopher's perspective, how should animal scientists meet the challenge of contentious issues? *J Anim Sci* 1999;77:372–377.
35. Webster J. *Animal welfare: a cool eye towards Eden*. Oxford, England: Blackwell, 1994.
36. Anderson E. Animal rights and the values of nonhuman life. In: Sunstein C, Nussbaum M, eds. *Animal rights: current debates and new directions*. Oxford, England: Oxford University Press, 2004;277–298.
37. Regan T. *Animal rights, human wrongs: an introduction to moral philosophy*. Lanham, Md: Rowman and Littlefield, 2003.
38. Pluhar E. *Beyond prejudice*. Durham, NC: Duke University Press, 1995.
39. Regan T. *The case for animal rights*. Berkeley, Calif: University of California Press, 1983.
40. Anthony R. Ethical implications of the human-animal bond on the farm. *Anim Welf* 2003;12:505–512.
41. Burgess-Jackson K. Doing right by our animal companions. *J Ethics* 1998;2:159–185.
42. Frey R. *Rights, killing and suffering*. Oxford, England: Blackwell, 1983.
43. Singer P. *Practical ethics*. Cambridge: Cambridge University Press, 1978.
44. Rollin BE. The use and abuse of Aesculapian authority in veterinary medicine. *J Am Vet Med Assoc* 2002;220:1144–1149.
45. Sanders RD, Tononi G, Laureys S, et al. Unresponsiveness ≠ unconsciousness. *Anesthesiology* 2012;116:946–959.
46. Hendrickx JF, Eger II EI, Sonner JM, et al. Is synergy the rule?

A review of anesthetic interactions producing hypnosis and immobility. *Anesth Analg* 2008;107:494–506.
47. Antognini JF, Barter L, Carstens E. Overview: movement as an index of anesthetic depth in humans and experimental animals. *Comp Med* 2005;55:413–418.
48. Alkire MT, Hudetz AG, Tononi G. Consciousness and anesthesia. *Science* 2008;322:876–880.
49. Gregory NG. Animal welfare at markets and during transport and slaughter. *Meat Sci* 2008;80:2–11.
50. Finnie JW. Neuropathologic changes produced by non-penetrating percussive captive bolt stunning of cattle. *N Z Vet J* 1995;43:183–185.
51. Blackmore DK, Newhook JC. The assessment of insensibility in sheep, calves and pigs during slaughter. In: Eikelenboom G, ed. *Stunning of animals for slaughter*. Boston: Martinus Nijhoff Publishers, 1983;13–25.
52. Gregory NG, Lee CJ, Widdicombe JP. Depth of concussion in cattle shot by penetrating captive bolt. *Meat Sci* 2007;77:499–503.
53. Vogel KD, Badtram G, Claus JR, et al. Head-only followed by cardiac arrest electrical stunning is an effective alternative to head-only electrical stunning in pigs. *J Anim Sci* 2011;89:1412–1418.
54. Blackmore DK, Newhook JC. Electroencephalographic studies of stunning and slaughter of sheep and calves. 3. The duration of insensibility induced by electrical stunning in sheep and calves. *Meat Sci* 1982;7:19–28.
55. Cartner SC, Barlow SC, Ness TJ. Loss of cortical function in mice after decapitation, cervical dislocation, potassium chloride injection, and $CO_2$ inhalation. *Comp Med* 2007;57:570–573.
56. Close B, Banister K, Baumans V, et al. Recommendations for euthanasia of experimental animals: part 2. DGXT of the European Commission. *Lab Anim* 1997;31:1–32.
57. Close B, Banister K, Baumans V, et al. Recommendations for euthanasia of experimental animals: part 1. DGXI of the European Commission. *Lab Anim* 1996;30:293–316.
58. Gregory NG, Wotton SB. Effect of slaughter on the spontaneous and evoked activity of the brain. *Br Poult Sci* 1986;27:195–205.
59. Bates G. Humane issues surrounding decapitation reconsidered. *J Am Vet Med Assoc* 2010;237:1024–1026.
60. Holson RR. Euthanasia by decapitation: evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. *Neurotoxicol Teratol* 1992;14:253–257.
61. Derr RF. Pain perception in decapitated rat brain. *Life Sci* 1991;49:1399–1402.
62. Vanderwolf CH, Buzak DP, Cain RK, et al. Neocortical and hippocampal electrical activity following decapitation in the rat. *Brain Res* 1988;451:340–344.
63. Mikeska JA, Klemm WR. EEG evaluation of humaneness of asphyxia and decapitation euthanasia of the laboratory rat. *Lab Anim Sci* 1975;25:175–179.
64. Muir WW. Considerations for general anesthesia. In: Tranquilli WJ, Thurmon JC, Grimm KA, eds. *Lumb and Jones' veterinary anesthesia and analgesia*. 4th ed. Ames, Iowa: Blackwell, 2007;7–30.
65. Erhardt W, Ring C, Kraft H, et al. $CO_2$ stunning of swine for slaughter from the anesthesiological viewpoint. *Dtsch Tierarztl Wochenschr* 1989;96:92–99.
66. Terlouw C, Bourguet C, Deiss V. Consciousness, unconsciousness and death in the context of slaughter. Part 1. Neurobiological mechanisms underlying stunning and killing. *Meat Sci* 2016;118:133–146.
67. Terlouw C, Bourguet C, Deiss V. Consciousness, unconsciousness and death in the context of slaughter. Part 2. Evaluation methods. *Meat Sci* 2016;118:147–156.
68. Verhoeven MTW, Gerritzen MA, Kluivers-Poodt M, et al. Validation of behavioural indicators used to assess unconsciousness in sheep. *Res Vet Sci* 2015;101:144–153.
69. Verhoeven MT, Gerritzen MA, Hellebrekers LJ, et al. Indicators used in livestock to assess unconsciousness after stunning: a review. *Animal* 2015;9:320–330.
70. Verhoeven MTW, Hellebrekers LJ, Gerritzen MA, et al. Validation of indicators used to assess unconsciousness in veal calves at slaughter. *Animal* 2016;10:1457–1465.
71. Marchant N, Sanders R, Sleigh J, et al. How electroencephalography serves the anesthesiologist. *Clin EEG Neurosci* 2014;45:22–32.
72. International Association for the Study of Pain. Pain terms. Available at: www.iasp-pain.org/AM/Template.cfm?Section=Pain_Definitions&Template=/CM/HTMLDisplay.cfm&ContentID=1728# Pain. Accessed Feb 7, 2011.
73. AVMA. AVMA guidelines on euthanasia. June 2007. Available at: www.avma.org/issues/animal_welfare/euthanasia.pdf. Accessed May 7, 2011.
74. Tarsitano MS, Jackson RR. Araneophagic jumping spiders discriminate between detour routes that do and do not lead to prey. *Anim Behav* 1997;53:257–266.
75. Jackson RR, Carter CM, Tarsitano MS. Trial-and-error solving of a confinement problem by a jumping spider, *Portia fibriata*. *Behaviour* 2001;138:1215–1234.
76. Dyakonova VE. Role of opioid peptides in behavior of invertebrates. *J Evol Biochem Physiol* 2001;37:335–347.
77. Sladky KK, Kinney ME, Johnson SM. Analgesic efficacy of butorphanol and morphine in bearded dragons and corn snakes. *J Am Vet Med Assoc* 2008;233:267–273.
78. Baker BB, Sladky KK, Johnson SM. Evaluation of the analgesic effects of oral and subcutaneous tramadol administration in red-eared slider turtles. *J Am Vet Med Assoc* 2011;238:220–227.
79. Sneddon LU, Braithwaite VA, Gentle JM. Do fish have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proc Biol Sci* 2003;270:1115–1121.
80. Sneddon LU. Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, *Oncorhynchus mykiss*. *Neurosci Lett* 2002;319:167–171.
81. Rose JD. The neurobehavioral nature of fishes and the question of awareness and pain. *Rev Fish Sci* 2002;10:1–38.
82. Nordgreen J, Horsberg TE, Ranheim B, et al. Somatosensory evoked potentials in the telencephalon of Atlantic salmon (*Salmo salar*) following galvanic stimulation of the tail. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2007;193:1235–1242.
83. Dunlop R, Laming P. Mechanoreceptive and nociceptive responses in the central nervous system of goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*). *J Pain* 2005;6:561–568.
84. Braithwaite VA. Cognition in fish. *Behav Physiol Fish* 2006;24:1–37.
85. Kitchen N, Aronson AL, Bittle JL, et al. Panel report on the Colloquium on Recognition and Alleviation of Animal Pain and Distress. *J Am Vet Med Assoc* 1987;191:1186–1191.
86. Wack R, Morris P, Sikarskie J, et al. Criteria for humane euthanasia and associated concerns. In: American Association of Zoo Veterinarians (AAZV). *Guidelines for euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;3–5.
87. National Research Committee on Pain and Distress in Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. *Recognition and alleviation of pain and distress in laboratory animals*. Washington, DC: National Academy Press, 1992.
88. Breazile JE. Physiologic basis and consequences of distress in animals. *J Am Vet Med Assoc* 1987;191:1212–1215.
89. McMillan FD. Comfort as the primary goal in veterinary medical practice. *J Am Vet Med Assoc* 1998;212:1370–1374.
90. Fraser D. Assessing animal welfare at the farm and group level: the interplay of science and values. *Anim Welf* 2003;12:433–443.
91. Coppola CL, Grandin T, Enns MR. Human interaction and cortisol: can human contact reduce stress in shelter dogs? *Physiol Behav* 2006;87:537–541.
92. Van Reenen CG, O'Connell NE, Van der Werf JT, et al. Response of calves to acute stress: individual consistency and

relations between behavioral and physiological measures. *Physiol Behav* 2005;85:557–570.
93. Dantzer R, Mormède P. Stress in farm animals: a need for reevaluation. *J Anim Sci* 1983;57:6–18.
94. Moberg GP, Wood VA. Effect of differential rearing on the behavorial and adrenocortical response of lambs to a novel environment. *Appl Anim Ethol* 1982;8:269–279.
95. Baran BE, Allen JA, Rogelberg SG, et al. Euthanasia-related strain and coping strategies in animal shelter employees. *J Am Vet Med Assoc* 2009;235:83–88.
96. Collette JC, Millam JR, Klasing KC, et al. Neonatal handling of Amazon parrots alters stress response and immune function. *Appl Anim Behav Sci* 2000;66:335–349.
97. Grandin T. Assessment of stress during handling and transport. *J Anim Sci* 1997;75:249–257.
98. Boandl KE, Wohlt JE, Carsia RV. Effect of handling, administration of a local anesthetic and electrical dehorning on plasma cortisol in Holstein calves. *J Dairy Sci* 1989;72:2193.
99. Lay DC, Friend TH, Bowers CL, et al. A comparative physiological and behavioral study of freeze and hot-iron branding using dairy cows. *J Anim Sci* 1992;70:1121–1125.
100. Lay DC, Friend TH, Randel RD, et al. Behavioral and physiological effects of freeze and hot-iron branding on crossbred cattle. *J Anim Sci* 1992;70:330–336.
101. Duke JL, Zammit TG, Lawson DM. The effects of routine cage-changing in cardiovascular and behavioral parameters in male Sprague-Dawley rats. *Contemp Top Lab Anim Sci* 2001;40:17–20.
102. Ramsay EC, Wetzel RW. Comparison of five regimens for oral administration of medication to induce sedation in dogs prior to euthanasia. *J Am Vet Med Assoc* 1998;213:240–242.
103. Wetzel RW, Ramsay EC. Comparison of four regimens for intraoral administration of medication to induce sedation in cats prior to euthanasia. *J Am Vet Med Assoc* 1998;213:243–245.
104. Houpt KA. *Domestic animal behavior for veterinarians and animal scientists*. 3rd ed. Ames, Iowa: Iowa State University Press, 1998.
105. Beaver BV. *Canine behavior: a guide for veterinarians*. Philadelphia: WB Saunders Co, 1998.
106. Beaver BV. *The veterinarian's encyclopedia of animal behavior*. Ames, Iowa: Iowa State University Press, 1994.
107. Schafer M. *The language of the horse: habits and forms of expression*. New York: Arco Publishing Co, 1975.
108. White RG, DeShazer JA, Tressler CJ, et al. Vocalization and physiological response of pigs during castration with and without a local anesthetic. *J Anim Sci* 1995;73:381–386.
109. Warriss PD, Brown SN, Adams M. Relationships between subjective and objective assessments of stress at slaughter and meat quality in pigs. *Meat Sci* 1994;38:329–340.
110. Dunn CS. Stress reactions of cattle undergoing ritual slaughter using two methods of restraint. *Vet Rec* 1990;126:522–525.
111. Grandin T. Cattle vocalizations are associated with handling and equipment problems in slaughter plants. *Appl Anim Behav Sci* 2001;71:191–201.
112. Grandin T. Objective scoring of animal handling and stunning practices at slaughter plants. *J Am Vet Med Assoc* 1998;212:36–39.
113. Jones RB. Experimental novelty and tonic immobility in chickens (*Gallas domesticus*). *Behav Processes* 1984;9:255–260.
114. Vieuille-Thomas C, Signoret JP. Pheromonal transmission of an aversive experience in domestic pig. *J Chem Ecol* 1992;18:1551–1557.
115. Stevens DA, Saplikoski NJ. Rats' reactions to conspecific muscle and blood evidence for alarm substances. *Behav Biol* 1973;8:75–82.
116. Grandin T. Effect of animal welfare audits of slaughter plants by a major fast food company on cattle handling and stunning practices. *J Am Vet Med Assoc* 2000;216:848–851.
117. Grandin T. Euthanasia and slaughter of livestock. *J Am Vet Med Assoc* 1994;204:1354–1360.
118. Grandin T. Pig behavior studies applied to slaughter-plant design. *Appl Anim Ethol* 1982;9:141–151.
119. Grandin T. Observations of cattle behavior applied to design of cattle handling facilities. *Appl Anim Ethol* 1980;6:19–31.
120. Knesl O, Hart B, Fine AH, et al. Veterinarians and humane endings: when is it the right time to euthanize a companion animal? *Front Vet Sci* 2017;4:45.
121. Nogueira Borden LJ, Adams CL, Bonnett BN, et al. Use of the measure of patient-centered communication to analyze euthanasia discussions in companion animal practice. *J Am Vet Med Assoc* 2010;237:1275–1287.
122. Martin F, Ruby KL, Deking TM, et al. Factors associated with client, staff, and student satisfaction regarding small animal euthanasia procedures at a veterinary teaching hospital. *J Am Vet Med Assoc* 2004;224:1774–1779.
123. Guntzelman J, Riegger MH. Helping pet owners with the euthanasia decision. *Vet Med* 1993;88:26–34.
124. Arluke A. Managing emotions in an animal shelter. In: Manning A, Serpell J, eds. *Animals and human society*. New York: Routledge, 1994;145–165.
125. Rhoades RH. *The Humane Society of the United States euthanasia training manual*. Washington, DC: Humane Society Press, 2002.
126. Manette CS. A reflection on the ways veterinarians cope with the death, euthanasia, and slaughter of animals. *J Am Vet Med Assoc* 2004;225:34–38.
127. Adams CL, Bonnett BN, Meek AH. Predictors of owner response to companion animal death in 177 clients from 14 practices in Ontario. *J Am Vet Med Assoc* 2000;217:1303–1309.
128. Shaw JR, Lagoni L. End-of-life communication in veterinary medicine: delivering bad news and euthanasia decision making. *Vet Clin North Am Small Anim Pract* 2007;37:95–108.
129. Frid MH, Perea AT. Euthanasia and thanatology in small animals. *J Vet Behav* 2007;2:35–39.
130. AVMA. Pet loss support hotlines (grief counseling). *J Am Vet Med Assoc* 1999;215:1805.
131. Hart LA, Mader B. Pet loss support hotline: the veterinary students' perspective. *Calif Vet* 1992;(Jan-Feb):19–22.
132. Neiburg HA, Fischer A. *Pet loss: a thoughtful guide for adults and children*. New York: Harper & Row, 1982.
133. Rogelberg SG, Reeve CL, Spitzmüller C, et al. Impact of euthanasia rates, euthanasia practices, and human resource practices on employee turnover in animal shelters. *J Am Vet Med Assoc* 2007;230:713–719.
134. Arluke A. Coping with euthanasia: a case study of shelter culture. *J Am Vet Med Assoc* 1991;198:1176–1180.
135. Reeve CL, Rogelberg SG, Spitzmuller C, et al. The caring-killing paradox: euthanasia-related strain among animal shelter workers. *J Appl Soc Psychol* 2005;35:119–143.
136. White DJ, Shawhan R. Emotional responses of animal shelter workers to euthanasia. *J Am Vet Med Assoc* 1996;208:846–849.
137. Wolfle T. Laboratory animal technicians: their role in stress reduction and human-companion animal bonding. *Vet Clin North Am Small Anim Pract* 1985;15:449–454.
138. Rohlf V, Bennett P. Perpetration-induced traumatic stress in persons who euthanize nonhuman animals in surgeries, animal shelters, and laboratories. *Soc Anim* 2005;13:201–219.
139. Bayne K. Development of the human-research bond and its impact on animal well-being. *ILAR J* 2002;43:4–9.
140. Chang FT, Hart LA. Human-animal bonds in the laboratory: how animal behavior affects the perspectives of caregivers. *ILAR J* 2002;43:10–18.
141. Overhulse KA. Coping with lab animal morbidity and mortality: a trainer's role. *Lab Anim (NY)* 2002;31:39–42.
142. Woods J, Shearer JK, Hill J. Recommended on-farm euthanasia practices. In: Grandin T, ed. *Improving animal welfare: a practical approach*. Wallingford, England: CABI Publishing, 2010.
143. Morrow WEM. Euthanasia hazards. In: Langley RL, ed. *Animal handlers. Occupational medicine: state of the art reviews*. Philadelphia: Hanley and Belfus, 1999;235–246.

144. Radek L, Kallionpää RE, Karvonen M, et al. Dreaming and awareness during dexmedetomidine- and propofol- induced unresponsiveness. *Br J Anaesth* 2018;121:260–269.
145. Reilly JS, ed. *Euthanasia of animals used for scientific purposes*. Adelaide, SA, Australia: Australia and New Zealand Council for the Care of Animals in Research and Teaching, Department of Environmental Biology, Adelaide University, 2001.
146. Murray MJ. Euthanasia. In: Lewbart GA, ed. *Invertebrate medicine*. Ames, Iowa: Blackwell, 2006;303–304.
147. Messenger JB, Nixon M, Ryan KP. Magnesium chloride as an anaesthetic for cephalopods. *Comp Biochem Physiol C* 1985;82:203–205.
148. Meyer RE, Morrow WEM. Euthanasia. In: Rollin BE, Benson GJ, eds. *Improving the well-being of farm animals: maximizing welfare and minimizing pain and suffering*. Ames, Iowa: Blackwell, 2004;351–362.
149. Fakkema D. *Operational guide for animal care and control agencies: euthanasia by injection*. Denver: American Humane Association, 2010.
150. Krueger BW, Krueger KA. US Fish and Wildlife Service fact sheet: secondary pentobarbital poisoning in wildlife. Available at: cpharm.vetmed.vt.edu/USFWS/. Accessed Mar 7, 2011.
151. O'Rourke K. Euthanatized animals can poison wildlife: veterinarians receive fines. *J Am Vet Med Assoc* 2002;220:146–147.
152. Otten DR. Advisory on proper disposal of euthanatized animals. *J Am Vet Med Assoc* 2001;219:1677–1678.

# Part II—Methods of Euthanasia

## M1 Inhaled Agents

### M1.1 COMMON CONSIDERATIONS

Inhaled vapors and gases require a critical concentration within the alveoli and blood for effect; thus, all inhaled methods have the potential to adversely affect animal welfare because onset of unconsciousness is not immediate. Distress may be created by properties of the agent (eg, pungency, hypoxia, hypercarbia) or by the conditions under which the agent is administered (eg, home cage or dedicated chamber, gradual displacement or prefilling of the container), and may manifest itself behaviorally (eg, overt escape behaviors, approach-avoidance preferences [aversion]) or physiologically (eg, changes in heart rate, SNS activity, HPA activity). Although SNS and HPA activation are well accepted as markers of a stress response, these systems are activated in response to both physical and psychological stressors and are not necessarily associated with higher-order CNS processing and conscious experience by the animal. Furthermore, use of SNS and HPA activation to assess distress during inhalation of euthanasia agents is complicated by continued exposure to the agents during the period between loss of consciousness and death.

Distress during administration of inhaled agents has been evaluated by means of both behavioral assessment and aversion testing. While overt behavioral signs of distress have been reported in some studies, others have not consistently found these effects. Through preference and approach-avoidance testing, all inhaled agents currently used for euthanasia have been identified as being aversive to varying degrees. Aversion is a measure of preference, and while aversion does not necessarily imply that the experience is painful, forcing animals into aversive situations creates distress. The conditions of exposure used for aversion studies, however, may differ from those used for stunning or killing. In addition, agents identified as being less aversive (eg, Ar or $N_2$ gas mixtures, inhaled anesthetics) can still produce overt signs of behavioral distress (eg, open-mouth breathing) in some species under certain conditions of administration (eg, gradual displacement). As previously noted in the section on consciousness, one of the characteristics of anesthesia in people is feeling as if one is having an out-of-body experience, suggesting a disconnection between one's sense of self and one's awareness of time and space.[1] Although we cannot know for certain the subjective experiences of animals, one can speculate similar feelings of disorientation may contribute to the observed signs of distress.

As for physical methods, the conditions under which inhaled agents are administered for euthanasia can have profound effects on an animal's response and, thus, agent suitability. Simply placing Sprague-Dawley rats into an unfamiliar exposure chamber containing room air produces arousal, if not distress.[2] Pigs are social animals and prefer not to be isolated from one another; consequently, moving them to the $CO_2$ stunning box in groups, rather than lining them up single file as needed for electric stunning, improves voluntary forward movement, reduces handling stress and electric prod use, and improves meat quality.[3]

That inhaled agents can produce distress and aversion in people raises concerns for their use in animals, in that the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training[4] state, "Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals." Interestingly, more than 40% of human children 2 to 10 years old display distress behaviors during sevoflurane induction, with 17% displaying significant distress and more than 30% physically resisting during induction.[5] Fear in children undergoing anesthesia may be due to odor, feel of the mask, or a true phobia of the mask.[6] Despite evidence of distress and aversion, inhaled anesthetics continue to be administered because the benefits associated with their use greatly outweigh any distress and/or aversion they may cause.

The suitability of any particular inhaled agent for euthanasia therefore depends largely on distress and/or pain experienced prior to loss of consciousness. Distress can be caused by handling, specific agent properties, or method of administration, such that a 1-size-fits-all approach cannot be easily applied. Suffering can be conceptualized as the product of severity, incidence, and duration. As a general rule, a gentle death that takes longer is preferable to a rapid, but more distressing death[7]; however, in some species and under some circumstances, the most humane and pragmatic option may be exposure to an aversive agent or condition that results in rapid unconsciousness with few or no outward signs of distress. Our goal is to identify best practices for administering inhaled agents, defining the optimal conditions for transport, handling, and agent selection and delivery to produce the least aversive and distressing experience for each species.

The following contingencies are common to all inhaled euthanasia agents:

(1) Time to unconsciousness with inhaled agents is dependent on the displacement rate, container volume, and concentration. An understanding of the principles governing delivery of gases or vapors into enclosed spaces is necessary for appropriate application of both prefill and gradual displacement methods. The desired final concentration will be achieved more quickly by using a greater displacement rate (see M1.2).

(2) Loss of consciousness will be more rapid if ani-

mals are initially exposed to a high concentration of the agent. However, for many agents and species, forced exposure to high concentrations can be aversive and distressing, such that gradual exposure may be the most pragmatic and humane option.

(3) Inhaled agents must be supplied in purified form without contaminants or adulterants, typically from a commercially supplied source, cylinder, or tank, such that an effective displacement rate and/or concentration can be readily quantified. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate.

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

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

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

(7) Neonatal animals appear to be resistant to hypoxia, and because all inhaled agents ultimately cause hypoxia, neonatal animals take longer to die than adults.[8] Inhaled agents can be used alone in unweaned animals to induce loss of consciousness, but prolonged exposure time or a secondary method may be required to kill the unconscious animal.

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

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

(10) When possible, inhaled agents should be administered under conditions where animals are most comfortable (eg, for rodents, in a darkened home cage[9]; for pigs, in small groups). If animals need to be combined, they should be of the same species and compatible cohorts, and, if needed, restrained or separated so that they will not hurt themselves or others. Chambers should not be overloaded and need to be kept clean to minimize odors that might cause distress in animals subsequently euthanized.

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

(12) Death must be verified following administration of inhaled agents. This can be done either by examination of individual animals or by adherence to validated exposure processes proven to result in death.[10] If an animal is not dead, exposure must be repeated or followed with another method of euthanasia.

## M1.2 PRINCIPLES GOVERNING ADMINISTRATION

Changes in gas concentration within any enclosed space involve 2 physical processes: 1) wash-in of new gas (or wash-out of existing gas) and 2) the time constant required for that change to occur within the container for a known flow rate. These processes are commonly combined in the practice of anesthesia to predict how quickly a change in concentration of an inhaled anesthetic will occur within a circle rebreathing circuit.[11] An understanding of how these processes work together is critical for the appropriate application of both gradual displacement and prefill immersion euthanasia methods.[12]

The rate of change of gas concentration within any enclosed space is a special form of nonlinear change known as an exponential process, and as such can be derived from the wash-in and wash-out exponential functions.[13] Briefly, for the wash-in exponential function the quantity under consideration rises toward a limiting value, at a rate that progressively decreases in proportion to the distance it still has to rise. In theory, the quantity approaches, but never reaches, 100%. Conversely, for the wash-out exponential function the quantity under consideration falls at a rate that progressively decreases in proportion to the distance it still has to fall. Again, in theory, the quantity approaches, but never reaches, zero.

The exponential wash-in and wash-out equations are used to derive the time constant ($\tau$) for an enclosed volume or space. This constant is mathematically equal to the enclosed volume or space undergoing wash-in or wash-out divided by the rate of flow, or displacement, into that space, where $\tau$ = volume/flow rate.[13,14] Thus, the time constant represents the

time at which the wash-in or wash-out process would have been complete had the initial rate of change continued as a linear function rather than an exponential function.[13] As such, the time constant is similar in concept to the half-life, although they are neither identical nor interchangeable.[14]

For the wash-in function, 1(τ) is required for the concentration of the inflowing gas to rise to 63.2% of the inflowing gas concentration, 2(τ) are required for the concentration to rise to 86.5%, and 3(τ) are required for the concentration to rise to 95%, with ∞(τ) required for the gas concentration within the container to equal the inflowing gas concentration. Conversely, for the wash-out function, 1(τ) is required for the remaining gas concentration to fall to 36.8% of the original value, 2(τ) are required for gas concentration to fall to 13.5%, 3(τ) are required for gas concentration to fall to 5%, with ∞(τ) required for gas concentration to fall to 0% **(Figure 3)**. The flow, or displacement rate, therefore determines the time constant for any given enclosed volume, such that increasing the flow rate will result in a proportional reduction of the wash-in and wash-out time constants for any size chamber (and vice versa).

Based on Figure 3, it can be shown that a gradual inflow or displacement rate of 20% of the chamber volume/minute represents a time constant (τ) value of 5 minutes (1 divided by 0.2/min) regardless of chamber volume. For example, $CO_2$ displacement rate equivalent to 20% of the chamber volume/min, as recommended by Hornett and Haynes[15] and Smith and Harrap,[16] is predicted to increase $CO_2$ concentration from zero to 63.2% in 5 minutes (1τ), to 86.5% in 10 minutes (2τ), and to 95% in 15 minutes (3τ). An examination of the published experimental data of Smith and Harrap confirms this, where $CO_2$ supplied at a displacement rate of 22% of chamber volume increased the $CO_2$ concentration to approximately 64% in 4.5 minutes (1τ for their chamber). Similarly, Niel and Weary[17] reported 65% after 340 seconds (1τ) and 87% after 600 seconds (2τ) for a $CO_2$ displacement rate of 17.5% of chamber volume/min. Prefill methods will require displacement rates of 3τ to attain 95% of the inflow gas concentration within the chamber.

Thus, gas displacement rate is critical to the humane application of inhaled methods, such that an appropriate pressure-reducing regulator and flow meter combination or equivalent equipment with demonstrated capability for generating the recommended displacement rate for the size container being utilized is absolutely necessary when compressed gases are used for euthanasia. Nitrogen, Ar, and CO are all commercially supplied in cylinders under high pressure, but $CO_2$ is unique in that it is supplied as a liquefied gas under high pressure. By reducing high pressure at the cylinder valve, gas flow is made constant to the flow meter as cylinder pressure decreases during use. With $CO_2$, the regulator also acts to prevent high gas flow rates that can lead to delivery of freezing gas and dry ice snow to the animals as well as regulator icing and cylinder freezing.

A distinction must be made between immersion, where animals are directly placed into a gas or vapor contained within a container, and the process of CAS as employed for the commercial stunning of poultry and hogs. Although a complete description of the operation of the commercial CAS systems currently in use is beyond the scope of this document, typically the entry point is open to the atmosphere with negligible concentrations of stunning gas present. Unlike immersion, animals are introduced at a controlled rate into a tightly controlled stunning atmospheric gradient, such that CAS can be considered to be a gradual displacement method.

## M1.3 INHALED ANESTHETICS

Overdoses of inhaled anesthetics (eg, ether, halothane, methoxyflurane, isoflurane, sevoflurane, desflurane, enflurane) have been used to euthanize many species.[18] Presently, only isoflurane, enflurane, sevoflurane, and desflurane are clinically available in the United States, although halothane and methoxyflurane are still available elsewhere in the world. Halothane induces anesthesia rapidly and is an effective inhaled agent for euthanasia. Enflurane is less soluble in blood than halothane, but, because of its lower vapor pressure and lower potency, induction rates may be similar to those for halothane. At deep anesthetic planes, convulsions may occur. Enflurane is an effective agent for euthanasia, but the associated seizure activity may be disturbing to personnel. Isoflurane is less soluble than halothane, and it induces anesthesia more rapidly. However, it has a pungent odor and onset of unconsciousness may be delayed due to breath holding. Due to lower potency, isoflurane also may require more drug to kill an animal, compared with halothane. Sevoflurane is less potent than either isoflurane or halothane and has a lower vapor pressure. Anesthetic concentrations can be achieved and maintained rapidly but more drug will be required to kill the animal. Although sevoflurane is reported to possess less of an objectionable odor than isoflurane, some species may struggle violently and experience apnea when sevoflurane is administered by face mask or induction chamber.[19] Like enflurane, sevoflurane induces epileptiform electrocortical activity.[20] Desflurane is currently the least soluble potent inhaled anesthetic, but the vapor is quite pungent, which may slow induction. This drug is so volatile that it could displace $O_2$ and induce hypoxemia during induction if supplemental $O_2$ is not provided. Both diethyl ether and methoxyflurane are highly soluble, and may be accompanied by agitation because anesthetic induction is quite slow. Diethyl ether is irritating to the eyes, nose, and respiratory airways; poses serious risks due to flammability and explosiveness; and has been used to create a model for stress.[21–24]

Although inhaled anesthetics are routinely used to produce general anesthesia in humans and animals,

these agents may be aversive and distressful under certain conditions. Flecknell et al[19] reported violent struggling accompanied by apnea and bradycardia in rabbits administered isoflurane, halothane, and sevoflurane by mask or induction chamber, and concluded these agents were aversive and should be avoided whenever possible. Leach et al[25–27] found inhaled anesthetic vapors to be associated with some degree of aversion in laboratory rodents, with increasing aversion noted as concentration increased; halothane was least aversive for rats, while halothane and enflurane were least aversive for mice. Makowska and Weary[28] also reported halothane and isoflurane to be aversive to male Wistar rats, but less so than $CO_2$. Aversion to inhaled anesthetics increases following initial exposure; rodents are more likely to leave the test chamber on second and subsequent exposures to inhaled anesthetics.[29–31] This may indicate the possibility of learned aversion to these agents.[32]

Anesthetic vapor is inhaled until respiration ceases and death ensues. Because the liquid state of most inhaled anesthetics is irritating, animals should be exposed only to vapors. With inhaled anesthetics, animals can be placed in a closed receptacle containing cotton or gauze soaked with an appropriate amount of liquid anesthetic[33] or anesthetic vapor can be introduced from a precision vaporizer.[34] Precision anesthetic vaporizers typically are limited to 5% to 7% maximum output between 0.5 and 10 L/min $O_2$ flow rate. Induction time will be influenced by dial setting, flow rate, and size of the container; time to death may be prolonged because $O_2$ is commonly used as the vapor carrier gas. The amount of liquid anesthetic required to produce a given concentration of anesthetic vapor within any closed container can be readily calculated[35]; in the case of isoflurane, a maximum of 33% vapor can be produced at 20°C. Sufficient air or $O_2$ must be provided during the induction period to prevent hypoxia.[33] In the case of small rodents placed in a large container, there will be sufficient $O_2$ in the chamber to prevent hypoxia. Larger species placed in small containers may initially need supplemental air or $O_2$.[33]

Nitrous oxide is the least potent of the inhalation anesthetics. In humans, the minimum alveolar concentration (defined as the median effective dose) for $N_2O$ is 104%; its potency in other species is less than half that in humans (ie, approx 200%). Because the effective dose for $N_2O$ is above 100%, it cannot be used alone at 1 atmosphere of pressure in any species without producing hypoxia prior to respiratory or cardiac arrest. As a result, animals may become distressed prior to loss of consciousness when $N_2O$ is used as the sole agent. Up to 70% $N_2O$ may be combined with other inhaled gases to speed the onset of anesthesia; however, the anesthetic contribution of $N_2O$ will be only half (20% to 30%) of that expected in humans due to its reduced potency in animals.[36]

The addition of $N_2O$ to inhaled gases may represent a refinement for euthanasia. Adding 75% $N_2O$ to 5% isoflurane in oxygen reduced time to loss of righting in mice by approximately 18%, while at 20% displacement rate administration of a mixture of 60% $N_2O$ with $CO_2$ reduced time to loss of righting by 10%.[37] However, while $N_2O$ narcosis prior to $CO_2$ in 0- to 7-day-old piglets reduced the amount of time the piglets were exposed to $CO_2$, it did not reduce the amount of distressful behaviors observed.[38]

Effective procedures should be in place to reduce animal worker exposure to anesthetic vapors.[39] Human workplace recommended exposure limits were issued in 1977 by the National Institute of Occupational Safety and Health; concentrations for halogenated inhaled anesthetics are not to exceed 2 ppm (1-hour ceiling) when used alone, or 0.5 ppm for halogenated anesthetics combined with 25-ppm $N_2O$ (time-weighted average during use). The American Conference of Government Industrial Hygienists has assigned a threshold limit value time-weighted average of 50 ppm for $N_2O$, 50 ppm for halothane, and 75 ppm for enflurane for an 8-hour time-weighted exposure. These concentrations were established because they were found to be attainable utilizing clinical scavenging techniques and there are no controlled studies proving exposure at these concentrations are safe. No National Institute of Occupational Safety and Health–recommended exposure limits exist for the 3 most currently used anesthetics (isoflurane, desflurane, and sevoflurane), and, at present, the Occupational Safety and Health Administration has no permissible exposure limits regulating these specific agents.

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

*Disadvantages*—(1) Inhaled anesthetics are aversive to rabbits and laboratory rodents and the same may be true for other species. Animals may struggle and become anxious during induction of anesthesia, with some animals exhibiting escape behaviors prior to onset of unconsciousness. Learned aversion to inhaled anesthetics occurs in rodents. Should apnea or excitement occur, time to loss of consciousness may be prolonged. (2) Ether is irritating, flammable, and explosive. Explosions have occurred when animals, euthanized with ether, were placed in an ordinary

(not explosion-proof) refrigerator or freezer and when bagged animals were placed in an incinerator. (3) Induction with methoxyflurane is unacceptably slow in some species. (4) Because of design limits on vapor output, precision anesthetic vaporizers may be associated with a longer wash-in time constant and, thus, longer induction time; time to death may be prolonged as $O_2$ is commonly used as the vapor carrier gas. (5) Nitrous oxide used alone will create a hypoxic atmosphere and will support combustion at high concentrations. (6) Personnel and animals may be injured by exposure to these agents. There is recognized potential for human abuse of inhaled anesthetics. (7) Because large amounts of inhaled anesthetics are absorbed and substantial amounts remain in the body for days, even after apparent recovery,[40] use of inhaled anesthetics for euthanasia is unsuitable for food-producing animals due to potential for tissue residues.

*General recommendations*—Inhaled anesthetics are acceptable with conditions for euthanasia of small animals (< 7 kg) where the following contingencies can be met: (1) In those species where aversion or overt escape behaviors have not been noted, exposure to high concentrations resulting in rapid loss of consciousness is preferred. Otherwise, gradual-fill methods can be used, keeping in mind the effect that chamber volume, flow rate, and anesthetic concentration will have on the time constant and rate of rise of anesthetic concentration. Inhaled anesthetics can be administered as the sole euthanasia agent or as part of a 2-step process, where animals are first rendered unconscious through inhaled anesthetic agent exposure and then subsequently killed by a secondary method. (2) Order of preference is isoflurane, halothane, sevoflurane, enflurane, methoxyflurane, and desflurane, with or without $N_2O$. Nitrous oxide should not be used alone. Methoxyflurane is acceptable with conditions only if other agents or methods are not available. Ether is not acceptable for euthanasia. (3) Although acceptable, inhaled anesthetics are generally not used for larger animals because of cost and difficulty of administration. (4) Exposure of workers to anesthetics must comply with state and federal occupational health and safety regulations. (5) Neonatal animals will require extended exposure times.[41]

## M1.4 CARBON MONOXIDE

Carbon monoxide is a colorless, odorless gas that is nonflammable and nonexplosive at concentrations < 12%. Carbon monoxide is a cumulative poison that produces fatal hypoxemia; it readily combines with hemoglobin and blocks uptake of $O_2$ by erythrocytes by forming carboxyhemoglobin.[42,43] Precisely because it is insidious, difficult to detect, and highly toxic even at low concentrations, the lethal properties of CO have long been recognized; indeed, approximately 50,000 emergency room visits for human CO poisoning occur in the United States annually.[44]

In people, the clinical presentation for CO inhalation is nonspecific, with headache, dizziness, and weakness the most common symptoms of low-level CO toxicosis. As concentrations of CO increase, these signs may be followed by decreased visual acuity, tinnitus, nausea, progressive depression, confusion, and collapse.[45] With higher-level exposure, coma, convulsions, and cardiorespiratory arrest may occur.[43] Carbon monoxide stimulates motor centers in the brain, such that loss of consciousness may be accompanied by convulsions and muscular spasms. Distinct signs of CO toxicosis are not evident until the CO concentration is 0.05% in air, and acute signs do not develop until CO concentration is approximately 0.2% in air. In humans, exposure to 0.32% CO and 0.45% CO for 1 hour will induce loss of consciousness and death, respectively.[46] Chronic exposure to low concentrations of CO may be a health hazard, especially with regard to cardiovascular disease and teratogenic effects.[43,44,47–49] An efficient exhaust or ventilation system is essential to prevent accidental exposure of humans.

In the past, mass euthanasia was accomplished by use of 3 different methods for generating CO: (1) chemical interaction of sodium formate and sulfuric acid, (2) exhaust fumes from gasoline internal combustion engines, and (3) commercially compressed CO in cylinders. The first 2 techniques are associated with substantial problems such as production of other gases, inadequate production of CO, inadequate gas cooling, inability to quantify delivery rate, and maintenance of equipment.

Ramsey and Eilmann[50] found that a concentration of 8% CO caused guinea pigs to collapse in 40 seconds to 2 minutes, and death occurred within 6 minutes. When used with mink and chinchillas, CO caused collapse in 1 minute, cessation of breathing in 2 minutes, and cardiac arrest in 5 to 7 minutes.[51,52] Chalifoux and Dallaire[53] evaluated the physiologic and behavioral characteristics of dogs exposed to 6% CO in air, and could not determine the precise time of loss of consciousness. Electroencephalographic recordings revealed 20 to 25 seconds of abnormal cortical function, and during this period the dogs became agitated and vocalized. It is not clear whether these behavioral responses are indicative of animal distress; however, humans in this phase reportedly are not distressed.[42] Subsequent studies[54] have revealed that tranquilization with acepromazine significantly decreases behavioral and physiologic responses of dogs euthanized with CO. Carbon monoxide is noted to be aversive to laboratory rats, but not as aversive as $CO_2$.[55]

In 1 study on cats,[56] CO from gasoline engine exhaust was compared with a combination of 70% $CO_2$ plus 30% $O_2$. Signs of agitation before loss of consciousness were greater for the $CO_2$-plus-$O_2$ combination. Time to complete immobilization was greater with $CO_2$ plus $O_2$ (approx 90 seconds) than with CO alone (approx 56 seconds).[56] In another study in neo-

natal pigs,[57] excitation was less likely to precede loss of consciousness if animals were exposed to a slow rise in CO concentration.

A study of an epidemic of avian influenza in the Netherlands in 2003 compared the use of $CO_2$ with CO for gassing whole houses of poultry.[58] The researchers noted that more convulsions were observed in the presence of CO and recommended that $CO_2$ was the preferred agent for this application due to safety regulations required for the use of CO.

*Advantages*—(1) Carbon monoxide induces loss of consciousness without pain and with minimal discernible discomfort, depending on species. (2) Hypoxemia induced by CO is insidious. (3) Death occurs rapidly if concentrations of 4% to 6% are used.

*Disadvantages*—(1) Carbon monoxide is an aversive agent for laboratory rodents and the same may be true for other species. (2) Safeguards must be taken to prevent and monitor exposure of personnel. (3) Electrical equipment exposed to CO (eg, lights and fans) must be spark free and explosion proof.

*General recommendations*—Carbon monoxide is acceptable with conditions for euthanasia, provided all of the following contingencies are met: (1) Personnel using CO must be instructed thoroughly in its use and must understand its hazards and limitations. (2) The CO chamber must be of the highest-quality construction and should allow for separation of individual animals. If animals need to be combined, they should be of the same species, and, if needed, restrained or separated so that they will not hurt themselves or others. Chambers should not be overloaded and need to be kept clean to minimize odors that might distress animals that are subsequently euthanized. (3) The CO source and chamber must be located in a well-ventilated environment, preferably out-of-doors. (4) The chamber must be well lighted and must allow personnel direct observation of animals. (5) The CO flow rate should be adequate to rapidly achieve a uniform CO concentration of at least 6% after animals are placed in the chamber, except for those species (eg, neonatal pigs) where it has been shown that less agitation occurs with a gradual rise in CO concentration.[57] (6) If the chamber is inside a room, CO monitors must be placed in the room to warn personnel of hazardous concentrations. (7) It is essential that CO use be in compliance with state and federal occupational health and safety regulations. (8) Carbon monoxide must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate. As gas displacement rate is critical to the humane application of CO, an appropriate pressure-reducing regulator and flow meter combination or equivalent equipment with demonstrated capability for generating the recommended displacement rate for the size container being utilized is absolutely necessary.

## M1.5 NITROGEN, ARGON

Nitrogen and Ar are odorless, colorless, and tasteless gases that are inert, nonflammable, and nonexplosive. Nitrogen normally comprises 78% of atmospheric air, whereas Ar comprises < 1%. These gases function in the current context by displacing air (and the $O_2$ it contains), causing anoxia. Exposure of Sprague-Dawley rats to severe hypoxic conditions (< 2% $O_2$) using either gas leads to unconsciousness around 90 seconds and death after 3 minutes using Ar or 7 minutes using $N_2$[2]; similar findings have been reported for dogs, rabbits, and mink.[51,52,59,60] Male Sprague-Dawley rats become hyperpneic, but can survive for more than 20 minutes in Ar or $N_2$ at an $O_2$ concentration of 4.9%.[61]

Rats are sensitive to even small changes in the concentration of $O_2$, and are able to detect concentrations both lower and higher than the 20.9% normally found in air.[62] Rats and mice allowed to travel between chambers containing different gases spent most of their time in the control chamber (containing air), but preferred a hypoxic chamber (containing Ar) to a chamber containing $CO_2$; however, the animals stayed only a few seconds in either gas.[25–27] Even when rats were trained to enter a chamber for a food reward they typically refused to enter, or left immediately after entering, when the atmosphere was hypoxic (< 2% $O_2$, 90% Ar).[63] When rats were exposed to gradually decreasing concentrations of $O_2$ and increasing concentrations of Ar, they always left the chamber before losing consciousness (typically when $O_2$ declined to about 7%).[64] With $N_2$ flowing at a rate of 39% of chamber volume/min ($\tau$ = 2 minutes 34 seconds), rats collapsed in approximately 3 minutes and stopped breathing in 5 to 6 minutes; regardless of flow rate, signs of panic and distress were evident before the rats collapsed and died.[15] During forced exposure to Ar gradually filling a chamber at a rate of 50% of the chamber volume/min ($\tau$ = 2 minutes), male Sprague-Dawley rats showed open-mouthed breathing and seizure-like behavior prior to loss of consciousness, suggesting similar potential for distress.[65] These observations are not surprising, as gradual displacement methods using $N_2$ or Ar, alone or mixed with other gases, are predicted by the wash-in and wash-out functions to result in prolonged exposure to hypoxic conditions.

In contrast, hypoxia produced by inert gases such as $N_2$ and Ar appears to cause little or no aversion in turkeys[66] or chickens[67]; these animals freely entered a chamber containing < 2% $O_2$ and > 90% Ar. When Ar was used to euthanize chickens, exposure to a chamber prefilled with Ar, with an $O_2$ concentration of < 2%, led to EEG changes and collapse in 9 to 12 seconds. Birds removed from the chamber at 15 to 17 seconds failed to respond to comb pinching. Continued exposure led to convulsions at 20 to 24 seconds. Somatosensory evoked potentials were lost at 24 to 34 seconds, and the EEG became isoelectric at 57 to 66 seconds.[68] With turkeys, immersion in 90% Ar

with 2% residual $O_2$ led to EEG suppression in 41 seconds, loss of Somatosensory evoked potential in 44 seconds, and isoelectric EEG in 101 seconds, leading the authors to conclude exposure times > 3 minutes were necessary to kill all birds.[69] Failure to maintain < 2% $O_2$ prolongs survival.[70,71] Gerritzen et al[72] also reported that chickens did not avoid chambers containing < 2% $O_2$; birds gradually became unconscious without showing signs of distress. Chickens[72–75] and turkeys[66] killed by hypoxia show less head shaking and open-beak breathing than birds exposed to $CO_2$.

Hypoxia produced by $N_2$ and Ar appears to reduce, but not eliminate, aversive responses in pigs. Pigs chose to place their head in a hypoxic (< 2% $O_2$, 90% Ar) chamber containing a food reward, remained with their head in the chamber until they became ataxic, and freely returned to the chamber once they regained posture.[76] In contrast, exposure to 90% Ar, 70% $N_2$/30% $CO_2$, and 85% $N_2$/15% $CO_2$ all resulted in signs of aversion, defined by the authors as escape attempts and gasping; however, the proportion of pigs showing these behaviors was lowest with Ar.[77] Early removal from the stunning atmosphere results in rapid regaining of consciousness, such that exposure times > 7 minutes are needed to ensure killing with these gases.[78]

Mink will also enter into a hypoxic chamber (< 2% $O_2$, 90% Ar), but will not remain until the point of unconsciousness. The duration of hypoxic exposure freely chosen is similar to the average duration of a dive for mink, suggesting they are able to detect hypoxia and modify their behavior to avoid detrimental effects.[79]

*Advantages*—(1) Nitrogen and Ar do not appear to be directly aversive to chickens or turkeys, and the resulting hypoxia appears to be nonaversive or only mildly aversive to these species. Similarly, $N_2$ and Ar gas mixtures do not appear to be directly aversive to pigs and appear to reduce, but not eliminate, the behavioral responses to hypoxia. (2) Nitrogen and Ar are nonflammable, nonexplosive, and readily available as compressed gases. (3) Hazards to personnel are minimal when used with properly designed equipment. (4) Argon and $N_2$-$CO_2$ gas mixtures are heavier than air and can be contained within an apparatus into which animals and birds can be lowered or immersed.[77]

*Disadvantages*—(1) Hypoxia resulting from exposure to these gases is aversive to rats, mice, and mink. (2) Based on the wash-in and wash-out functions, gradual displacement methods using $N_2$ or Ar, alone or mixed with other gases, may result in exposure to hypoxic conditions prior to loss of consciousness. Loss of consciousness will be preceded by open-mouth breathing and hyperpnea, which may be distressing for nonavian species. (3) Reestablishing a low concentration of $O_2$ (ie, 6% or greater) in the chamber before death will allow immediate recovery.[76,78,80] (4) Exposure times > 7 minutes are needed to ensure killing of pigs. (5) As with $CO_2$, rats euthanized with Ar demonstrate alveolar hemorrhage consistent with terminal asphyxiation.[65] (6) Argon costs about 3 times as much as $N_2$. (7) These gases tend to cause more convulsive wing flapping in poultry than $CO_2$ in air mixtures.

*General recommendations*—Hypoxia resulting from exposure to Ar or $N_2$ gas mixtures is acceptable with conditions for euthanasia of chickens and turkeys. Likewise, hypoxia resulting from Ar or $N_2$-$CO_2$ gas mixtures is acceptable with conditions for euthanasia of pigs, provided animals can be directly placed into a < 2% $O_2$ atmosphere and exposure times > 7 minutes are used. Use of Ar or $N_2$ is unacceptable for other mammals. These gases create an anoxic environment that is distressing for some species and aversive to laboratory rodents and mink; other methods of euthanasia are preferable for these species. Argon or $N_2$ hypoxia, defined as $O_2$ < 2%, could be used to kill these animals after they are rendered unconscious via an acceptable method, although prolonged exposure may be necessary to ensure death.

Nitrogen, Ar, and gas mixtures containing these gases must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition or displacement rate. As gas displacement rate is critical to the humane application of these gases, an appropriate pressure-reducing regulator and flow meter combination or equivalent equipment with demonstrated capability for generating the recommended displacement rate for the size container being utilized is absolutely necessary.

## M1.6 CARBON DIOXIDE

Inhalation of $CO_2$ causes respiratory acidosis and produces a reversible anesthetic state by rapidly decreasing intracellular pH.[81] Both basal and evoked neural activity are depressed soon after inhalation of 100% $CO_2$.[81–84] Inhalation of $CO_2$ at a concentration of 7.5% increases pain threshold, and concentrations of 30% and higher cause deep anesthesia and death with prolonged exposure.[16,17,85–87] Methods to administer $CO_2$ include placing animals directly into a closed, prefilled chamber containing $CO_2$, or exposure to a gradually increasing concentration of $CO_2$.

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

Carbon dioxide may cause pain due to the formation of carbonic acid when it contacts moisture on the respiratory and ocular membranes. In humans, rats, and cats, most nociceptors begin to respond at

$CO_2$ concentrations of approximately 40%.[88–91] Humans report discomfort begins at 30% to 50% $CO_2$ and intensifies to overt pain with higher concentrations.[92–94] Inhaled irritants are known to induce a reflex apnea and heart rate reduction, and these responses are thought to reduce transfer of harmful substances into the body.[95] In rats, 100% $CO_2$ elicits apnea and bradycardia, but $CO_2$ at concentrations of 10%, 25%, and 50% does not,[96] suggesting that gradual displacement methods are less likely to produce pain prior to unconsciousness in rodents where unconsciousness occurs before chamber concentration reaches levels associated with nociceptor activation.[32] On the other hand, bradycardia associated with $CO_2$ exposure in rats is reported to occur prior to loss of consciousness.[97]

Carbon dioxide has a key role as a respiratory stimulant, and elevated concentrations are known to cause profound effects on the respiratory system, cardiovascular system, and SNS.[98–100] In humans, air hunger begins at concentrations as low as 8% and this sensation intensifies with higher concentrations, becoming severe at approximately 15%.[101–103] With mild increases in inspired $CO_2$, increased ventilation results in a reduction or elimination of air hunger, but there are limits to this compensatory mechanism such that air hunger may reoccur during spontaneous breathing with moderate hypercarbia and hypoxemia.[104–106] Adding $O_2$ to $CO_2$ may or may not preclude signs of distress.[94,107–109] Supplemental $O_2$ will, however, prolong time to hypoxemic death and may delay onset of unconsciousness.

Although $CO_2$ exposure has the potential to produce a stress response, interpretation of the subjective experiences of animals is complicated. Borovsky[110] found an increase in norepinephrine in rats following 30 seconds of exposure to 100% $CO_2$. Similarly, Reed[111] exposed rats to 20 to 25 seconds of $CO_2$, which was sufficient to render them recumbent, unconscious, and unresponsive, and observed 10-fold increases in vasopressin and oxytocin concentrations. Indirect measures of SNS activation, such as elevated heart rate and blood pressure, have been complicated by the rapid depressant effects of $CO_2$ exposure. Activation of the HPA has also been examined during $CO_2$ exposure. Prolonged exposure to low concentrations of $CO_2$ (6% to 10%) has been found to increase corticosterone in rats[112,113] and cortisol in dogs.[114] In a single-blind study in healthy human volunteers, a single breath of 35% $CO_2$ was found to result in elevated cortisol concentrations and exposure was associated with an increase in fear.[115] It has been suggested that responses to systemic stressors associated with immediate survival, such as hypoxia and hypercapnia, are likely directly relayed from brainstem nuclei and are not associated with higher-order CNS processing and conscious experience.[116] In fact, Kc et al[117] found that hypothalamic vasopressin-containing neurons are similarly activated in response to $CO_2$ exposure in both awake and anesthetized rats. As stated previously, assessment of the animal's response to inhaled agents, such as $CO_2$, is complicated by continued exposure during the period between loss of consciousness and death.

Distress during $CO_2$ exposure has also been examined by means of behavioral assessment and aversion testing. Variability in behavioral responses to $CO_2$ has been reported for rats and mice,[15–17,65,108,118–120] pigs,[76,121–124] and poultry.[66,72–75,125–128] While signs of distress have been reported as occurring in animals in some studies, other researchers have not consistently observed these effects. This may be due to variations in methods of gas exposure and types of behaviors assessed, as well as strain variability.

Using preference and approach-avoidance testing, rats and mice show aversion to $CO_2$ concentrations sufficient to induce unconsciousness,[25,26] and are willing to forgo a palatable food reward to avoid exposure to $CO_2$ concentrations of approximately 15% and higher[28,63] after up to 24 hours of food deprivation.[107] Powell et al[9] reported a significant increase in anxious behaviors in mice with exposure to isoflurane, high-flow $CO_2$, and brightly lighted chambers. Mink will avoid a chamber containing a desirable novel object when it contains 100% $CO_2$.[129] In contrast to other species, a large proportion of chickens and turkeys will enter a chamber containing moderate concentrations of $CO_2$ (60%) to gain access to food or social contact.[67,72,121] Following incapacitation and prior to loss of consciousness, birds in these studies show behaviors such as open-beak breathing and head-shaking; these behaviors, however, may not be associated with distress because birds do not withdraw from $CO_2$ when these behaviors occur.[73] Thus, it appears that birds are more willing than rodents and mink to tolerate $CO_2$ at concentrations that are sufficient to induce loss of posture, and that loss of consciousness follows shortly afterwards. Using an approach-avoidance model, a preliminary study by Withrock et al[130] suggests that dairy goat kids exhibit no avoidance behaviors to 10% to 30% $CO_2$ and do not develop conditioned aversion.

Genetics may play a role in $CO_2$ response variability. Panic disorder in humans is genetically linked to enhanced sensitivity to $CO_2$.[131] The fear network, comprising the hippocampus, the medial prefrontal cortex, the amygdala, and its brainstem projections, appears to be abnormally sensitive to $CO_2$ in these patients.[132] The genetic background of some pigs, especially excitable lines such as the Hampshire and German Landrace, has been associated with animals that react poorly to $CO_2$ stunning, while calmer lines combining the Yorkshire or Dutch Landrace conformations show much milder reactions.[133] Given a choice, Duroc and Large White pigs will tolerate 30% $CO_2$ to gain access to a food reward, but will forgo the reward to avoid exposure to 90% $CO_2$, even after a 24-hour period of food deprivation.[76,121] A shock with an electric prod, however, is more aversive to Landrace × Large White pigs than inhaling 60% or 90%

$CO_2$, with pigs inhaling 60% $CO_2$ willing to reenter the crate containing $CO_2$.[122] Until further research is conducted, one can conclude that use of $CO_2$ may be humane for certain genetic lines of pigs and stressful for others.[133]

Recent studies involving mice have found regions of the amygdala associated with fear behavior to contain ASICs sensitive to elevated $CO_2$.[134] Fear behaviors and aversion in response to $CO_2$ exposure were reduced in mice in which the ASIC receptors were eliminated or inhibited, suggesting that aversive responses to $CO_2$ in rodents, and potentially other species, are mediated in part by an innate fear response. Further studies defining the presence of ASICs and their role in $CO_2$-induced fear in other rodent strains, as well as other animal species, are warranted.

As with other inhaled agents, time to unconsciousness with $CO_2$ is dependent on the displacement rate, container volume, and concentration used. In rats, unconsciousness is induced in approximately 12 to 33 seconds with 80% to 100% $CO_2$ and 40 to 50 seconds with 70% $CO_2$.[108,135] Similarly, a rapidly increasing concentration (flow rate > 50% of the chamber volume/minute) induces unconsciousness in only 26 to 48 seconds.[16,17,29,65,109,118,136] Leake and Waters[87] found that dogs exposed to 30% to 40% $CO_2$ were anesthetized in 1 to 2 minutes. For cats, inhalation of 60% $CO_2$ results in loss of consciousness within 45 seconds, and respiratory arrest within 5 minutes.[137] For pigs, exposure to 60% to 90% $CO_2$ causes unconsciousness in 14 to 30 seconds,[80–82,121] with unconsciousness occurring prior to onset of signs of excitation.[80,84] Euthanasia via exposure to $CO_2$ has been described for individual birds and small groups,[138] and its application to euthanasia of chickens, turkeys, ducks, and rabbits has been studied, resulting in information about times to collapse, unconsciousness and death, loss of somatosensory evoked potentials, and changes in EEG. Leghorn chicks 7 days of age collapsed in 12 seconds after exposure to 97% $CO_2$.[119] Raj[71] found that 2 minutes' exposure to 90% $CO_2$ was sufficient to kill day-old chicks exposed in batches. Broilers 5 weeks of age collapsed an average of 17 seconds after entering a tunnel filled with 60% $CO_2$.[72]

Unlike $N_2$ and Ar, which must be held within a very tight range of concentration for effective euthanasia, $CO_2$ can render poultry unconscious and kill over a wide range of concentrations. In tests where it took 8 seconds to achieve the target gas concentration, broilers and mature hens collapsed in 19 to 21 seconds at 65% $CO_2$ and 25 to 28 seconds at 35% $CO_2$.[139] In a gradual-fill study, ducks and turkeys lost consciousness before 25% $CO_2$ was reached and died after the concentration reached 45%.[125] At 49% $CO_2$, EEG suppression, loss of somatosensory evoked potentials, and EEG silence occurred in 11, 26, and 76 seconds in chickens.[140] In turkeys, EEG suppression took place in an average of 21 seconds at 49% $CO_2$, but was reduced to 13 seconds at 86% $CO_2$. In the same report, time to loss of somatosensory evoked potentials was not affected by gas concentration, averaging 20, 15, and 21 seconds, but time to EEG silence was concentration dependent (ie, 88, 67, and 42 seconds for 49%, 65%, and 86% $CO_2$, respectively).[141]

In rabbits, a 58% $CO_2$ displacement rate resulted in a significantly shorter time to insensibility and death than did a 28% displacement rate, with neither rate reported as inducing significant distress behaviors.[142] In contrast, Dalmau et al[143] compared immersion of meat rabbits into a commercial stunning system prefilled with 70%, 80%, or 90% $CO_2$. Loss of posture occurred within the first 30 seconds following initial exposure to $CO_2$; however, aversion (as nasal discomfort and vocalizations) was observed for 15 seconds prior to loss of posture. Dalmau et al concluded that despite the advantages their system provides in terms of prestunning handling and irreversibility, it is not free of animal welfare problems.

As a general rule, a gentle death that takes longer is preferable to a rapid, but more distressing death.[7] Gradual-fill $CO_2$ exposure causes aversion in rodents beginning at approximately a 15% concentration and lasting to onset of unconsciousness. If an appropriate gradual displacement rate is used, animals will lose consciousness before $CO_2$ concentrations become painful.[32,65] A 20%/min gradual displacement produces unconsciousness in 106 seconds at a $CO_2$ concentration of 30%[15,17,94,109]; a slower 10%/min displacement increases time to onset of unconsciousness to 156 seconds at a $CO_2$ concentration of 21%.[65] A 50%/min $CO_2$ displacement rate, while holding the chamber concentration just below 40% $CO_2$, minimizes the interval between onset of labored breathing and recumbency in mice; however, even at these rates, mice experienced > 30 seconds between onset of dyspnea and insensibility.[29] For poultry, immersion into relatively low concentrations or exposure to $CO_2$ concentrations producing a gradual induction of unconsciousness reduces convulsions compared with immersion into $N_2$ or Ar.[74,144] Carbon dioxide may invoke involuntary (unconscious) motor activity in birds, such as flapping of the wings or other terminal movements, which can damage tissues and be disconcerting for observers[119,145]; wing flapping is less with $CO_2$ than with $N_2$ or Ar.[144]

Due to respiratory adaptations in immature animals, reptiles, amphibians, and some burrowing and diving species (eg, lagomorphs, mustelids, aquatic birds, nonhatched birds, newly hatched chicks), high $CO_2$ concentrations, combined with extended exposure times, follow-up exposure to hypoxemia, or a secondary euthanasia method, may be required to ensure unconsciousness and death. High $CO_2$ concentrations (> 60%) and extended exposure times (> 5 minutes) are required for effective euthanasia of newly hatched chickens.[71,146] On the day of birth, rats and mice exposed to 100% $CO_2$ required exposure times of 35 and 50 minutes, respectively, to ensure death. By 10 days of age, exposure times of 5 minutes were sufficient to ensure death.[147,148] For adult mink,

5 minutes of exposure is sufficient to ensure death using 100% $CO_2$, but not using 70% $CO_2$.[51] Rabbits of the genus *Oryctolagus* also have prolonged survival times when exposed to $CO_2$.[149]

Inhaled halocarbon anesthetics have been proposed as alternatives to $CO_2$ for rodent euthanasia.[7,28,34] Inhaled anesthetics also produce various degrees of aversion in rodents[25–28] and are associated in other animals and humans with aversion, distress, and escape behaviors during anesthetic induction. Both mice and rats will always choose exposure to light (an aversive condition) over exposure to $CO_2$ (more aversive) in approach-avoidance tests, while > 50% of mice and rats will tolerate first exposure to an inhalant anesthetic until they become recumbent. However, mice and rats also show evidence of learned aversion to inhaled anesthetic agents and are more likely to escape the test chamber, and to do so more quickly, on second and subsequent exposures to inhaled anesthetics; in contrast, aversion to $CO_2$ does not increase with subsequent exposures.[32] Time to death may be prolonged as $O_2$ is commonly used as the vapor carrier gas with precision anesthetic vaporizers. Because large amounts of inhaled anesthetics are absorbed and substantial amounts remain in the body for days, even after apparent recovery,[40] euthanasia via inhaled anesthetics is unsuitable for food-producing animals because of the potential for tissue residues. Effective procedures should be in place to reduce worker exposure to anesthetic vapors. While incorporating inhaled anesthetics can be considered a potential refinement to $CO_2$ and anoxic methods, further consideration of the consequences associated with this strategy is warranted.

*Advantages*—(1) The rapid depressant, analgesic, and anesthetic effects of $CO_2$ are well established. (2) Carbon dioxide is readily available in compressed gas cylinders. (3) Carbon dioxide is inexpensive, nonflammable, and nonexplosive and poses minimal hazard to personnel when used with properly designed equipment. (4) Carbon dioxide does not result in accumulation of toxic tissue residues in animals from which food is produced.

*Disadvantages*—(1) Substantial and conflicting differences in response to $CO_2$ inhalation exist between and within species, strains, and breeds, making broad generalizations difficult. (2) Carbon dioxide, whether administered by prefill or gradual displacement methods, can be aversive to some species, and therefore potential exists to cause distress. (3) Because $CO_2$ is heavier than air, layering of gas or incomplete filling of a chamber may permit animals to climb or raise their heads above the effective concentrations and avoid exposure. (4) Immature individuals and some aquatic and burrowing species may have extraordinary tolerance for $CO_2$. (5) Reptiles and amphibians may breathe too slowly for the use of $CO_2$. (6) Euthanasia by exposure to $CO_2$ with $O_2$ supplementation may take longer than euthanasia by other means.[94,108,109] (7) Induction of loss of consciousness at concentrations < 80% may produce postmortem pulmonary and upper respiratory tract lesions.[94,150] (8) Dry ice and liquid $CO_2$ are potential sources of distress or injury if permitted to directly contact animals. (9) If animals are anesthetized with inhaled agents prior to completing the euthanasia process with $CO_2$, sufficient time should be allowed to prevent rapid recovery during the wash-in of $CO_2$ and the subsequent wash-out of inhaled agent.[32,151]

*General recommendations*—Carbon dioxide is acceptable with conditions for euthanasia in those species where aversion or distress can be minimized. Carbon dioxide exposure using a gradual-fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness; a displacement rate from 30% to 70% of the chamber volume/min is recommended for rodents.[15,63,65,142] Consideration should be given to the benefits of using a darkened home cage, while also keeping in mind the need to have the animal under observation.[9] Whenever gradual displacement methods are used, $CO_2$ flow should be maintained for at least 1 minute after respiratory arrest.[16] If animals need to be combined, they should be of the same species and, if needed, restrained so that they will not hurt themselves or others. Immature animals must be exposed to high concentrations of $CO_2$ for an extended period of time to ensure death. Oxygen administered together with $CO_2$ appears to provide little advantage and is not recommended for euthanasia. There is no apparent welfare advantage to killing animals with $CO_2$ when prior exposure to inhaled anesthetics has occurred.[32]

The practice of immersion, where conscious rodents are placed directly into a container prefilled with 100% $CO_2$, is unacceptable. A 2-step process, where animals are first rendered unconscious and then immersed into 100% $CO_2$, is preferred when gradual displacement methods cannot be used. Further studies are necessary before $CO_2$ immersion can be recommended for rabbits.[143] Immersion of poultry in lesser concentrations is acceptable with conditions as it does not appear to be distressing.

Carbon dioxide and $CO_2$ gas mixtures must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate. As gas displacement rate is critical to the humane application of $CO_2$, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size container being utilized is absolutely necessary.

# 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 possible[153] 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 nonirritating[160] 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

more than 200 µL of injectable anesthetic solution (ketamine:xylazine) is acceptable with conditions, resulting in death within 5 seconds of cessation of injection.[163] Intraosseous administration of some euthanasia solutions to awake animals may cause pain due to the viscosity of the agent, chemical irritation, or other reasons.[164] Administration of analgesics, slower injection of euthanasia agent, and other strategies that may reduce discomfort should be used where possible when administering euthanasia agents through preexisting intraosseous catheters.[165] Placement of intraosseous (greater trochanter of the femur, greater tubercle of the humerus, medial aspect of the proximal tibia) catheters for administration of euthanasia agents and intracardiac, intrahepatic, intrasplenic, or intrarenal injections are acceptable only when performed on anesthetized or unconscious animals (with the exception of intrahepatic injections in cats as discussed in the Companion Animals section of the text). These routes are not acceptable in awake mammals and birds due to the difficulty and unpredictability of performing the techniques accurately with minimal discomfort. In some poikilotherms for which intracardiac puncture is the standard means of vascular access (eg, some snakes and other reptiles), intracardiac administration of euthanasia solutions in awake animals is acceptable. With the exceptions of IM delivery of ultrapotent opioids (ie, etorphine and carfentanil) and IM delivery of select injectable anesthetics, IM, SC, intrathoracic, intrapulmonary, intrathecal, and other nonvascular injections are not acceptable routes of administration for injectable euthanasia agents in awake animals.

### M2.2.2 Immersion

Euthanasia of fish and some aquatic amphibians and invertebrates must take into account the vast differences in metabolism, respiration, and tolerance to cerebral hypoxia among the various aquatic species. Because aquatic animals have diverse physiologic and anatomic characteristics, optimal methods for delivery of euthanasia agents will vary. In many situations, the immersion of aquatic animals in water containing euthanasia agents is the best way to minimize pain and distress. The response of aquatic animals to immersion agents can vary with species, concentration of agent, and quality of water; consideration of these factors should be made when selecting an appropriate euthanasia agent. Immersion agents added to water may be absorbed by multiple routes, including across the gills, via ingestion, and/or through the skin.

Ideally, immersion agents added to water will be nonirritating to skin, eyes, and oral and respiratory tissues and will result in rapid loss of consciousness (often, but not always, measured as a loss of righting response) with minimal signs of distress or avoidance behavior. Currently there are no US FDA-approved drugs for the euthanasia of aquatic animals. United States EPA–registered agents for poisoning fish (eg, rotenone, antimycin) are not recommended as euthanasia agents, because their mechanisms of action and times to death do not fit the criteria for euthanasia. Additionally, the use of these agents requires a restricted pesticide applicator's license and extralabel use of these agents is a violation of federal law. Agents approved by the FDA as tranquilizers and anesthetics for fish (eg, MS 222, metomidate) have been used extralabel as euthanasia agents for aquatic animals.

### M2.2.3 Topical Application

Absorption of topically applied agents is slow and variable, making topical application an unacceptable means of efficient delivery of euthanasia agents for most animals. Exceptions include animals with highly permeable skin to which nonirritating, rapidly absorbed agents are applied (eg, amphibians euthanized with benzocaine gel). Currently there are no topical euthanasia agents that are US FDA approved for any species.

### M2.2.4 Oral Administration

The oral route has several disadvantages when considered for administration of euthanasia agents, including lack of established drugs and doses, variability in agent bioavailability and rate of absorption, potential difficulty of administration (including potential for aspiration), and potential for loss of agent through vomiting or regurgitation (in species that are capable of these functions). For these reasons, the oral route is unacceptable as a sole means of euthanasia. However, the oral route is an appropriate means to deliver sedatives prior to administration of parenteral euthanasia agents.

## M2.3 BARBITURIC ACID DERIVATIVES

Barbiturates depress the CNS in descending order, beginning with the cerebral cortex, with loss of consciousness progressing to anesthesia. With an overdose, deep anesthesia progresses to apnea due to depression of the respiratory center, and this is followed by cardiac arrest.

All barbituric acid derivatives used for anesthesia are acceptable for euthanasia when administered IV. There is a rapid onset of action, and loss of consciousness induced by barbiturates results in minimal or transient pain associated with venipuncture. Desirable barbiturates are those that are potent, nonirritating, long acting, stable in solution, and inexpensive. Sodium pentobarbital best fits these criteria and is most widely used, although others such as secobarbital are also acceptable. More research into the efficacy, speed of action, and nociceptive responses of nonvascular routes of barbiturate euthanasia solutions is needed before changes in recommendations for these alternate routes can be made.

*Advantages*—(1) A primary advantage of barbiturates is speed of action. This effect depends on the dose, concentration, route, and rate of the injec-

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

dogs provided that it is administered IV by a highly skilled person at recommended dosages and at proper injection rates. If barbiturates are not available, its extralabel use in cats is also acceptable. Routes of administration of Tributame other than IV injection are not acceptable.

## M2.6 T-61

T-61 is an injectable, nonbarbiturate, nonnarcotic mixture of embutramide, mebozonium (mebenzonium) iodide, and tetracaine hydrochloride.[172] Embutramide induces narcosis and respiratory depression, while mebozonium causes nondepolarizing muscular paralysis.[173] Concern has been expressed that the paralytic effect of mebozonium occurs before embutramide-induced unconsciousness, creating a potential for animal distress prior to loss of consciousness, as manifested by muscular activity and/or vocalization during injection. However, electrophysiologic studies in dogs and rabbits have shown that loss of consciousness and loss of motor activity occur simultaneously following T-61 injection.[174] Although many consider the aesthetically unpleasant reactions of dogs to T-61 injection to be similar to dysphoria seen during the induction phases of anesthesia, the behavior demonstrated during these reactions can cause distress in personnel witnessing euthanasia. Because of these concerns, T-61 has been voluntarily withdrawn from the market by the manufacturer and is no longer manufactured or commercially available in the United States, although it is available in Canada and other countries. T-61 should be administered only IV and at carefully monitored rates of injection to avoid dysphoria during injection.

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

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

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

## M2.7 ULTRAPOTENT OPIOIDS

Etorphine hydrochloride and carfentanil citrate are ultrapotent opioids (10,000 times as potent as morphine sulfate) that are FDA approved for the immobilization of wildlife.[175] These opioids have been used as immobilization and extralabel euthanasia drugs primarily for large animals, particularly wildlife. Carfentanil has been used transmucosally in a lollipop form to euthanize captive large apes.[176] These drugs act on μ opioid receptors to cause profound CNS depression, with death secondary to respiratory arrest.

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

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

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

## M2.8 DISSOCIATIVE AGENTS AND $\alpha_2$-ADRENERGIC RECEPTOR AGONISTS

Injectable dissociative agent and $\alpha_2$-adrenergic receptor agonists induce rapid loss of consciousness, and sometimes muscle relaxation, prior to surgery, dentistry, or other procedures. These agents are sometimes given prior to administration of euthanasia solutions to minimize animal distress, facilitate restraint, and/or provide a more aesthetic euthanasia environment for owner-attended euthanasia. In overdose situations, these agents can cause death; however, doses that consistently will produce death have not been established for most species. In mice, injection of 100 μL of a 10:1 (mg:mg) solution of ketamine:xylazine resulted in death within 3 to 5 seconds after completion of the injection.[163] Intraperitoneal injection of dissociative agents in combination with $\alpha_2$-adrenergic receptor agonists at 5 times the anesthetic dose has been used as a means of euthanizing laboratory animals.[177]

*Advantages*—(1) These agents are readily available. (2) The combination of these agents causes rapid loss of consciousness. (3) Although IV injection for euthanasia is preferred, these combinations can be delivered IM in situations where IV administration is not feasible or is dangerous.

*Disadvantages*—(1) These agents are not FDA approved for use as agents of euthanasia. (2) Doses that consistently produce rapid death have not been established for most drugs and species. (3) The cost

of the higher doses of agents required to cause death may substantially exceed that of an approved euthanasia agent. (4) Many dissociative agents are controlled substances and their acquisition, storage, and use are restricted. (5) Some injectable agents can be hazardous for human personnel if accidental exposure occurs. (6) The environmental impact of residues of injectable anesthetics in the remains of euthanized animals has not been determined.

*General recommendations*—In species for which effective euthanasia doses and routes have been established, overdose of dissociative agent–$\alpha_2$-adrenergic combinations is an acceptable method of euthanasia. These agents are acceptable with conditions in situations where approved euthanasia drugs are not available or as secondary means of euthanasia in already anesthetized animals provided utmost care is taken to ensure that death has occurred prior to disposing of animal remains. These combinations are also acceptable as the first step in a 2-step euthanasia method. Until the environmental impact of tissue residues is determined, special care must be taken in the disposal of animal remains. Injectable anesthetics should not be used in animals intended for consumption.

## M2.9 POTASSIUM CHLORIDE AND MAGNESIUM SALTS

Although unacceptable when used in conscious vertebrate animals, a solution of potassium chloride, magnesium chloride, or magnesium sulfate injected IV or intracardially in an animal that is unconscious or under general anesthesia is an acceptable way to induce cardiac arrest and death. The potassium ion is cardiotoxic, and rapid IV or intracardiac administration of 1 to 2 mmol/kg (0.5 to 0.9 mmol/lb) of body weight (1 to 2 mEq $K^+$/kg; 75 to 150 mg of potassium chloride/kg [34.1 to 68.2 mg/lb]) will cause cardiac arrest.[178] This is an injectable technique for euthanasia of livestock or wildlife species that may reduce the risk of toxicosis for predators or scavengers in situations where the remains of euthanized animals may be consumed.[179,180] Potassium chloride injected IV at 3 mg/kg (1.4 mg/lb) into parrots anesthetized with isoflurane caused mild vocalization in 1 of 6 birds and resulted in asystole in 68 seconds.[181] Use of 10 mg/kg (4.5 mg/lb) IV in anesthetized parrots resulted in involuntary muscle tremors in 5 of 6 birds and caused asystole in 32.8 seconds. Neither dosage resulted in histologic artifacts.

Magnesium salts may also be mixed in water for use as immersion euthanasia agents for some aquatic invertebrates. In these animals, magnesium salts induce death through suppression of neural activity.[181]

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

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

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

## M2.10 CHLORAL HYDRATE AND α-CHLORALOSE

Chloral hydrate (1,1,1-trichloro-2,2,-dihydroxyethane) was once used in combination with magnesium sulfate and sodium pentobarbital as an economical anesthesia and euthanasia agent for large animals, but now is rarely used for this application in veterinary medicine. α-Chloralose is a longer-acting derivative of chloral hydrate that has been used for anesthesia of laboratory animals, particularly for study of cerebrovasculature.[182,183] When administered IV, these agents have almost immediate sedative action, but unless combined with other anesthetics, the onset of anesthesia is delayed. Death is caused by hypoxemia resulting from progressive depression of the respiratory center, and may be preceded by gasping, muscle spasms, and vocalization.

*Advantages*—(1) Historically, chloral hydrate was an inexpensive anesthetic and euthanasia agent, making it economical for large animals. (2) Schedule IV status makes chloral hydrate somewhat simpler to obtain, store, and administer than schedule II or III drugs, such as sodium pentobarbital.

*Disadvantages*—(1) Chloral hydrate depresses the cerebrum slowly; therefore, restraint may be a problem for some animals. (2) Chloral hydrate is no longer available as an FDA-approved drug in the United States, so it must be compounded from bulk drug.

This is problematic because of the lack of manufacturing controls, tests for potency, and potential illegality of using products made from bulk drugs.

*General recommendations*—Chloral hydrate and α-chloralose are not acceptable euthanasia agents because the associated adverse effects may be severe, reactions can be aesthetically objectionable, and other products are better choices.

## M2.11 ALCOHOLS

Ethanol and other alcohols increase cell membrane fluidity, alter ion channels within neural cells, and decrease nerve cell activity.[184] Alcohols induce death through nervous system and respiratory depression, resulting in anesthesia and anoxia. Alcohols have been used as secondary euthanasia methods for some fish species[185] and as primary injectable euthanasia agents in laboratory mice 35 days of age or older.[186] Intraperitoneal injection of ethanol has been used for the euthanasia of laboratory mice (*Mus musculus*) and rats (*Rattus norvegicus*).[187] Mice receiving IP injections of 0.5 mL of 70% ethanol developed gradual loss of muscle control, coma, and death within 2 to 6 minutes. However, mice < 35 days of age experienced prolonged time to death following IP ethanol administration, so this method is not appropriate in mice in this age group or for neonatal mice (*M musculus*).[188] This method is not appropriate for euthanasia of rats due to volume of ethanol required and prolonged time to respiratory effects.[187] Tribromoethanol is used as an anesthetic agent in laboratory rodents.

*Advantages*—(1) Alcohol is inexpensive and readily available.

*Disadvantages*—(1) Alcohols produce dose-related irritation to tissue. (2) Onset of insensibility and death can be delayed following alcohol administration. (3) The volume required to euthanize animals larger than mice renders most alcohols impractical as euthanasia agents. (4) Alcohols are not FDA approved as euthanasia agents. (5) Tribromoethanol is not commercially available as a pharmaceutical-grade product and must be compounded.

*General recommendations*—Ethanol in low concentrations is an acceptable secondary means of euthanasia in fish rendered insensible by other means and as a primary or secondary means of euthanasia of some invertebrates. Immersion in high concentrations (eg, 70%) of ethanol is not acceptable. Ethanol is acceptable with conditions as an agent of euthanasia for mice 35 days of age and older, but is unacceptable as an agent of euthanasia for larger species. Tribromoethanol is acceptable with conditions as a method for euthanasia of laboratory rodents when approved by the IACUC and prepared, stored, and administered at the appropriate dosage.

## M2.12 MS 222 (TMS)

Tricaine methanesulfonate, commonly referred to as MS 222, is an anesthetic agent that is FDA approved for temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals.[189] Tricaine methanesulfonate has been used for euthanasia of reptiles, amphibians, and fish. Tricaine is a benzoic acid derivative and, in water of low alkalinity (< 50 mg/L as $CaCO_3$), the solution should be buffered with sodium bicarbonate.[190] A 10 g/L stock solution can be made, and sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5 for the solution. The stock solution should be protected from light and refrigerated or frozen if possible. The solution should be replaced monthly and any time a brown color is observed.[191] Potency is increased in warm water and decreased in cold water.[190] Immersion of fish in solutions of MS 222 for 30 minutes following loss of rhythmic opercular movement is sufficient for euthanasia of most fish. Due to species differences in response to MS 222, a secondary method of euthanasia is recommended in many fish to ensure death.[190,192] In the United States, there is a 21-day withdrawal time for MS 222; therefore, it is not appropriate for euthanasia of animals intended for consumption.

MS 222 rapidly enters the CNS and alters nerve conduction through blockade of voltage-sensitive sodium channels.[192] Additionally, accumulation within ventricular myocardium results in decreased cardiovascular function. Death is due to decreased nervous and cardiovascular function.

Studies[193] with *Xenopus laevis* (African clawed frog or platanna) have shown that the concentrations of MS 222 traditionally used for amphibian euthanasia (0.25 to 0.5 g/L) are not sufficient to induce reliable euthanasia in this species. Immersion of frogs in 5 g/L of MS 222 resulted in deep anesthesia within 4 minutes, but at least 1 hour of immersion at this concentration was required to reliably euthanize 100% of frogs. The authors of that study recommended that if a concentration of MS 222 < 5 g/L or a shorter time frame than 1 hour is allowed, a secondary euthanasia method should be used for *X laevis.* Intracoelomic injection of MS 222 at the highest possible dosage (2,590 mg/kg [1,177 mg/lb]) did not result in euthanasia, with 6 of 20 frogs regaining mobility within 3 hours after injections. Consequently, intracoelomic injection of MS 222 is not considered to be an acceptable method of euthanasia for *X laevis* and possibly other amphibians.

A 2-stage euthanasia method for reptiles using MS 222 has been described.[172] The first stage entails intracoelomic injection of 250 to 500 mg/kg (113.6 to 227.3 mg/lb) of a pH-neutralized solution (0.7% to 1.0% MS 222), which results in rapid loss of consciousness (< 30 seconds to 4 minutes). Once unconsciousness occurs, a second intracoelomic injection of unbuffered 50% MS 222 is administered.

*Advantages*—(1) Tricaine methanesulfonate is soluble in both fresh and salt water and can be used for a wide variety of fish, amphibians, and reptiles. (2) Tricaine methanesulfonate is commercially available and is not a controlled substance, which increases ease of acquisition, storage, and administration.

*Disadvantages*—(1) Tricaine methanesulfonate is expensive and may be cost prohibitive for use for large fish, amphibians, and reptiles or for large populations. (2) There appears to be substantial species variability in response to MS 222, with some species requiring higher doses or secondary measures to ensure death. (3) Injection of MS 222 is not appropriate for fish as rapid excretion via the gills renders it ineffective by this route.[190] (4) Tricaine methanesulfonate cannot be used in animals intended for human consumption. (5) Occupational exposure to MS 222 has been associated with retinal toxicity in humans.[194] (6) Tricaine methanesulfonate is not FDA approved for use as an agent of euthanasia. (7) The impact of MS 222 residues in euthanized fish on the environment or scavenger species has not been determined.

*General recommendations*—Tricaine methanesulfonate is an acceptable method of euthanasia for fish and for some amphibians and reptiles. When used for some fish and some amphibians (eg, *Xenopus* spp), a secondary method should be used to ensure death. By itself, intracoelomic injection of MS 222 is not an acceptable euthanasia method for *X laevis* and possibly other amphibians. Animals euthanized with MS 222 should not be used as food sources for humans or other animals.

## M2.13 BENZOCAINE HYDROCHLORIDE

Benzocaine base, a compound similar to MS 222, is not water soluble and therefore is prepared as a stock solution (100 g/L) with acetone or ethanol; the presence of these solvents can be irritating to tissues. Conversely, benzocaine hydrochloride is water soluble and can be used directly for either anesthesia or euthanasia of fish and amphibians.[172,191] Benzocaine-containing products should be protected from light and protected from freezing or excessive heat (> 40°C). Topical application of 7.5% or 20% benzocaine hydrochloride gel on an amphibian's ventrum is effective and does not require buffering. Similarly to MS 222, benzocaine acts through blockade of voltage-sensitive sodium channels within the CNS and heart, resulting in depression of the nervous and cardiovascular systems.

The application of benzocaine hydrochloride gel to the ventral abdomen of amphibians (20% concentration; 2.0-cm X 1.0-mm application) is an effective means of anesthesia and euthanasia for some species.[193,195,196] Following application of the gel to the ventrum of *X laevis* and placement in a wet bucket, righting and withdrawal reflexes subsided within 7 minutes, and death occurred within 5 hours.[193] No evidence of dermal injury, loss of dermal hydration, or difficulty breathing was associated with topical application of benzocaine hydrochloride gel to amphibians. A recent investigation on euthanasia of adult *X laevis* describes a dose of 182 mg/kg (82.7 mg/lb) of benzocaine hydrochloride gel as effective.[193] A comparison of benzocaine hydrochloride application with ice-slurry immersion for euthanasia of bony bream (*Nematalosa erebi*) indicated that, for certain warm water fish species, an ice-slurry elicits less motor response than benzocaine overdose as a method of euthanasia, but additional work is needed to determine the most humane method.[197]

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

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

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

## M2.14 EUGENOL

Cloves contain a number of essential oils, including eugenol, isoeugenol, and methyleugenol.[198] Eugenol comprises 85% to 95% of the essential oils in cloves, and has been used as a food flavoring and a local anesthetic for human dentistry. Eugenol is classified as a "generally regarded as safe" food additive by the FDA and as an exempted least-toxic pesticide active ingredient by the US EPA. Eugenol exhibits antifungal, antibacterial, antioxidant, and anticonvulsant activity. Some other components of clove oil, such as isoeugenol, are equivocal carcinogens based on studies in rodents.[199] Clove oil and its extracts have become popular as anesthetic agents for freshwater and marine fish because of their wide availability, low cost, and shorter induction times when compared with MS 222.[200,201] When compared with MS 222 as an anesthetic agent, eugenol was found to have a more rapid induction, prolonged recovery, and narrow margin of safety, as it can cause rapid onset of ventilatory failure at high concentrations (> 400 mg/L).[202]

The anesthetic mechanism of clove oil and its derivatives has been poorly studied, but they appear to act similarly to other local anesthetics by inhibition of voltage-sensitive sodium channels within the nervous system.[175] Studies[203–205] of rodents indicate this class of agents may cause paralysis in addition to their anesthetic effects.

*Advantages*—(1) Clove oil and its derivatives are widely available, are relatively inexpensive, and are not controlled substances. (2) These agents have a short induction time. (3) Clove oil and its derivatives are effective at a wide range of water temperatures. (4) Eugenol has low risk for human toxicity at concentrations used for euthanasia of fish.

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

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

## M2.15 2-PHENOXYETHANOL

Immersion in 2-phenoxyethanol has been used for anesthesia and euthanasia of fish at concentrations of 0.3 to 0.5 mg/L or higher.[200] The solubility of 2-phenoxyethanol is reduced in colder water. The mechanism of action of 2-phenoxyethanol is poorly understood, but death is thought to occur from hypoxia secondary to CNS depression. Fish should be kept in the 2-phenoxyethanol solution for at least 10 minutes after cessation of opercular movement.

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

*Disadvantages*—(1) Induction times can be prolonged. (2) There are species variations in dosage levels and duration of exposure required for euthanasia. (3) Some species exhibit hyperactivity prior to loss of consciousness. (4) 2-phenoxyethanol is not FDA approved for use as an agent of euthanasia. (5) The impact of 2-phenoxyethanol residues in euthanized fish on the environment or scavenger species has not been determined.

*General recommendations*—Although there are probably more efficient immersion agents available, 2-phenoxyethanol is an acceptable method of euthanasia for fish under certain circumstances. 2-phenoxyethanol is not an acceptable means of euthanasia in animals intended for consumption.

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

Quinaldine has low solubility in water and therefore must first be dissolved in acetone or alcohol and then buffered with bicarbonate.[200] The potency of quinaldine varies with species, water temperature, water pH, and mineral content of water. Quinaldine acts through depression of sensory centers of the CNS.

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

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

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

## M2.17 METOMIDATE

Metomidate is a highly water-soluble, nonbarbiturate hypnotic that acts by causing CNS depression. It is a rapidly acting euthanasia compound when used at 10 times the upper limit of the recommended anesthetic dose. Some species of fish require higher concentrations of metomidate to achieve anesthesia.[206] Fish should be kept in the solution for at least 10 minutes following cessation of opercular movement. Metomidate is not an acceptable means of euthanasia in animals intended for human consumption. Metomidate is currently listed as a fish sedative and anesthetic in the FDA Index of Legally Marketed Unapproved New Animal Drugs for Minor Species,[207] which prohibits any off-label use of metomidate.

*Advantages*—(1) Metomidate can be utilized in a 1-step immersion method for euthanasia of fish. (2) Metomidate is not a controlled substance.

*Disadvantages*—(1) Extralabel use of metomidate for euthanasia is prohibited due to its status as an FDA-indexed drug. (2) Some species of fish require higher concentrations of metomidate for anesthesia, making metomidate a poor choice for euthanasia agent for those species. (3) Fish euthanized using metomidate are not acceptable for consumption. (4) The impact of metomidate residues in euthanized fish on the environment or scavenger species has not been determined.

*General recommendations*—Should its regulatory status change to allow its use for euthanasia, metomidate is an acceptable method for euthanasia of some species of fish under certain circumstances. Metomidate is not an acceptable means of euthanasia in animals intended for consumption.

## M2.18 SODIUM HYPOCHLORITE

Sodium hypochlorite (bleach) and solutions made from calcium hypochlorite granules act as solvents and oxidants in tissue, resulting in saponification of fatty acids, denaturation of proteins, and derangement of cellular processes.[208] Hypochlorite has been used to euthanize unhatched and hatched zebrafish up to 7 days after fertilization, after which time hatchlings are considered developed beyond an

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 chloride[212]). 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.

Since most physical methods involve trauma, there is inherent risk for animals and people. If the method is not performed correctly, personnel may be injured or the animal may not be effectively euthanized; personnel skill and experience are essential. Inexperienced persons should be trained by experienced persons and should practice on euthanized animals or anesthetized animals to be euthanized until they are proficient in performing the method properly and humanely. After the method has been applied, death must be confirmed before disposal of the remains.

## M3.2 PCB

Penetrating captive bolts have been used for euthanasia of ruminants, horses, swine, laboratory rabbits, dogs, and alpacas.[215,216] Standard captive bolts may be inadequate for euthanizing water buffalo.[217] Their mode of action is concussion and trauma to the cerebral hemisphere and brainstem. More recent research[218] in cattle indicates that changing the location of the shot to be a slightly higher location increases the probability of brainstem disruption. Adequate restraint is important to ensure proper placement of captive bolts. A cerebral hemisphere and the brainstem must be sufficiently disrupted by the projectile to induce sudden loss of consciousness and subsequent death. Young steers and heifers were successfully rendered unconscious without disruption of the brainstem with a high-powered, pneumatic PCB.[219]

Appropriate placement of captive bolts for various species has been described.[220–224] Signs of effective captive bolt penetration and death are immediate collapse and a several-second period of tetanic spasm, followed by slow hind limb movements of increasing frequency.[225,226] The corneal reflex must be absent and the eyes must open into a wide blank stare and not be rotated.[227]

There are 2 types of PCBs: a regular PCB and an air injection PCB. In both cases, the bolts penetrate the brain. In the air injection PCB, air under high pressure is injected through the bolt into the brain to increase the extent of tissue destruction. Powder-activated guns that use the traditional captive bolt are available in 9 mm, .22 caliber, and .25 caliber.[220] Captive bolt guns powered by compressed air (pneumatic) are also available in regular and air injection types. All captive bolt guns require careful maintenance and cleaning after each day of use. Lack of maintenance is a major cause of captive bolt gun failure for both powder-activated and pneumatic captive bolt guns.[228] Repeated firing of a captive bolt for extended periods may reduce effectiveness.[229] This is due to the gun becoming overheated.

*Advantages*—(1) Both regular and air injection PCBs may be used effectively for euthanasia of animals in research facilities and on the farm, when the use of drugs for this purpose is inappropriate or impractical. (2) They do not chemically contaminate tissues.

*Disadvantages*—(1) Euthanasia by captive bolt can be aesthetically displeasing. (2) Death may not occur if equipment is not maintained and used properly. (3) The air injection captive bolt must never be used on ruminants that will be used for food because of concerns about contamination of meat with specified risk materials (neurologic tissue). (4) Because the PCB is destructive, brain tissue may not be able to be examined for evidence of rabies infection or chronic wasting disease. Leg-paddling motions that occur after an animal is shot with a captive bolt are spinal reflexes that occur in completely unconscious animals after the spinal cord has been severed at the base of the skull.[230,231]

*General recommendations*—Use of the PCB is acceptable with conditions and is a practical method of euthanasia for horses, ruminants, swine, rabbits, and poultry. To ensure death, it is recommended that animals be immediately exsanguinated or pithed (see adjunctive methods) unless a powerful captive bolt gun designed for euthanasia is used. These guns have recently become available and reduce the need to apply an adjunctive method. Ruminants used for food should not be pithed to avoid contamination of the carcass with specified risk materials. Captive bolt guns used for larger species must have an extended bolt.

## M3.3 NPCB

Research has shown that NPCBs are less effective on cattle than PCBs.[232,233] The NPCB has a wide, mushroom-shaped head that does not penetrate the brain of large mammals, such as adult cattle, slaughter-weight pigs, sows, and adult sheep. In general, NPCB guns only stun animals and should not be used as a sole method of euthanasia. Correct positioning is critical for an effective stun of an adult cow. Nonpenetrating captive bolts are not effective for stunning bulls, adult swine, or cattle with long hair.

Purpose-built or powder-activated pneumatic NPCB guns have recently been developed and successfully used for euthanasia of suckling pigs up to 9 kg (20 lb).[234]

*Advantage*—Less damage to the brain.

*Disadvantages*—(1) Nonpenetrating captive bolt guns only stun animals and therefore are generally not effective as a sole means of euthanasia. The exception is nonpenetrating pneumatic captive bolt guns that have been purpose-built for euthanasia of suckling pigs,[b] neonatal ruminants,[220] and turkeys.[235] (2) Depending on degree of destruction, use of an NPCB may preclude postmortem diagnostics for diseases of the brain, including rabies and chronic wasting disease.

*General recommendations*—In general, NPCB guns should not be used as a sole method of euthanasia. However, pneumatic purpose-built NPCB guns have been used successfully to euthanize suckling pigs,[b] neonatal ruminants,[220] and turkeys.[236]

## M3.4 MANUALLY APPLIED BLUNT FORCE TRAUMA TO THE HEAD

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

Manually applied blunt force trauma to the head has been used primarily to euthanize small laboratory animals with thin craniums.[223,237,238] It has also been applied for euthanasia of young piglets. The anatomic features of neonatal calves make manually applied blunt force trauma to the head unacceptable as a method of euthanasia for this species.

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

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

*Disadvantages*—(1) Manually applied blunt force trauma is displeasing for personnel who have to perform it. (2) Repeatedly performing manually applied blunt force trauma can result in personnel fatigue, loss of efficacy, and humane concerns. (3) Trauma to the cranium can damage tissues and interfere with diagnosis of brain diseases.

*General recommendations*—Replace, as much as possible, manually applied blunt force trauma to the head with alternate methods. Manually applied blunt force trauma is not acceptable for neonatal calves, because of their anatomic features.

## M3.5 GUNSHOT

A properly placed gunshot can cause immediate insensibility and a humane death. Under some conditions, a gunshot may be the only practical method of euthanasia. Shooting should only be performed by highly skilled personnel trained in the use of firearms and only in jurisdictions that allow for legal firearm use. The safety of personnel, the public, and other animals that are nearby should be considered. The procedure should be performed outdoors and in areas where public access is restricted.

In applying gunshot to the head as a method of euthanasia for captive animals, the firearm should be aimed so that the projectile enters the brain, causing instant loss of consciousness.[33,224,239–242] This must take into account differences in brain position and skull conformation between species, as well as the energy requirement for penetration of the skull and sinus.[221,240] Accurate targeting for a gunshot to the head in various species has been described.[240,241,243] For wildlife and other freely roaming animals, the preferred target area should be the head. It may, however, not be possible or appropriate to target the head when killing is attempted from large distances (missed shots may result in jaw fractures or other nonfatal injuries) or when diagnostic samples of brain tissue are needed for diagnosis of diseases (eg, rabies, chronic wasting disease) important to public health. The appropriate firearm should be selected for the situation, with the goal being penetration and destruction of brain tissue without emergence from the contralateral side of the head.[220,244] A gunshot to the heart or neck does not immediately render animals unconscious, but may be required when it is not possible to meet the POE's definition of euthanasia.[245]

### M3.5.1 Basic Principles of Firearms

To determine whether a firearm or type of ammunition is appropriate for euthanizing animals, some basic principles must be understood. The kinetic energy of an object increases as the speed and weight or mass of the object increase. In reference to firearms, the bullet's kinetic energy (muzzle energy) is the energy of a bullet as it leaves the end of the barrel when the firearm is discharged. Muzzle energy is frequently used as an indicator of a bullet's destructive potential. The heavier the bullet and the greater its velocity, the higher its muzzle energy and capacity for destruction of objects in its path.

Muzzle energy (E) can be expressed as the mass of the bullet (M) times its velocity (V) squared, divided by 2.[246] However, to accommodate units of measure commonly used in the United States for civilian firearms, energy (E) is expressed in foot-pounds. This is calculated by multiplying the bullet's weight (W) times its velocity in feet per second (V) squared and dividing the result by 450,450. The International System of Units expresses muzzle energy in joules (J).

The muzzle energy of commercially available ammunition varies greatly. For example, the difference in muzzle energy generated from a .357 Magnum handgun loaded with a 180 grain compared with a 110 grain bullet may differ by as much as 180 foot-pounds.[246] Velocity has an even greater impact on bullet energy than bullet mass. Selection of an appropriate bullet and firearm is critical to good performance when conducting euthanasia procedures. Lighter-weight, higher-velocity bullets can have high

muzzle energy, but decreased penetration, which can be an issue when penetrating thick bones.

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

### M3.5.2 Muzzle Energy Requirements

For euthanasia, the combination of firearm and ammunition[247] selected must achieve a muzzle energy of at least 300 feet-lb (407 J) for animals weighing up to 400 lb (180 kg). For animals larger than 400 lb, 1,000 feet-lb (1,356 J) is required.[220] Handguns do not typically achieve the muzzle energy required to euthanize animals weighing more than 400 lb (180 kg), and therefore rifles must be used to euthanize these animals.

Some would argue that the muzzle energies recommended are well beyond what is necessary to achieve satisfactory results. Anecdotal comment suggests that the .22 LR is one of the most frequently used firearms for euthanasia of livestock with varying degrees of success. There is little doubt that success or failure is partially related to firearm and bullet characteristics, but probably more so to selection of the ideal anatomic site (ie, a site more likely to affect the brainstem) for conducting the procedure. The Humane Slaughter Association lists multiple firearms for euthanasia of livestock, including shotguns (12, 16, 20, 28, and .410 gauges), handguns (.32 to .45 caliber), and rifles (.22, .243, .270, and .308). In general, when comparing handguns with rifles, the longer the barrel, the higher the muzzle velocity. Therefore, if a .22 is used for euthanasia it is best fired from a rifle. The .22 should never be used on aged bulls, boars, or rams.[248]

### M3.5.3 Bullet Selection

While much of the emphasis in euthanasia by gunshot is placed on choice of the most appropriate firearm, it should be remembered that the gun is only the means of delivery. Bullet selection is quite possibly the most important consideration for euthanasia of livestock by gunshot. There are 3 basic types of bullets pertinent to this discussion: solid points, hollow points, and full metal jacket bullets. Solid-point bullets are preferred for euthanasia since they are designed for greater penetration of their targets. Under ideal conditions this type of bullet will also undergo moderate expansion to a mushroom shape that increases its destructive characteristics. Hollow-point bullets are designed with a hollowed-out tip that causes rapid expansion and fragmentation of the bullet on impact. The hollow-point design allows maximum transfer of energy without risk of overpenetration. For applications where it may be desirable to control or reduce the degree of bullet penetration, hollow-point bullets are preferred. However, for the purposes of euthanasia of livestock the first requirement is that the bullet possesses sufficient energy to penetrate the skull and enter the underlying brain tissue. The concern with hollow-point bullets is that, since the majority of their energy is released on impact through fragmentation, they may not have sufficient energy to traverse the skull. The other extreme is represented by full metal jacket bullets, which do not expand or fragment on impact with their targets. These bullets have a lead core with a thin metal jacket cover that completely covers (surrounds) the bullet. Full metal jacket bullets generally achieve maximum penetration, which may have benefits for euthanasia but also creates additional safety hazards for bystanders. Shotguns loaded with shot shells (number 4, 5, or 6) have sufficient energy to traverse the skull but, unlike the possibility of bullets from either a handgun or rifle, rarely exit the skull. These are important considerations when selecting a firearm for on-farm euthanasia. Probably the most important point to be made relative to the use of gunshot for euthanasia is that scientific information on firearm and bullet selection is lacking. This is an area of urgent need in euthanasia research.

### M3.5.4 Firearm Safety

Firearm safety cannot be overemphasized. Guns are inherently dangerous and must be handled with caution at all times. This needs to become the mindset in handling and use of firearms. Common recommendations include the following: 1) assume that all firearms are loaded, 2) always know where the muzzle is and never allow it to point in the direction of oneself or bystanders, 3) keep fingers away from the trigger and out of the trigger guard until ready to fire, 4) be sure of the target and what lies beyond it, and 5) always be sure that the gun is unloaded when not in use. Readers desiring more information or training on proper use of firearms are advised to contact local hunter safety programs. These programs offer training in firearm safety and also provide information on rules and regulations for firearm use.

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

oblongata, which will assure immediate loss of consciousness and rapid death.

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

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

*General recommendations*—When other methods cannot be used, an accurately delivered gunshot is acceptable with conditions for euthanasia.[241,249] When an animal can be appropriately restrained, the PCB, preferably one designed for euthanasia, is preferred to a gunshot because it is safer for personnel. Prior to shooting, animals accustomed to the presence of humans should be treated in a calm and reassuring manner to minimize anxiety. In the case of wild animals, gunshots should be delivered with the least amount of prior human contact necessary. Gunshot should not be used for routine euthanasia of animals in animal control situations, such as municipal pounds or shelters.

## M3.6 CERVICAL DISLOCATION

Cervical dislocation has been used for many years for euthanasia and, when performed by well-trained individuals on appropriate animals, appears to be humane. However, there are few scientific studies available to confirm this observation. The method has been used to euthanize small birds, poultry, mice, immature rats (< 200 g [7.1 oz]), and rabbits. For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull. For immature rabbits, the head is held in one hand and the hind limbs in the other. The animal is stretched and the neck is hyperextended and dorsally twisted to separate the first cervical vertebra from the skull.[223,250] For poultry and other birds, the legs of the bird should be grasped (or wings if grasped at the base) and the neck stretched by pulling on the head while applying a ventrodorsal rotational force to the skull. Crushing of cervical vertebrae and spinal cord is not acceptable unless the bird is first rendered unconscious. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency.

Data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation in rats,[251] and unlike decapitation, rapid exsanguination does not contribute to loss of consciousness.[252,253] For some classes of poultry there is evidence that cervical dislocation may not cause immediate unconsciousness.[235,236,254,255]

*Advantages*—(1) Cervical dislocation is a method that may induce rapid loss of consciousness.[150,251] (2) It does not chemically contaminate tissue. (3) It is rapidly accomplished.

*Disadvantages*—(1) Cervical dislocation may be aesthetically displeasing to personnel performing or observing the method. (2) Cervical dislocation requires mastering technical skills to ensure loss of consciousness is rapidly induced. (3) Its use for euthanasia is limited to small birds, poultry, mice, immature rats (< 200 g), and rabbits.

*General recommendations*—Manual cervical dislocation is acceptable with conditions for euthanasia of small birds, poultry, mice, rats weighing < 200 g, and rabbits when performed by individuals with a demonstrated high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be unconscious or anesthetized prior to cervical dislocation. For heavy rats and rabbits, the large muscle mass in the cervical region makes manual cervical dislocation physically more difficult.[256] When performed on poultry, cervical dislocation must result in luxation of the cervical vertebrae without primary crushing of the vertebrae and spinal cord. In some classes of poultry, there is evidence that cervical dislocation may not cause immediate unconsciousness.[235,236,254,255] In these cases, other physical methods such as blunt force trauma or decapitation may be more humane[257] and should be employed when available or practicable.

Those responsible for the use of this method must ensure that personnel performing cervical dislocation have been properly trained and consistently apply it humanely and effectively.

## M3.7 DECAPITATION

Decapitation can be used to euthanize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides a means of obtaining anatomically undamaged brain tissue for study.[258]

Although it has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation,[259] more recent studies and reports[251–253] indicate this activity does not imply that pain is perceived, and in fact conclude that loss of consciousness develops rapidly. Visually evoked potentials in mice were reduced more quickly after cervical dislocation compared with decapitation.[260]

Guillotines designed to accomplish decapitation of adult rodents and small rabbits in a uniformly instantaneous manner are commercially available. Guil-

lotines are not commercially available for neonatal rodents, but sharp blades can be used for this purpose.

*Advantages*—(1) Decapitation appears to induce rapid loss of consciousness.[251–254] (2) It does not chemically contaminate tissues. (3) It is rapidly accomplished.

*Disadvantages*—(1) Handling and restraint required to perform decapitation may be distressful for animals.[261] (2) The interpretation of the presence of electrical activity in the brain following decapitation has created controversy, and its importance may still be open to debate.[251–254,259] (3) Personnel performing this method should recognize the inherent danger of the guillotine and take precautions to prevent personal injury. (4) Decapitation may be aesthetically displeasing to personnel performing or observing the method.

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

## M3.8 ELECTROCUTION

Alternating current has been used to euthanize dogs, cattle, sheep, goats, swine, chickens, foxes, mink, and fish.[227,239,242,262–270] Fifty- or 60-cycle electrical current is more effective than higher frequencies.[271,272] Electrocution induces death by cardiac fibrillation, which causes cerebral hypoxia.[269,270,273] However, animals do not lose consciousness for 10 to 30 seconds or more after onset of cardiac fibrillation. It is imperative that animals be unconscious and insensible to pain before being electrocuted. Unconsciousness can be induced by any method that is acceptable or acceptable with conditions, including passing a current through the brain.[274]

Parameters for use of electricity to induce unconsciousness are readily available.[239,275] When electricity is used to induce unconsciousness, a current is passed through the brain, which will induce a grand mal epileptic seizure.[267,270,276,277] Signs of effective induction of the seizure are extension of the limbs, opisthotonus, downward rotation of the eyeballs, and a tonic (rigid) spasm changing to a clonic (paddling) spasm with eventual muscle flaccidity.

There are 3 approaches to the use of electricity for euthanasia. They are head only, 1-step head to body, and 2-step head and body. To be effective for euthanasia all 3 of these methods must induce a grand mal epileptic seizure.

For the head-only procedure, an electrical current is passed through the head to induce a seizure. This causes a temporary loss of consciousness of 15 to 30 seconds' duration,[276–278] but does not induce cardiac fibrillation. For this reason, head-only application must be immediately followed by a secondary procedure to cause death. When the head-only procedure is applied, the grand mal seizure is easily observable. Electrically induced cardiac fibrillation, exsanguination, or other appropriate adjunctive methods may be used to achieve death and should be performed within 15 seconds of when the animal becomes unconscious.

In the 1-step head-to-body approach an electrical current is simultaneously passed through both the brain and the heart. This simultaneously induces a grand mal seizure and electrocutes the animal by inducing cardiac arrest.[263,276,279–281] Because electricity passes through the spinal column, clinical signs of the grand mal seizure may be masked; however, it is usually possible to see a weak tonic phase and weak clonic phase after a 3-second application. If current is applied for more than 3 seconds, tonic and clonic spasms may be blocked. The 1-step approach must be used with amperage settings that have been scientifically verified to induce a seizure. Recommended amperages are 1.25 amps for pigs, 1 amp for sheep, and 1.25 amps for cattle.[238,281] Denicourt et al[282] report that 110 V at 60 Hz applied for 3 seconds was effective for pigs up to 125 kg (275 lb).

In the 2-step method an electrical current is passed through the head to induce unconsciousness, then a second current is passed through either the side of the body or the brisket to induce cardiac arrest.[283,284] Applying the second current by an electrode placed on the side of the body behind the forelimb has been reported to be effective.[283]

A common cause of failure to induce unconsciousness is incorrect placement of the electrodes.[279] Experiments with dogs revealed that electrode positions where the brain is bypassed do not cause instantaneous unconsciousness. When electricity passes only between the forelimbs and hind limbs or neck and feet, it causes the heart to fibrillate but does not induce sudden loss of consciousness.[273] The animal will be electrocuted, but will remain conscious until it dies from cardiac fibrillation.

Three options are available for correct electrode placement for the head-only method, including on both sides of the head between the eye and ear, the base of the ear on both sides of the head, and diagonally below one ear and above the eye on the opposite side of the head. For the 1-step (head-to-back) method, the head electrode may be placed on the forehead or immediately behind the ear. The head electrode should never be placed on the neck because the brain

will be bypassed.[285] Diagonal movement of the electrical current through the body can be accomplished by placing the head electrode behind one ear and the body electrode on the opposite side. When the 2-step procedure is used, placement of the body electrode behind the forelimb is effective.[283]

Electrodes consisting of a metal band or chain around the nose and a band or chain around the thorax appear to be effective for pigs weighing up to 125 kg.[282]

When electrical methods of euthanasia are used, the following signs of return to consciousness must be absent: rhythmic breathing, righting reflex, vocalization, eyeblink, and tracking of a moving object.[283] Gasping and nystagmus may be present in animals that have been successfully rendered unconscious with electricity. Gasping should not be confused with rhythmic breathing, and nystagmus (a rapid vibrating or fluttering of the eye) should not be confused with eyeblink (complete closure and then complete opening of the eye, which occurs without touching).

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

*Disadvantages*—(1) Electrocution may be hazardous to personnel. (2) It is not useful for dangerous, intractable animals that are difficult to restrain. (3) It is aesthetically objectionable because of violent extension and stiffening of the limbs, head, and neck. (4) It may not result in death in small animals (< 5 kg [11 lb]) because ventricular fibrillation and circulatory collapse do not always persist after cessation of current flow. (5) Sometimes it is not effective in dehydrated animals.[275] (6) Personnel must be familiar with appropriate placement of electrodes and use of equipment. (7) Purpose-built equipment must be used.

*General recommendations*—Euthanasia by electrocution is acceptable with conditions. It requires special skills and equipment that will ensure passage of sufficient current through the brain to induce loss of consciousness and induce tonic and clonic epileptic spasms. Unconsciousness must be induced before cardiac fibrillation or simultaneously with cardiac fibrillation. Cardiac fibrillation must never occur before the animal is rendered unconscious. Methods that apply electric current from head to tail, head to foot, or head to moistened metal plates on which the animal stands are unacceptable. The 2-step method should be used in situations where there may be questions about sufficient current to induce a grand mal seizure with tonic and clonic spasms. This approach enables observation of tonic and clonic spasms before a second current is applied to induce cardiac arrest. Although acceptable with conditions if the aforementioned requirements are met, the method's disadvantages outweigh its advantages in most applications. Electroimmobilization that paralyzes an animal without first inducing unconsciousness is extremely aversive and is unacceptable.[274,275] For both humane and safety reasons, the use of household electrical cords is not acceptable.

## M3.9 KILL TRAPS

Mechanical kill traps are used for the collection and killing of small, free-ranging mammals for commercial purposes (fur, skin, or meat), scientific purposes, to stop property damage, and to protect human safety. Their use remains controversial and kill traps do not always render a rapid or stress-free death consistent with the criteria established for euthanasia by the POE.[286] For this reason, use of live traps followed by other methods of euthanasia is preferred. There are a few situations when that is not possible (eg, pest control) or when it may actually be more stressful for the animals or dangerous for humans to use live traps.

Although newer technologies are improving kill trap performance in achieving loss of consciousness quickly, individual testing is recommended to be sure the trap is working properly.[287] If kill traps must be used, the most humane option available must be chosen,[288–290] as evaluated by use of International Organization for Standardization testing procedures,[291] or by the methods of Gilbert,[292] Proulx et al,[293,294] or Hiltz and Roy.[295]

To reach the required level of efficacy, traps may need to be modified from manufacturers' production standards. In addition, as specified in scientific studies, trap placement (ground vs tree sets), bait type, set location, selectivity apparatus, body placement modifying devices (eg, sidewings, cones), trigger sensitivity, and trigger type, size, and conformation are essential considerations that could affect a kill trap's ability to reach these standards. Several kill traps, modifications, and set specifics have been scientifically evaluated and found to meet standards for various species.[293,294,296–309,c]

*Advantage*—(1) Free-ranging small mammals may be killed with minimal distress associated with handling and human contact. (2) Multiple animals may be effectively killed in situations where public health, animal behavior, or other constraints exist.

*Disadvantages*—(1) Traps may not kill within acceptable time periods. (2) Selectivity and efficiency is dependent on the skill and proficiency of the operator. (3) Nontarget species may be trapped and injured.

*General recommendations*—Kill traps do not consistently meet the POE's criteria for euthanasia, and may be best characterized as humane killing under some circumstances. At the same time, it is recognized they can be practical and effective for scientific animal collection or pest control when used in a manner that ensures selectivity, a swift kill, and no damage to body parts needed for field research.[310,311] Care must be taken to avoid trapping and injuring nontarget species.

Traps need to be checked at least once daily. In those instances when an animal is wounded or cap-

tured but not dead, the animal must be killed quickly and humanely. Kill traps should be used only when other acceptable methods are not practical or have failed. Traps for nocturnal species should not be activated during the day to avoid capture of diurnal species.[310] Trap manufacturers should strive to meet their responsibility of minimizing pain and suffering in target species. Traps that entrap a conscious animal in glue or other sticky substance are not acceptable for euthanasia, but may be required for pest control. Glue traps are acceptable for insects or spiders.

## M3.10 MACERATION

Maceration, via use of a specially designed mechanical apparatus having rotating blades or projections, causes immediate fragmentation and death of poultry up to 72 hours old and embryonated eggs. A review[312] 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 $CO_2$ 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,[313] Agriculture Canada,[314] World Organization for Animal Health,[239] and European Union.[315]

*Advantages*—(1) Death is almost instantaneous. (2) The method is safe for workers. (3) Large numbers of animals can be killed quickly.

*Disadvantages*—(1) Special equipment is required and it must be kept in excellent working condition. (2) Personnel must be trained to ensure proper operation of equipment. (3) Macerated tissues may present biosecurity risks.

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

## M3.11 FOCUSED BEAM MICROWAVE IRRADIATION

Heating by focused beam microwave irradiation is used primarily by neurobiologists to fix brain metabolites in vivo while maintaining the anatomic integrity of the brain.[316] Microwave instruments have been specifically designed for use in euthanasia of laboratory mice and rats. The instruments differ in design from kitchen units and may vary in maximal power output from 1.3 to 10 kW. All units direct their microwave energy to the head of the animal. The power required to rapidly halt brain enzyme activity depends on the efficiency of the unit, the ability to tune the resonant cavity, and the size of the rodent head.[317] There is considerable variation among instruments in the time required for loss of consciousness and euthanasia. A 10-kW, 2,450-MHz instrument operated at a power of 9 kW will increase the brain temperature of 18- to 28-g (0.6- to 1.0-oz) mice to 79°C in 330 milliseconds, and the brain temperature of 250- to 420-g (8.8- to 14.8-oz) rats to 94°C in 800 milliseconds.[318]

*Advantages*—(1) Loss of consciousness is achieved in < 100 milliseconds, and death in < 1 second. (2) This is the most effective method to fix brain tissue in vivo for subsequent assay of enzymatically labile chemicals.

*Disadvantages*—(1) Instruments are expensive. (2) Only animals the size of mice and rats can be euthanized with commercial instruments that are currently available.

*General recommendations*—Focused beam microwave irradiation is a humane method for euthanizing small laboratory rodents if instruments that induce rapid loss of consciousness are used. Only instruments that are designed for this use and have appropriate power and microwave distribution can be used. Microwave ovens designed for domestic and institutional kitchens are unacceptable for euthanasia.

## M3.12 THORACIC (CARDIOPULMONARY, CARDIAC) COMPRESSION

Thoracic (cardiopulmonary, cardiac) compression is a method that has been used by biologists to terminate the lives of wild small mammals and birds, mainly under field conditions.[319] Although it has been used extensively in the field, data supporting this method are limited, including degree of distress induced and time to unconsciousness or death.[320] Given our current knowledge of the physiology of both small mammals and birds, it cannot be assured that thoracic compression does not result in pain and distress before animals become unconscious. Consequently, thoracic compression is an unacceptable means of euthanizing animals that are not deeply anesthetized or insentient due to other reasons, but is appropriate as a secondary method for animals that are insentient.

The consensus of veterinarians with field biology training and expertise is that portable equipment and alternate methods are currently available to field biologists for euthanasia of wildlife under field conditions, in accordance with current standards for good animal welfare. Anesthetics can be administered prior to application of thoracic compression. Depending on taxa, open-drop methods or injectable agents that do not require DEA registration can be used. These alternate methods are generally practical to use with minimal training and preparation as standard procedures prior to embarking upon fieldwork.

## M3.13 ADJUNCTIVE METHODS

### M3.13.1 Exsanguination

Exsanguination can be used to ensure death subsequent to stunning, or in otherwise unconscious animals. Because anxiety is associated with extreme hypovolemia, exsanguination must not be used as a sole means of euthanasia.[321] Animals may be exsanguinated to obtain blood products, but only when they are sedated, stunned, or anesthetized.[322]

### M3.13.2 Pithing

In general, pithing is used as an adjunctive procedure to ensure death in an animal that has been rendered unconscious by other means. For some species, such as frogs, with anatomic features that facilitate easy access to the CNS, pithing may be used as a sole means of euthanasia, but an anesthetic overdose is more suitable.

Pithing in ruminants is performed by inserting a pithing rod or tool through the entry site produced in the skull by a PCB or free bullet.[323] The operator manipulates the pithing tool to substantially destroy both brainstem and spinal cord tissue. Muscular activity during pithing can be considerable, but is followed by quiescence that facilitates exsanguination or other procedures. Pithing is sometimes used in advance of exsanguination to reduce involuntary movement in stunned animals.[324] This method should not be used in ruminants intended for food because of possible contamination of the meat with specified risk materials.

Disposable pithing rods are available for purchase. The rod must be somewhat rigid, yet flexible, and of sufficient length to reach the brain and spinal column through the access point in the skull.

## M4 Footnotes

a. MS 222, Argent Laboratories Inc, Redmond, Wash.
b. T-61, Intervet Canada Corp, Kirkland, QC, Canada.
c. Twitchell C, Roy LD, Gilbert FF, et al. Effectiveness of rotating-jaw killing traps for beaver (*Castor Canadensis*) (oral presentation). North Am Aquat Furbearer Symp, Starkville, Miss, May 1999.

## M5 References


1. Alkire MT. General anesthesia. In: Banks WP, ed. *Encyclopedia of consciousness*. San Diego: Elsevier/Academic Press, 2009;296–313.
2. Sharp J, Azar T, Lawson D. Comparison of carbon dioxide, argon, and nitrogen for inducing unconsciousness or euthanasia of rats. *J Am Assoc Lab Anim Sci* 2006;45:21–25.
3. Christensen L, Barton Gade P. Danish Meat Research Institute. Transportation and pre-stun handling: CO2-systems. Available at: www.butina.eu/fileadmin/user_upload/images/articles/transport.pdf. Accessed Dec 13, 2010.
4. Interagency Research Animal Committee. US government principles for the utilization and care of vertebrate animals used in testing, research and training. Available at: grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples. Accessed Dec 13, 2010.
5. Chorney JM, Kain ZN. Behavioral analysis of children's response to induction of anesthesia. *Anesth Analg* 2009;109:1434–1440.
6. Przybylo HJ, Tarbell SE, Stevenson GW. Mask fear in children presenting for anesthesia: aversion, phobia, or both? *Paediatr Anaesth* 2005;15:366–370.
7. Hawkins P, Playle L, Golledge H, et al. *Newcastle consensus meeting on carbon dioxide euthanasia of laboratory animals*. London: National Centre for the Replacement, Refinement and Reduction of Animals in Science, 2006. Available at: www.nc3rs.org.uk/downloaddoc.asp?id=416&page=292&skin=0. Accessed Jan 20, 2011.
8. Glass HG, Snyder FF, Webster E. The rate of decline in resistance to anoxia of rabbits, dogs, and guinea pigs from the onset of viability to adult life. *Am J Physiol* 1944;140:609–615.
9. Powell K, Ethun K, Taylor DK. The effect of light level, $CO_2$ flow rate, and anesthesia on the stress response of mice during $CO_2$ euthanasia. *Lab Anim (NY)* 2016;45:386–395.
10. Garnett N. PHS policy on humane care and use of laboratory animals clarification regarding use of carbon dioxide for euthanasia of small laboratory animals. Release date: July 17, 2002. Available at: grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html. Accessed Dec 14, 2010.
11. Meyer RE, Morrow WEM. Carbon dioxide for emergency on-farm euthanasia of swine. *J Swine Health Prod* 2005;13:210–217.
12. Meyer RE. Principles of carbon dioxide displacement. *Lab Anim (NY)* 2008;37:241–242.
13. Nunn JF. *Nunn's applied respiratory physiology*. 4th ed. Oxford, England: Butterworth-Heinemann, 1993;583–593.
14. Davis PD, Kenny GNC. *Basic physics and measurement in anaesthesia*. 5th ed. Edinburgh: Butterworth-Heinemann, 2003;57–58.
15. Hornett TD, Haynes AP. Comparison of carbon dioxide/air mixture and nitrogen/air mixture for the euthanasia of rodents: design of a system for inhalation euthanasia. *Anim Technol* 1984;35:93–99.
16. Smith W, Harrap SB. Behavioural and cardiovascular responses of rats to euthanasia using carbon dioxide gas. *Lab Anim* 1997;31:337–346.
17. Niel L, Weary DM. Behavioural responses of rats to gradual-fill carbon dioxide euthanasia and reduced oxygen concentrations. *Appl Anim Behav Sci* 2006;100:295–308.
18. Booth NH. Inhalant anesthetics. In: Booth NH, McDonald LE, eds. *Veterinary pharmacology and therapeutics*. 6th ed. Ames, Iowa: Iowa State University Press, 1988;181–211.
19. Flecknell PA, Roughan JV, Hedenqvist P. Induction of anaesthesia with sevoflurane and isoflurane in the rabbit. *Lab Anim* 1999;33:41–46.
20. Voss LJ, Sleigh JW, Barnard JP, et al. The howling cortex: seizures and general anesthetic drugs. *Anesth Analg* 2008;107:1689–1703.
21. Knigge U, Søe-Jensen P, Jorgensen H, et al. Stress-induced release of anterior pituitary hormones: effect of H3 receptor-mediated inhibition of histaminergic activity or posterior hypothalamic lesion. *Neuroendocrinology* 1999;69:44–53.
22. Tinnikov AA. Responses of serum corticosterone and corticosteroid-binding globulin to acute and prolonged stress in the rat. *Endocrine* 1999;11:145–150.
23. Zelena D, Kiem DT, Barna I, et al. Alpha-2-adenoreceptor subtypes regulate ACTH and beta-endorphin secretions during stress in the rat. *Psychoneuroendocrinology* 1999;24:333–343.
24. van Herck H, Baumans V, de Boer SF, et al. Endocrine stress response in rats subjected to singular orbital puncture while under diethyl-ether anesthesia. *Lab Anim* 1991;25:325–329.
25. Leach MC, Bowell VA, Allan TF, et al. Aversion to gaseous euthanasia agents in rats and mice. *Comp Med* 2002;52:249–257.
26. Leach MC, Bowell VA, Allan TF, et al. Degrees of aversion shown by rats and mice to different concentrations of inhalational anaesthetics. *Vet Rec* 2002;150:808–815.
27. Leach MC, Bowell VA, Allan TF, et al. Measurement of aversion to determine humane methods of anaesthesia and euthanasia. *Anim Welf* 2004;13:S77–S86.
28. Makowska LJ, Weary DM. Rat aversion to induction with inhaled anaesthetics. *Appl Anim Behav Sci* 2009;119:229–235.

29. Moody CM, Chua B, Weary DM. The effect of carbon dioxide flow rate on the euthanasia of laboratory mice. *Lab Anim* 2014;48:298–304.
30. Wong D, Makowska IJ, Weary DM. Rat aversion to isoflurane versus carbon dioxide. *Biol Lett* 2012;9:20121000.
31. Bertolus JB, Nemeth G, Makowska IJ, e al. Rat aversion to sevoflurane and isoflurane. *Appl Anim Behav Sci* 2015;164:73–80.
32. Hawkins P, Prescott MJ, Carbone L, et al. A good death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. *Animals (Basel)* 2016;6:50.
33. Universities Federation for Animal Welfare. *Humane killing of animals*. 4th ed. South Mimms, Potters Bar, England: Universities Federation for Animal Welfare, 1988;16–22.
34. Makowska IJ, Vickers L, Mancell J, et al. Evaluating methods of gas euthanasia for laboratory mice. *Appl Anim Behav Sci* 2009;121:230–235.
35. Schmid RD, Hodgson DS, McMurphy RM. Comparison of anesthetic induction in cats by use of isoflurane in an anesthetic chamber with a conventional vapor or liquid injection technique. *J Am Vet Med Assoc* 2008;233:262–266.
36. Steffey EP, Mama KR. Inhalation anesthetics. In: Tranquilli WJ, Thurmon JC, Grimm KA, eds. *Lumb and Jones' veterinary anesthesia and analgesia*. 4th ed. Ames, Iowa: Blackwell, 2007;355–393.
37. Thomas AA, Flecknell PA, Golledge HDR. Combining nitrous oxide with carbon dioxide decreases the time to loss of consciousness during euthanasia in mice—refinement of animal welfare? *PLoS One* 2012;7:e32290.
38. Smith RK, Rault JL, Gates RS, et al. A two-step process of nitrous oxide before carbon dioxide for humanely euthanizing piglets: on-farm trials. *Animals (Basel)* 2018;8:52.
39. Occupational Safety and Health Administration. Anesthetic gases: guidelines for workplace exposures. Available at: www.osha.gov/dts/osta/anestheticgases/index.html#A. Accessed Dec 5, 2010.
40. Lockwood G. Theoretical context-sensitive elimination times for inhalational anaesthetics. *Br J Anaesth* 2010;104:648–655.
41. Seymour TL, Nagamine CM. Evaluation of isoflurane overdose for euthanasia of neonatal mice. *J Am Assoc Lab Anim Sci* 2016;55:321–323.
42. Haldane J. The action of carbonic oxide in man. *J Physiol* 1895;18:430–462.
43. Raub JA, Mathieu-Nolf M, Hampson NB, et al. Carbon monoxide poisoning—a public health perspective. *Toxicology* 2000;145:1–14.
44. Hampson NB, Weaver LK. Carbon monoxide poisoning: a new incidence for an old disease. *Undersea Hyperb Med* 2007;34:163–168.
45. Lowe-Ponsford FL, Henry JA. Clinical aspects of carbon monoxide poisoning. *Adverse Drug React Acute Poisoning Rev* 1989;8:217–240.
46. Bloom JD. Some considerations in establishing divers' breathing gas purity standards for carbon monoxide. *Aerosp Med* 1972;43:633–636.
47. Norman CA, Halton DM. Is carbon monoxide a workplace teratogen? A review and evaluation of the literature. *Ann Occup Hyg* 1990;34:335–347.
48. Wojtczak-Jaroszowa J, Kbow S. Carbon monoxide, carbon disulfide, lead and cadmium—four examples of occupational toxic agents linked to cardiovascular disease. *Med Hypotheses* 1989;30:141–150.
49. Fechter LD. Neurotoxicity of prenatal carbon monoxide exposure. *Res Rep Health Eff Inst* 1987;12:3–22.
50. Ramsey TL, Eilmann HJ. Carbon monoxide acute and chronic poisoning and experimental studies. *J Lab Clin Med* 1932;17:415–427.
51. Enggaard Hansen N, Creutzberg A, Simonsen HB. Euthanasia of mink (*Mustela vison*) by means of carbon dioxide ($CO_2$), carbon monoxide (CO) and nitrogen ($N_2$). *Br Vet J* 1991;147:140–146.
52. Vinte FJ. *The humane killing of mink*. London: Universities Federation for Animal Welfare, 1957.
53. Chalifoux A, Dallaire A. Physiologic and behavioral evaluation of CO euthanasia of adult dogs. *Am J Vet Res* 1983;44:2412–2417.
54. Dallaire A, Chalifoux A. Premedication of dogs with acepromazine or pentazocine before euthanasia with carbon monoxide. *Can J Comp Med* 1985;49:171–178.
55. Weary DM, Makowska IJ. Rat aversion to carbon monoxide. *Appl Anim Behav Sci* 2009;121:148–151.
56. Simonsen HB, Thordal-Christensen AA, Ockens N. Carbon monoxide and carbon dioxide euthanasia of cats: duration and animal behavior. *Br Vet J* 1981;137:274–278.
57. Lambooy E, Spanjaard W. Euthanasia of young pigs with carbon monoxide. *Vet Rec* 1980;107:59–61.
58. Gerritzen MA, Lambooij E, Stegeman JA, et al. Slaughter of poultry during the epidemic of avian influenza in the Netherlands in 2003. *Vet Rec* 2006;159:39–42.
59. Herin RA, Hall P, Fitch JW. Nitrogen inhalation as a method of euthanasia in dogs. *Am J Vet Res* 1978;39:989–991.
60. Noell WK, Chinn HI. Time course of failure of the visual pathway in rabbits during anoxia. *Fed Proc* 1949;8:1–19.
61. Altland PD, Brubach HF, Parker MG. Effects of inert gases on tolerance of rats to hypoxia. *J Appl Physiol* 1968;24:778–781.
62. Arieli R. Can the rat detect hypoxia in inspired air? *Respir Physiol* 1990;79:243–253.
63. Niel L, Weary DM. Rats avoid exposure to carbon dioxide and argon. *Appl Anim Behav Sci* 2007;107:100–109.
64. Makowska IJ, Niel L, Kirkden RD, et al. Rats show aversion to argon-induced hypoxia. *Appl Anim Behav Sci* 2008;114:572–581.
65. Burkholder TH, Niel L, Weed JL, et al. Comparison of carbon dioxide and argon euthanasia: effects on behavior, heart rate, and respiratory lesions in rats. *J Am Assoc Lab Anim Sci* 2010;49:448–453.
66. Raj ABM. Aversive reactions to argon, carbon dioxide and a mixture of carbon dioxide and argon. *Vet Rec* 1996;138:592–593.
67. Webster AB, Fletcher DL. Assessment of the aversion of hens to different gas atmospheres using an approach-avoidance test. *Appl Anim Behav Sci* 2004;88:275–287.
68. Raj ABM, Gregory NG, Wotton SB. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of hens during stunning in argon-induced anoxia. *Br Vet J* 1991;147:322–330.
69. Raj M, Gregory NG. Time to loss of somatosensory evoked potentials and onset of changes in the spontaneous electroencephalogram of turkeys during gas stunning. *Vet Rec* 1993;133:318–320.
70. Mohan Raj AB, Gregory NG, Wotton SB. Effect of carbon dioxide stunning on somatosensory evoked potentials in hens. *Res Vet Sci* 1990;49:355–359.
71. Raj ABM, Whittington PE. Euthanasia of day-old chicks with carbon dioxide and argon. *Vet Rec* 1995;136:292–294.
72. Gerritzen MA, Lambooij E, Hillebrand SJW, et al. Behavioral responses of broilers to different gaseous atmospheres. *Poult Sci* 2000;79:928–933.
73. McKeegan DEF, McIntyre J, Demmers TGM, et al. Behavioural responses of broiler chickens during acute exposure to gaseous stimulation. *Appl Anim Behav Sci* 2006;99:271–286.
74. Webster AB, Fletcher DL. Reactions of laying hens and broilers to different gases used for stunning poultry. *Poult Sci* 2001;80:1371–1377.
75. Lambooij E, Gerritzen MA, Engel B, et al. Behavioural responses during exposure of broiler chickens to different gas mixtures. *Appl Anim Behav Sci* 1999;62:255–265.
76. Raj ABM, Gregory NG. Welfare implications of the gas stunning of pigs: 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. *Anim Welf* 1995;4:273–280.
77. Dalmau A, Llonch P, Rodriguez P, et al. Stunning pigs with different gas mixtures: gas stability. *Anim Welf* 2010;19:315–323.
78. Raj AB. Behaviour of pigs exposed to mixtures of gases and

the time required to stun and kill them: welfare implications. *Vet Rec* 1999;144:165–168.
79. Raj M, Mason G. Reaction of farmed mink (*Mustela vison*) to argon-induced hypoxia. *Vet Rec* 1999;145:736–737.
80. Dalmau A, Rodriguez P, Llonch P, et al. Stunning pigs with different gas mixtures: aversion in pigs. *Anim Welf* 2010;19:325–333.
81. Martoft L, Lomholt L, Kolthoff C, et al. Effects of $CO_2$ anaesthesia on central nervous system activity in swine. *Lab Anim* 2002;36:115–126.
82. Raj AB, Johnson SP, Wotton SB, et al. Welfare implications of gas stunning pigs: 3. the time to loss of somatosensory evoked potentials and spontaneous electrocorticogram of pigs during exposure to gases. *Vet J* 1997;153:329–339.
83. Ring C, Erhardt W, Kraft H, et al. $CO_2$ anaesthesia of slaughter pigs. *Fleischwirtschaft (Frankf)* 1988;68:1304–1307.
84. Forslid A. Transient neocortical, hippocampal, and amygdaloid EEG silence induced by one minute inhalation of high $CO_2$ concentration in swine. *Acta Physiol Scand* 1987;130:1–10.
85. Mattsson JL, Stinson JM, Clark CS. Electroencephalographic power-spectral changes coincident with onset of carbon dioxide narcosis in rhesus monkey. *Am J Vet Res* 1972;33:2043–2049.
86. Woodbury DM, Rollins LT, Gardner MD, et al. Effects of carbon dioxide on brain excitability and electrolytes. *Am J Physiol* 1958;192:79–90.
87. Leake CD, Waters RM. The anesthetic properties of carbon dioxide. *Curr Res Anesth Anal* 1929;8:17–19.
88. Chen X, Gallar J, Pozo MA, et al. $CO_2$ stimulation of the cornea—a comparison between human sensation and nerve activity in polymodal nociceptive afferents of the cat. *Eur J Neurosci* 1995;7:1154–1163.
89. Peppel P, Anton F. Responses of rat medullary dorsal horn neurons following intranasal noxious chemical stimulation—effects of stimulus intensity, duration and interstimulus interval. *J Neurophysiol* 1993;70:2260–2275.
90. Thürauf N, Hummel T, Kettenmann B, et al. Nociceptive and reflexive responses recorded from the human nasal mucosa. *Brain Res* 1993;629:293–299.
91. Anton F, Peppel P, Euchner I, et al. Noxious chemical stimulation—responses of rat trigeminal brain stem neurons to $CO_2$ pulses applied to the nasal mucosa. *Neurosci Lett* 1991;123:208–211.
92. Feng Y, Simpson TL. Nociceptive sensation and sensitivity evoked from human cornea and conjunctiva stimulated by $CO_2$. *Invest Ophthalmol Vis Sci* 2003;44:529–532.
93. Thürauf N, Günther M, Pauli E, et al. Sensitivity of the negative mucosal potential to the trigeminal target stimulus $CO_2$. *Brain Res* 2002;942:79–86.
94. Danneman PJ, Stein S, Walshaw SO. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. *Lab Anim Sci* 1997;47:376–385.
95. Widdicombe JG. Reflexes from the upper respiratory tract. In: Cherniak NS, Widdicombe JG, eds. *Handbook of physiology: the respiratory system*. Bethesda, Md: American Physiological Society, 1986;363–394.
96. Yavari P, McCulloch PF, Panneton WM. Trigeminally-mediated alteration of cardiorespiratory rhythms during nasal application of carbon dioxide in the rat. *J Auton Nerv Syst* 1996;61:195–200.
97. Chisholm JM, Pang DS. Assessment of carbon dioxide, carbon dioxide/oxygen, isoflurane and pentobarbital killing methods in adult female Sprague-Dawley rats. *PLoS One* 2016;11:e0162639.
98. Moosavi SH, Golestanian E, Binks AP, et al. Hypoxic and hypercapnic drives to breathe generate equivalent levels of air hunger in humans. *J Appl Physiol* 2003;94:141–154.
99. Millar RA. Plasma adrenaline and noradrenaline during diffusion respiration. *J Physiol* 1960;150:79–90.
100. Nahas GG, Ligou JC, Mehlman B. Effects of pH changes on $O_2$ uptake and plasma catecholamine levels in the dog. *Am J Physiol* 1960;198:60–66.
101. Liotti M, Brannan S, Egan G, et al. Brain responses associated with consciousness of breathlessness (air hunger). *Proc Natl Acad Sci USA* 2001;98:2035–2040.
102. Dripps RD, Comroe JH. The respiratory and circulatory response of normal man to inhalation of 7.6 percent $CO_2$ and 10.4 percent $CO_2$ with a comparison of the maximal ventilation produced by severe muscular exercise, inhalation of $CO_2$ and maximal voluntary hyperventilation. *Am J Physiol* 1947;149:43–51.
103. Hill L, Flack M. The effect of excess of carbon dioxide and of want of oxygen upon the respiration and the circulation. *J Physiol* 1908;37:77–111.
104. Banzett RB, Lansing RW, Evans KC, et al. Stimulus-response characteristics of $CO_2$-induced air hunger in normal subjects. *Respir Physiol* 1996;103:19–31.
105. Shea SA, Harty HR, Banzett RB. Self-control of level of mechanical ventilation to minimize $CO_2$-induced air hunger. *Respir Physiol* 1996;103:113–125.
106. Fowler WS. Breaking point of breath-holding. *J Appl Physiol* 1954;6:539–545.
107. Kirkden RD, Niel L, Stewart SA, et al. Gas killing of rats: the effect of supplemental oxygen on aversion to carbon dioxide. *Anim Welf* 2008;17:79–87.
108. Coenen AM, Drinkenburg WH, Hoenderken R, et al. Carbon dioxide euthanasia in rats: oxygen supplementation minimizes signs of agitation and asphyxia. *Lab Anim* 1995;29:262–268.
109. Hewett TA, Kovacs MS, Artwohl JE, et al. A comparison of euthanasia methods in rats, using carbon dioxide in prefilled and fixed flow-rate filled chambers. *Lab Anim Sci* 1993;43:579–582.
110. Borovsky V, Herman M, Dunphy G, et al. $CO_2$ asphyxia increases plasma norepinephrine in rats via sympathetic nerves. *Am J Physiol* 1998;274:R19–R22.
111. Reed B, Varon J, Chait BT, et al. Carbon dioxide-induced anesthesia result in a rapid increase in plasma levels of vasopressin. *Endocrinology* 2009;150:2934–2939.
112. Raff H, Roarty TP. Renin, ACTH, and aldosterone during acute hypercapnia and hypoxia in conscious rats. *Am J Physiol* 1988;254:R431–R435.
113. Marotta SF, Sithichoke N, Garcy AM, et al. Adrenocortical responses of rats to acute hypoxic and hypercapnic stresses after treatment with aminergic agents. *Neuroendocrinology* 1976;20:182–192.
114. Raff H, Shinsako J, Keil LC, et al. Vasopressin, ACTH, and corticosteroids during hypercapnia and graded hypoxia in dogs. *Am J Physiol* 1983;244:E453–E458.
115. Argyropoulos SV, Bailey JE, Hood SD, et al. Inhalation of 35% $CO_2$ results in activation of the HPA axis in healthy volunteers. *Psychoneuroendocrinology* 2002;27:715–729.
116. Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci* 1997;20:78–84.
117. Kc P, Haxhiu MA, Trouth CO, et al. $CO_2$-induced c-Fos expression in hypothalamic vasopressin containing neurons. *Respir Physiol* 2002;129:289–296.
118. Hackbarth H, Kuppers N, Bohnet W. Euthanasia of rats with carbon dioxide—animal welfare aspects. *Lab Anim* 2000;34:91–96.
119. Blackshaw JK, Fenwick DC, Beattie AW, et al. The behavior of chickens, mice and rats during euthanasia with chloroform, carbon dioxide and ether. *Lab Anim* 1988;22:67–75.
120. Britt DP. The humaneness of carbon dioxide as an agent of euthanasia for laboratory rodents. In: *Euthanasia of unwanted, injured or diseased animals or for educational or scientific purposes*. Potters Bar, England: Universities Federation for Animal Welfare, 1987;19–31.
121. Raj ABM, Gregory NG. Welfare implications of the gas stunning of pigs: 2. Stress of induction of anaesthesia. *Anim Welf* 1996;5:71–78.
122. Jongman EC, Barnett JL, Hemsworth PH. The aversiveness of carbon dioxide stunning in pigs and a comparison of the $CO_2$ stunner crate vs the V-restrainer. *Appl Anim Behav Sci* 2000;67:67–76.

123. Troeger K, Woltersdorf W. Gas anesthesia of slaughter pigs. 1. Stunning experiments under laboratory conditions with fat pigs of known halothane reaction type—meat quality, animal protection. *Fleischwirtschaft (Frankf)* 1991;72:1063–1068.
124. Dodman NH. Observations on use of Wernberg dip-lift carbon dioxide apparatus for pre-slaughter anesthesia of pigs. *Br Vet J* 1977;133:71–80.
125. Gerritzen MA, Lambooij E, Reimert HG, et al. Susceptibility of duck and turkey to severe hypercapnic hypoxia. *Poult Sci* 2006;85:1055–1061.
126. Gerritzen M, Lambooij B, Reimert H, et al. A note on behaviour of poultry exposed to increasing carbon dioxide concentrations. *Appl Anim Behav Sci* 2007;108:179–185.
127. McKeegan DEF, McIntyre JA, Demmers TGM, et al. Physiological and behavioural responses of broilers to controlled atmosphere stunning: implications for welfare. *Anim Welf* 2007;16:409–426.
128. Abeyesinghe SM, McKeegan DEF, McLeman MA, et al. Controlled atmosphere stunning of broiler chickens. I. Effects on behaviour, physiology and meat quality in a pilot scale system at a processing plant. *Br Poult Sci* 2007;48:406–423.
129. Cooper J, Mason G, Raj M. Determination of the aversion of farmed mink (*Mustela vison*) to carbon dioxide. *Vet Rec* 1998;143:359–361.
130. Withrock IC. The use of carbon dioxide (CO2) as an alternative euthanasia method for goat kids. Available at: search.proquest.com/docview/1733971790/abstract/C0605E819BD543A6PQ/1. Accessed Nov 11, 2019.
131. Battaglia M, Ogliari A, Harris J, et al. A genetic study of the acute anxious response to carbon dioxide stimulation in man. *J Psychiatr Res* 2007;41:906–917.
132. Nardi AE, Freire RC, Zin WA. Panic disorder and control of breathing. *Respir Physiol Neurobiol* 2009;167:133–143.
133. Grandin T. Effect of genetics on handling and $CO_2$ stunning of pigs (updated July 2008). *Meat Focus Int* 1992;July:124–126. Available at: www.grandin.com/humane/meatfocus7–92.html. Accessed Dec 13, 2010.
134. Ziemann AE, Allen JE, Dahdaleh NS, et al. The amygdala is a chemosensor that detects carbon dioxide and acidosis to elicit fear behavior. *Cell* 2009;139:1012–1021.
135. Kohler I, Moens Y, Busato A, et al. Inhalation anaesthesia for the castration of piglets: $CO_2$ compared to halothane. *Zentralbl Veterinarmed A* 1998;45:625–633.
136. Boivin GP, Bottomley MA, Dudley ES, et al. Physiological, behavioral, and histological responses of male C57BL/6N mice to different $CO_2$ chamber replacement rates. *J Am Assoc Lab Anim Sci* 2016;55:451–461.
137. Glen JB, Scott WN. Carbon dioxide euthanasia of cats. *Br Vet J* 1973;129:471–479.
138. Franson JC. Euthanasia. In: Friend M, Franson JC, eds. *Field manual of wildlife diseases. General field procedures and diseases of birds*. Biological Resources Division information and technology report 1999–001. Washington, DC: US Department of the Interior and US Geological Survey, 1999;49–53.
139. Mohan Raj AB, Gregory NG. Effect of rate of induction of carbon dioxide anaesthesia on the time of onset of unconsciousness and convulsions. *Res Vet Sci* 1990;49:360–363.
140. Mohan Raj AB, Wotton SB, Gregory NG. Changes in the somatosensory evoked potentials and spontaneous electoencephalogram of hens during stunning with a carbon dioxide and argon mixture. *Br Vet J* 1992;148:147–156.
141. Raj M, Gregory NG. An evaluation of humane gas stunning methods for turkeys. *Vet Rec* 1994;135:222–223.
142. Walsh JL, Percival A, Turner PV. Efficacy of blunt force trauma, a novel mechanical cervical dislocation device, and a non-penetrating captive bolt device for on-farm euthanasia of pre-weaned kits, growers, and adult commercial meat rabbits. *Animals (Basel)* 2017;7:100.
143. Dalmau AJ, Pallisera C, Pedernera I, et al. Use of high concentrations of carbon dioxide for stunning rabbits reared for meat production. *World Rabbit Sci* 2016;24:25–37.
144. Poole GH, Fletcher DL. A comparison of argon, carbon dioxide, and nitrogen in a broiler killing system. *Poult Sci* 1995;74:1218–1223.
145. Latimer KS, Rakich PM. Necropsy examination. In: Ritchie BW, Harrison GJ, Harrison LR, eds. *Avian medicine: principles and application*. Lake Worth, Fla: Wingers Publishing Inc, 1994;355–379.
146. Jaksch W. Euthanasia of day-old male chicks in the poultry industry. *Int J Study Anim Probl* 1981;2:203–213.
147. Pritchett-Corning KR. Euthanasia of neonatal rats with carbon dioxide. *J Am Assoc Lab Anim Sci* 2009;48:23–27.
148. Pritchett K, Corrow D, Stockwell J, et al. Euthanasia of neonatal mice with carbon dioxide. *Comp Med* 2005;55:275–281.
149. Hayward JS, Lisson PA. Carbon dioxide tolerance of rabbits and its relation to burrow fumigation. *Aust Wildl Res* 1978;5:253–261.
150. Iwarsson K, Rehbinder C. A study of different euthanasia techniques in guinea pigs, rats, and mice. Animal response and postmortem findings. *Scand J Lab Anim Sci* 1993;20:191–205.
151. Valentine H, Williams WO, Maurer KJ. Sedation or inhalant anesthesia before euthanasia with $CO_2$ does not reduce behavioral or physiologic signs of pain and stress in mice. *J Am Assoc Lab Anim Sci* 2012;51:50–57.
152. US FDA. Animal drug compounding. Available at: www.fda.gov/animal-veterinary/unapproved-animal-drugs/animal-drug-compounding. Accessed Jan 13, 2020.
153. Campbell VL, Butler AL, Lunn KF. Use of a point-of-care urine drug test in a dog to assist in diagnosing barbiturate toxicosis secondary to ingestion of a euthanized carcass. *J Vet Emerg Crit Care San Antonio* 2009;19:286–291.
154. Jurczynski K, Zittlau E. Pentobarbital poisoning in Sumatran tigers (*Panthera tigris sumatrae*). *J Zoo Wildl Med* 2007;38:583–584.
155. O'Rourke K. Euthanatized animals can poison wildlife: veterinarians receive fines. *J Am Vet Med Assoc* 2002;220:146–147.
156. US FDA. 21 CFR Part 522. Injectable or implantable dosage form new animal drugs; euthanasia solution; technical amendment. *Fed Regist* 2003;68:42968–42969.
157. Wilkins III JR, Bowman ME. Needlestick injuries among female veterinarians: frequency, syringe contents and side-effects. *Occup Med (Lond)* 1997;47:451–457.
158. Lewbart GA, ed. *Invertebrate medicine*. Oxford, England: Blackwell, 2006.
159. Schwartz JA, Warren RJ, Henderson DW, et al. Captive and field tests of a method for immobilization and euthanasia of urban deer. *Wildl Soc Bull* 1997;25:532–541.
160. Bucher K, Bucher KE, Waltz D. Irritant actions of unphysiological pH values. A controlled procedure to test for topical irritancy. *Agents Actions* 1979;9:124–132.
161. Khoo SY, Lay BPP, Joya J, et al. Local anaesthetic refinement of pentobarbital euthanasia reduces abdominal writhing without affecting immunohistochemical endpoints in rats. *Lab Anim* 2018;52:152–162.
162. Grier RL, Schaffer CB. Evaluation of intraperitoneal and intrahepatic administration of a euthanasia agent in animal shelter cats. *J Am Vet Med Assoc* 1990;197:1611–1615.
163. Schoell AR, Heyde BR, Weir DE, et al. Euthanasia method for mice in rapid time-course pulmonary pharmacokinetic studies. *J Am Assoc Lab Anim Sci* 2009;48:506–511.
164. Philbeck TE, Miller LJ, Montez D, et al. Hurts so good. Easing IO pain and pressure. *JEMS* 2010;35:58–62, 65–66, 68, 69.
165. Montez D, Miller LJ, Puga T, et al. Pain management with the use of IO: easing IO pain and pressure. Available at: www.jems.com/article/intraosseous/pain-management-use-io. Accessed Jun 13, 2011.
166. US FDA. *Tributame euthanasia solution: embutramide/chloroquine phosphate/lidocaine*. Freedom of Information summary. NADA 141–245. Silver Spring, Md: FDA, 2005.
167. US FDA. 21 CFR Part 522. Implantation or injectable dosage form new animal drugs; embutramide, chloroquine, and lidocaine solution. *Fed Regist* 2005;70:36336–36337.

168. US FDA. 21 CFR Part 1308. Schedules of controlled substances: placement of embutramide into schedule III. *Fed Regist* 2006;71:51115–51117.
169. Sodfola OA. The cardiovascular effect of chloroquine in anesthetized dogs. *Can J Physiol Pharmacol* 1980;58:836–841.
170. Don Michael TA, Alwassadeh S. The effects of acute chloroquine poisoning with special references to the heart. *Am Heart J* 1970;79:831–842.
171. Webb AI. Euthanizing agents. In: Reviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Ames, Iowa: Wiley Blackwell, 2009;401–408.
172. Webb AI, Pablo LS. Local anesthetics. In: Reviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Ames, Iowa: Wiley Blackwell, 2009;381–400.
173. Hellebrekers LJ, Baumans V, Bertens APMG, et al. On the use of T61 for euthanasia of domestic and laboratory animals; an ethical evaluation. *Lab Anim* 1990;24:200–204.
174. Park CK, Kim K, Jung SJ, et al. Molecular mechanism for local anesthetic action of eugenol in the rat trigeminal system. *Pain* 2009;144:84–94.
175. Kearns KS, Swenson B, Ramsay EC. Dosage trials with transmucosal carfentanil citrate in non-human primates. *Zoo Biol* 1999;18:397–402.
176. Flecknell PA. *Laboratory animal anaesthesia*. 2nd ed. San Diego: Elsevier Academic Press, 1996;168–171.
177. Saxena K. Death from potassium chloride overdose. *Postgrad Med* 1988;84:97–98, 101–102.
178. Lumb WV. Euthanasia by noninhalant pharmacologic agents. *J Am Vet Med Assoc* 1974;165:851–852.
179. Ciganovich E. Barbiturates. In: *Field manual of wildlife diseases. General field procedures and diseases of birds*. Biological Resources Division information and technology report 1999–001. Washington, DC: US Department of the Interior and US Geological Survey, 1999;349–351.
180. Raghav R, Taylor M, Guincho M, et al. Potassium chloride as a euthanasia agent in psittacine birds: clinical aspects and consequences for histopathologic assessment. *Can Vet J* 2011;52:303–306.
181. Messenger JB, Nixon M, Ryan KP. Magnesium chloride as an anaesthetic for cephalopods. *Comp Biochem Physiol C* 1985;82:203–205.
182. Luckl J, Keating J, Greenberg JH. Alpha-chloralose is a suitable anesthetic for chronic focal cerebral ischemia studies in the rat: a comparative study. *Brain Res* 2008;1191:157–167.
183. Belant JL, Tyson LA, Seamans TW. Use of alpha-chloralose by the Wildlife Services program to capture nuisance birds. *Wildl Soc Bull* 1999;27:938–942.
184. Cobaugh DJ. Ethanol. In: Brent J, Phillips SD, Wallace KL, et al, eds. *Critical care toxicology*. Philadelphia: Mosby, 2005;1553–1558.
185. Harms C. Anesthesia in fish. In: Fowler ME, Miller RE, eds. *Zoo and wild animal medicine: current therapy 4*. Philadelphia: WB Saunders Co, 1999;158–163.
186. Lord R. Use of ethanol for euthanasia of mice. *Aust Vet J* 1989;66:268.
187. Allen-Worthington KH, Brice AK, Marx JO, et al. Intraperitoneal injection of ethanol for the euthanasia of laboratory mice (*Mus musculus*) and rats (*Rattus norvegicus*). *J Am Assoc Lab Anim Sci* 2015;54:769–778.
188. de Souza Dyer C, Brice AK, Marx JO. Intraperitoneal administration of ethanol as a means of euthanasia for neonatal mice (*Mus musculus*). *J Am Assoc Lab Anim Sci* 2017;56:299–306.
189. US FDA. ANADA 200–226 Tricaine-S—original approval. Available at: www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm132992.htm. Accessed May 16, 2011.
190. Noga EJ. Pharmacopoeia. In: *Fish disease: diagnosis and treatment*. 2nd ed. Ames, Iowa: Wiley-Blackwell, 2010;375–420.
191. Stoskopf MK. Anesthesia. In: Brown LA, ed. *Aquaculture for veterinarians: fish husbandry and medicine*. Oxford, England: Pergamon Press, 1993;161–167.
192. Committee for Veterinary Medicinal Products. *Tricaine mesilate: summary report*. EMEA/MRL/586/99-FINAL. London: European Agency for the Evaluation of Medicinal Products, 1999. Available at www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015660.pdf. Accessed Sep 9, 2010.
193. Torreilles SL, McClure DE, Green SL. Evaluation and refinement of euthanasia methods for *Xenopus laevis*. *J Am Assoc Lab Anim Sci* 2009;48:512–516.
194. Bernstein PS, Digre KB, Creel DJ. Retinal toxicity associated with occupations exposure to the fish anesthetic MS 222 (ethyl-m-aminobenzoic acid methanesulfonate). *Am J Ophthalmol* 1997;124:843–844.
195. Kaiser H, Green DM. Keeping the frogs still: Orajel is a safe anesthetic in amphibian photography. *Herpetol Rev* 2001;32:93–94.
196. Chen MH, Combs CA. An alternative anesthesia for amphibians: ventral application of benzocaine. *Herpetol Rev* 1999;30:34.
197. Blessing JJ, Marshal JC, Balcombe SR. Humane killing of fishes for scientific research: a comparison of two methods. *J Fish Biol* 2010;76:2571–2577.
198. US FDA Center for Veterinary Medicine. *Enforcement priorities for drug use in aquaculture*. Silver Spring, Md: US FDA, 2011. Available at: www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/PoliciesProceduresManual/UC M046931.pdf. Accessed Jan 10, 2011.
199. National Toxicology Program. *NTP technical report on the toxicology and carcinogenesis studies of isoeugenol (CAS No. 97–54–1) in F344/N rats and B6C3F1 mice (gavage studies)*. NTP TR 551. NIH publication No. 08–5892. Washington, DC: US Department of Health and Human Services, 2008. Available at: ntp.niehs.nih.gov/files/TR551board_web.pdf. Accessed May 16, 2011.
200. Grush J, Noakes DL, Moccia RD. The efficacy of clove oil as an anesthetic for the zebrafish, *Danio rerio* (Hamilton). *Zebrafish* 2004;1:46–53.
201. Borski RJ, Hodson RG. Fish research and the institutional animal care and use committee. *ILAR J* 2003;44:286–294.
202. Sladky KK, Swanson CR, Stoskopf MK, et al. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). *Am J Vet Res* 2001;62:337–342.
203. Brodin P, Roed A. Effects of eugenol on rat phrenic nerve and phrenic-diaphragm preparations. *Arch Oral Biol* 1984;29:611–615.
204. Ingvast-Larsson JC, Axén VC, Kiessling AK. Effects of isoeugenol on in vitro neuromuscular blockade of rat phrenic nerve-diaphragm preparations. *Am J Vet Res* 2003;64:690–693.
205. Meyer RE, Fish R. Pharmacology of injectable anesthetics, sedatives, and tranquilizers. In: Fish RE, Danneman PJ, Brown M, et al, eds. *Anesthesia and analgesia of laboratory animals*. 2nd ed. San Diego: Academic Press, 2008;27–82.
206. Neiffer DL, Stamper A. Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. *ILAR J* 2009;50:343–360.
207. US FDA. The index of legally marketed unapproved new animal drugs for minor species. Available at: www.fda.gov/animal-veterinary/minor-useminor-species/index-legally-marketed-unapproved-new-animal-drugs-minor-species. Accessed Dec 27, 2019.
208. Estrela C, Estrela CR, Barbin EL, et al. Mechanism of action of sodium hypochlorite. *Braz Dent J* 2002;13:113–117.
209. National Institutes of Health. *Guidelines for use of zebrafish in the NIH intramural research program*. Bethesda, Md: National Institutes of Health, 2009. Available at: oacu.od.nih.gov/arac/documents/Zebrafish.pdf. Accessed Nov 25, 2010.
210. Agency for Toxic Substances and Disease Registry. Toxological profile for formaldehyde. July 1999. Available at: www.atsdr.cdc.gov/toxprofiles/tp111.pdf. Accessed Aug 13, 2012.
211. National Toxicology Program. *Report on carcinogens*. 12th ed. Research Triangle Park, NC: US Department of Health

and Human Services, Public Health Service, National Toxicology Program, 2011.
212. Murray MJ. Invertebrates. In: *Guidelines for the euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;25–27.
213. Vickroy TW. Local anesthetics. In: Riviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 10th ed. Hoboken, NJ: Wiley Blackwell, 2018;369–386.
214. Aleman M, Davis E, Kynch H, et al. Drug residues after intravenous anesthesia and intrathecal lidocaine hydrochloride euthanasia in horses. *J Vet Intern Med* 2016;30:1322–1326.
215. Dennis MB, Jr., Dong WK, Weisbrod KA, et al. Use of captive bolt as a method of euthanasia in larger laboratory animal species. *Lab Anim Sci* 1988;38:459–462.
216. Gibson TJ, Whitehead C, Taylor R, et al. Pathophysiology of penetrating captive bolt stunning in Alpacas (*Vicugna pacos*). *Meat Sci* 2015;100:227–231.
217. Schwenk BK, Lechner I, Ross SG, et al. Magnetic resonance imaging and computer tomography of brain lesions in water buffaloes and cattle stunned with handguns or captive bolts. *Meat Sci* 2016;113:35–40.
218. Gilliam JN, Shearer JK, Bahr RJ, et al. Evaluation of brainstem disruption following penetrating captive-bolt shot in isolated cattle heads: comparison of traditional and alternative shot-placement landmarks. *Anim Welf* 2016;25:347–353.
219. Kline HC, Wagner DR, Edwards-Callaway LN, et al. Effect of captive bolt gun length on brain trauma and post-stunning hind limb activity in finished cattle *Bos taurus*. *Meat Sci* 2019;155:69–73.
220. Woods J, Shearer JK, Hill J. Recommended on-farm euthanasia practices. In: Grandin T, ed. *Improving animal welfare: a practical approach*. Wallingford, England: CABI Publishing, 2010.
221. Blackmore DK. Energy requirements for the penetration of heads of domestic stock and the development of a multiple projectile. *Vet Rec* 1985;116:36–40.
222. Daly CC, Whittington PE. Investigation into the principal determinants of effective captive bolt stunning of sheep. *Res Vet Sci* 1989;46:406–408.
223. Clifford DH. Preanesthesia, anesthesia, analgesia, and euthanasia. In: Fox JG, Cohen BJ, Loew FM, eds. *Laboratory animal medicine*. New York: Academic Press Inc, 1984;528–563.
224. Australian Veterinary Association. *Guidelines for humane slaughter and euthanasia. Member's directory and policy compendium*. Lisarow, NSW: Veritage Press, 1997.
225. Finnie JW. Neuropathologic changes produced by non-penetrating percussive captive bolt stunning of cattle. *N Z Vet J* 1995;43:183–185.
226. Blackmore DK, Newhook JC. The assessment of insensibility in sheep, calves and pigs during slaughter. In: Eikelenboom G, ed. *Stunning of animals for slaughter*. Boston: Martinus Nijhoff Publishers, 1983;13–25.
227. Gregory NG. Animal welfare at markets and during transport and slaughter. *Meat Sci* 2008;80:2–11.
228. Grandin T. Objective scoring of animal handling and stunning practices at slaughter plants. *J Am Vet Med Assoc* 1998;212:36–39.
229. Gibson TJ, Mason CW, Spence JY, et al. Factors affecting penetrating captive bolt gun performance. *J Appl Anim Welf Sci* 2015;18:222–238.
230. Terlouw C, Bourguet C, Deiss V. Consciousness, unconsciousness and death in the context of slaughter. Part 1. Neurobiological mechanisms underlying stunning and killing. *Meat Sci* 2016;118:133–146.
231. Terlouw C, Bourguet C, Deiss V. Consciousness, unconsciousness and death in the context of slaughter. Part 2. Evaluation methods. *Meat Sci* 2016;118:147–156.
232. Oliveira SEO, Gregory NG, Dalla Costa FA, et al. Effectiveness of pneumatically powered penetrating and non-penetrating captive bolts in stunning cattle. *Meat Sci* 2018;140:9–13.
233. Gibson TJ, Oliveira SEO, Dalla Costa FA, et al. Electroencephalographic assessment of pneumatically powered penetrating and non-penetrating captive-bolt stunning of bulls. *Meat Sci* 2019;151:54–59.
234. Casey-Trott TM, Millman ST, Turner PV, et al. Effectiveness of a nonpenetrating captive bolt for euthanasia of 3 kg to 9 kg pigs. *J Anim Sci* 2014;92:5166–5174.
235. Erasmus MA, Turner PV, Niekamp SG, et al. Brain and skull lesions resulting from use of percussive bolt, cervical dislocation by stretching, cervical dislocation by crushing and blunt trauma in turkeys. *Vet Rec* 2010;167:850–858.
236. Erasmus MA, Turner PV, Widowski TM. Measures of insensibility used to determine effective stunning and killing of poultry. *J Appl Poult Res* 2010;19:288–298.
237. Canadian Council on Animal Care. *Guide to the care and use of experimental animals*. Vol 1. 2nd ed. Ottawa: Canadian Council on Animal Care, 1993.
238. Green CJ. Euthanasia. In: *Animal anesthesia*. London: Laboratory Animals Ltd, 1979;237–241.
239. World Organisation for Animal Health (OIE). Chapter 7.6: killing of animals for disease control purposes. In: *Terrestrial animal health code*. 20th ed. Paris: OIE, 2011. Available at: www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.7.6.htm. Accessed May 16, 2011.
240. Finnie JW. Neuroradiological aspects of experimental traumatic missile injury in sheep. *N Z Vet J* 1994;42:54–57.
241. Longair JA, Finley GG, Laniel MA, et al. Guidelines for the euthanasia of domestic animals by firearms. *Can Vet J* 1991;32:724–726.
242. Carding T. Euthanasia of dogs and cats. *Anim Regul Stud* 1977;1:5–21.
243. Blackmore DK, Bowling MC, Madie P, et al. The use of a shotgun for the emergency slaughter or euthanasia of large mature pigs. *N Z Vet J* 1995;43:134–137.
244. Finnie IW. Traumatic head injury in ruminant livestock. *Aust Vet J* 1997;75:204–208.
245. Blackmore DK, Madie P, Bowling MC, et al. The use of a shotgun for emergency slaughter of stranded cetaceans. *N Z Vet J* 1995;43:158–159.
246. Nelson JM. Bullet energy in foot pounds. Available at: web.stcloudstate.edu/jmnelson/web/gun/benergy/index.html. Accessed Jun 15, 2011.
247. Baker HJ, Scrimgeour HJ. Evaluation of methods for the euthanasia of cattle in a foreign animal disease outbreak. *Can Vet J* 1995;36:160–165.
248. Humane Slaughter Association. *Humane killing of livestock using firearms: guidance notes #3*. 2nd ed. Wheathampstead, England: Humane Slaughter Association, 2005.
249. National Pork Board, American Association of Swine Practitioners. *On-farm euthanasia of swine*. 2nd edition. Des Moines, Iowa: National Pork Board, 2009.
250. Hughes HC. Euthanasia of laboratory animals. In: Melby EC, Altman NH, eds. *Handbook of laboratory animal science*. Vol 3. Cleveland: CRC Press, 1976;553–559.
251. Vanderwolf CH, Buzak DP, Cain RK, et al. Neocortical and hippocampal electrical activity following decapitation in the rat. *Brain Res* 1988;451:340–344.
252. Holson RR. Euthanasia by decapitation: evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. *Neurotoxicol Teratol* 1992;14:253–257.
253. Derr RF. Pain perception in decapitated rat brain. *Life Sci* 1991;49:1399–1402.
254. Gregory NG, Wotton SB. Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. *Vet Rec* 1990;126:570–572.
255. Erasmus MA, Lawlis P, Duncan IJ, et al. Using time to insensibility and estimated time of death to evaluate a nonpenetrating captive bolt, cervical dislocation, and blunt trauma for on-farm killing of turkeys. *Poult Sci* 2010;89:1345–1354.
256. Keller GL. Physical euthanasia methods. *Lab Anim (NY)* 1982;11:20–26.
257. Webster AB, Fletcher DL, Savage SI. Humane on-farm killing of spent hens. *J Appl Poult Res* 1996;5:191–200.
258. Feldman DB, Gupta BN. Histopathologic changes in labora-

tory animals resulting from various methods of euthanasia. *Lab Anim Sci* 1976;26:218–221.
259. Mikeska JA, Klemm WR. EEG evaluation of humaneness of asphyxia and decapitation euthanasia of the laboratory rat. *Lab Anim Sci* 1975;25:175–179.
260. Cartner SC, Barlow SC, Ness TJ. Loss of cortical function in mice after decapitation, cervical dislocation, potassium chloride injection, and $CO_2$ inhalation. *Comp Med* 2007;57:570–573.
261. Urbanski HF, Kelley ST. Sedation by exposure to gaseous carbon dioxide-oxygen mixture: application to studies involving small laboratory animal species. *Lab Anim Sci* 1991;41:80–82.
262. Gregory NG, Wotton SB. Effect of slaughter on the spontaneous and evoked activity of the brain. *Br Poult Sci* 1986;27:195–205.
263. Anil MH, McKinstry JL. Reflexes and loss of sensibility following head-to-back electrical stunning in sheep. *Vet Rec* 1991;128:106–107.
264. Hatch RC. Euthanatizing agents. In: Booth NH, McDonald LE, eds. *Veterinary pharmacology and therapeutics*. 6th ed. Ames, Iowa: Iowa State University Press, 1988;1143–1148.
265. Lambooy E, van Voorst N. Electrocution of pigs with notifiable diseases. *Vet Q* 1986;8:80–82.
266. Eikelenboom G, ed. *Stunning of animals for slaughter*. Boston: Martinus Nijhoff Publishers, 1983.
267. Warrington R. Electrical stunning, a review of the literature. *Vet Bull* 1974;44:617–628.
268. Roberts TDM. Electrocution cabinets. *Vet Rec* 1974;95:241–242.
269. Loftsgard G, Rraathen S, Helgebostad A. Electrical stunning of mink. *Vet Rec* 1972;91:132–134.
270. Croft PG, Hume CW. Electric stunning of sheep. *Vet Rec* 1956;68:318–321.
271. Anil MH, McKinstry JL. The effectiveness of high frequency electrical stunning in pigs. *Meat Sci* 1992;31:481–491.
272. Croft PS. Problems with electric stunning. *Vet Rec* 1952;64:255–258.
273. Roberts TDM. Cortical activity in electrocuted dogs. *Vet Rec* 1954;66:561–567.
274. Pascoe PJ. Humaneness of an electroimmobilization unit for cattle. *Am J Vet Res* 1986;47:2252–2256.
275. Grandin T, American Meat Institute Animal Welfare Committee. *Recommended animal handling guidelines and audit guide: a systematic approach to animal welfare*. Washington, DC: American Meat Institute, 2010;19–22.
276. Grandin T. Euthanasia and slaughter of livestock. *J Am Vet Med Assoc* 1994;204:1354–1360.
277. Lambooy E. Electrical stunning of sheep. *Meat Sci* 1982;6:123–135.
278. Blackmore DK, Newhook JC. Insensibility during slaughter of pigs in comparison to other domestic stock. *N Z Vet J* 1981;29:219–222.
279. Grandin T. Solving return-to-sensibility problems after electrical stunning in commercial pork slaughter plants. *J Am Vet Med Assoc* 2001;219:608–611.
280. Anil MH. Studies on the return of physical reflexes in pigs following electrical stunning. *Meat Sci* 1991;30:13–21.
281. Hoenderken R. Electrical and carbon dioxide stunning of pigs for slaughter. In: Eikelenboom G, ed. *Stunning of animals for slaughter*. Boston: Martinus Nijhoff Publishers, 1983;59–63
282. Denicourt M, Klopfenstein C, DuFour V, et al. *Developing a safe and acceptable method for on-farm euthanasia of pigs by electrocution. Final report*. Montreal: Faculty of Veterinary Medicine, University of Montreal, 2009.
283. Vogel KD, Badtram G, Claus JR, et al. Head-only followed by cardiac arrest electrical stunning is an effective alternative to head-only electrical stunning in pigs. *J Anim Sci* 2011;89:1412–1418.
284. Weaver AL, Wotton SB. The Jarvis Beef Stunner: effect of a prototype chest electrode. *Meat Sci* 2009;81:51–56.
285. Grandin T. Cattle vocalizations are associated with handling and equipment problems in slaughter plants. *Appl Anim Behav Sci* 2001;71:191–201.
286. Meerburg BGH, Brom FWA, Kijlstra A. The ethics of rodent control. *Pest Manag Sci* 2008;64:1205–1211.
287. Federal Provincial Committee for Humane Trapping. *Final report: Committee of the Federal Provincial Wildlife Conference*. Ottawa: Canadian Wildlife Service, 1981.
288. Department of Foreign Affairs and International Trade. *Agreement on international humane trapping standards between the European Community, Canada, and the Russian Federation*. Ottawa: Department of Foreign Affairs and International Trade, 1997;1–32.
289. Canadian General Standards Board. *Animal (mammal) traps—mechanically powered, trigger-activated killing traps for use on, land*. No. CAN/CGSB-144.1–96. Ottawa: Canadian General Standards Board, 1996;1–36.
290. Nolan JW, Barrett MW. *Description and operation of the humane trapping research facility at the Alberta Environmental Centre*. AECV90–R3. Vegreville, AB, Canada: Alberta Environmental Centre, 1990.
291. International Organization for Standardization. *Animal (mammal) traps-part 4: methods for testing killing trap systems used on land or underwater*. TC 191, ISO/DIS 19009–4E. Geneva: International Organization for Standardization, 2000;1–15.
292. Gilbert FF. Assessment of furbearer response to trapping devices, in *Proceedings*. Worldw Furbearer Conf 1981;1599–1611.
293. Proulx G, Barrett MW. Evaluation of the Bionic trap to quickly kill fisher (*Martes pennanti*) in simulated natural environments. *J Wildl Dis* 1993;29:310–316.
294. Proulx G, Barrett MW, Cook SR. The C120 Magnum with pan trigger: a humane trap for mink (*Mustela vison*). *J Wildl Dis* 1990;26:511–517.
295. Hiltz M, Roy LD. Rating of killing traps against humane trapping standards using computer simulations, in *Proceedings*. 19th Vertebr Pest Conf 2000;197–201.
296. Association of Fish and Wildlife Agencies. Best management practices. Available at: jjcdev.com/~fishwild/?section=best_management_practices Accessed Jul 22, 2012.
297. International Association of Fish and Wildlife Agencies. *Summary of progress. 1999–2000 field season: testing restraining and body-gripping traps for development of best management practices for trapping in the United States*. Washington, DC: International Association of Fish and Wildlife Agencies, 2003.
298. Warburton B, Gregory NG, Morriss G. Effect of jaw shape in kill-traps on time to loss of palpebral reflexes in brushtail possums. *J Wildl Dis* 2000;36:92–96.
299. King CM. The effects of two types of steel traps upon captured stoats (*Mustela erminea*). *J Zool (Lond)* 1981;195:553–554.
300. Proulx G, Kolenosky AJ, Cole PJ, et al. A humane killing trap for lynx (*Felis lynx*): the Conibear 330 with clamping bars. *J Wildl Dis* 1995;31:57–61.
301. Warburton B, Hall JV. Impact momentum and clamping force thresholds for developing standards for possum kill traps. *NZ J Zool* 1995;22:39–44.
302. Naylor BJ, Novak M. Catch efficiency and selectivity of various traps and sets used for capturing American martens. *Wildl Soc Bull* 1994;22:489–496.
303. Proulx G, Barrett MW. Field testing of the C120 magnum trap for mink. *Wildl Soc Bull* 1993;21:421–426.
304. Proulx G, Kolenosky AJ, Badry MJ, et al. Assessment of the Savageau 2001–8 trap to effectively kill artic fox. *Wildl Soc Bull* 1993;21:132–135.
305. Proulx G, Kolenosky AJ, Cole PJ. Assessment of the Kania trap to humanely kill red squirrels (*Tamiasciurus hudsonicus*) in enclosures. *J Wildl Dis* 1993;29:324–329.
306. Proulx G, Pawlina IM, Wong RK. Re-evaluation of the C120 magnum and Bionic traps to humanely kill mink (lett). *J Wildl Dis* 1993;29:184.
307. Cooper JE, Ewbank R, Platt C, et al. *Euthanasia of amphib-*

*ians and reptiles*. London: Universities Federation for Animal Welfare and World Society for the Protection of Animals, 1989.
308. Proulx G, Cook SR, Barrett MW. Assessment and preliminary development of the rotating jaw Conibear 120 trap to effectively kill marten (*Martes americana*). *Can J Zool* 1989;67:1074–1079.
309. Hill EP. *Evaluation of improved traps and trapping techniques*. Project report W-44–6, Job IV-B. Montgomery, Ala: Alabama Department of Conservation and Natural Resources, 1981;1–19.
310. Sikes RS, Gannon WL, Animal Care and Use Committee of the American Society of Mammalogists. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J Mammal* 2011;92:235–253.
311. *Improving animal welfare in US trapping programs*. Washington, DC: International Association of Fish and Wildlife Agencies, 1997.
312. American Association of Avian Pathologists (AAAP) Animal Welfare and Management Practices Committee. *Review of mechanical euthanasia of day-old poultry*. Athens, Ga: American Association of Avian Pathologists, 2005.
313. Federation of Animal Science Societies (FASS). *Guide for the care and use of agricultural animals in agricultural research and teaching*. Champaign, Ill: Federation of Animal Science Societies, 2010.
314. Agriculture Canada. *Recommended code of practice for the care and handling of poultry from hatchery to processing plant*. Publication 1757/E.1989. Ottawa: Agriculture Canada, 1989.
315. European Council. *European Council Regulation No. 1099/2009 of 24 December 2009 on the protection of animals at the time of killing*. Brussels: The Council of the European Union, 2009.
316. Stavinoha WR. Study of brain neurochemistry utilizing rapid inactivation of brain enzyme activity by heating and microwave irradiation. In: Black CL, Stavinoha WB, Marvyama Y, eds. *Microwave irradiation as a tool to study labile metabolites in tissue*. Elmsford, NY: Pergamon Press, 1983;1–12.
317. Stavinoha WB, Frazer J, Modak AT. Microwave fixation for the study of acetylcholine metabolism. In: Jenden DJ, ed. *Cholinergic mechanisms and psychopharmacology*. New York: Plenum Publishing Corp, 1978;169–179.
318. Ikarashi Y, Marvyama Y, Stavinoha WB. Study of the use of the microwave magnetic field for the rapid inactivation of brain enzymes. *Jpn J Pharmacol* 1984;35:371–387.
319. Engilis A Jr, Engilis IE, Paul-Murphy J. Rapid cardiac compression: an effective method of avian euthanasia. *Condor* 2018;120:617–621.
320. Paul-Murphy JR, Engilis, Jr. A, Pascoe PJ, et al. Comparison of intraosseous pentobarbital administration and thoracic compression for euthanasia of anesthetized sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*). *Am J Vet Res* 2017;78:887–899.
321. Blackmore DK. Differences in behavior between sheep and cattle during slaughter. *Res Vet Sci* 1984;37:223–226.
322. Gregory NG, Wotton SB. Time to loss of brain responsiveness following exsanguination in calves. *Res Vet Sci* 1984;37:141–143.
323. Appelt M, Sperry J. Stunning and killing cattle humanely and reliably in emergency situations—a comparison between a stunning-only and stunning and pithing protocol. *Can Vet J* 2007;48:529–534.
324. Leach TM, Wilkins LJ. Observations on the physiological effects of pithing cattle at slaughter. *Meat Sci* 1985;15:101–106.

# Part III—Methods of Euthanasia by Species and Environment

## S1 Companion Animals

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

### S1.1 GENERAL CONSIDERATIONS

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

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

For individually owned companion animals, euthanasia will often be conducted in a private room in a veterinary clinic or in the home, to minimize animal and owner distress.[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.

## S1.2 ACCEPTABLE METHODS

### S1.2.1 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 references[8] 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.

## S1.3 ACCEPTABLE WITH CONDITIONS METHODS

### S1.3.1 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.

### S1.3.2 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 $CO_2$. 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.

### S1.3.3 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.

## S1.4 ADJUNCTIVE METHODS

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

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

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

## S1.5 UNACCEPTABLE METHODS

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

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

Other unacceptable approaches to euthanasia include hypothermia and drowning.

## S1.6 SPECIAL CONSIDERATIONS

### S1.6.1 Dangerous or Fractious Animals

Animals that are unable to be safely and humanely restrained should be sedated by means of drugs delivered orally (eg, gelatin capsules for delivery of drugs in food,[20] liquid formulations squirted into mouths[21]) 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]

### S1.6.2 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.

## S1.7 FETUSES AND NEONATES

Scientific data[23] 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 $D_2$, 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 ovariohysterectomy of pregnant dogs and cats and small mammals with altricial neonates, ligation of the uterine blood vessels with retention of the fetuses inside the uterus will result in death of the fetuses. The resistance of altricial neonates (eg, cats, dogs, mice, rats) to euthanasia methods whose mechanisms rely on hypoxia suggests that the uterus should not be opened for substantially longer periods than for precocial neonates,[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).

## S1.8 EUTHANASIA IN SPECIFIC ENVIRONMENTS

### S1.8.1 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.

### S1.8.2 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.

### S1.8.3 Animal Control, Sheltering, and Rescue Facilities

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

derivative with appropriate animal handling. When euthanizing animals that are well socialized without pre-euthanasia sedation or anesthesia, appropriate handling usually involves 2 trained people. One individual restrains the animal and the other administers the euthanasia agent.[25]

When euthanizing distressed, dangerous, or fractious animals, a sedative or anesthetic should be administered prior to attempting euthanasia. When the necessary restraint can be performed safely (appropriate handling techniques and equipment must be used), a pre-euthanasia sedative or anesthetic can be delivered IM or PO. After administration of the sedative or anesthetic, the animal is released so that it can return to a comfortable low-stress location (eg, dimly lighted cage or area) while the drug takes effect.[22] Because of the diversity of animals received by shelters, technicians performing euthanasia must have a good understanding of animal behavior and restraint, the proper use of equipment, and the variety of euthanasia drugs available and their effects.[26]

### S1.8.4 Laboratory Animal Facilities

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

## S2 Laboratory Animals

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

### S2.1 GENERAL CONSIDERATIONS

General comments about companion animals, farm animals, poikilotherms, and birds are provided elsewhere in the Guidelines, and usually apply to these species in the laboratory setting. Some other commonly used laboratory animal species are addressed later in the text. Most laboratory mammals currently used in biomedical research are small rodents that are maintained in large numbers. Venous access in these animals is typically difficult, and injectable agents are usually delivered via the IP route.

In addition to humane outcomes, an important consideration in the choice of method for euthanasia of laboratory animals is the research objectives for the animals being euthanized. Euthanasia methods can lead to metabolic and histologic artifacts that may affect research outcomes. For example, isoflurane may artificially elevate blood glucose concentrations,[27] while IP injection of barbiturates can create artifacts in intestinal tissues[28] and euthanasia by inhalation of $CO_2$ may elevate serum potassium concentrations.[29] Time elapsed between euthanasia and tissue collection can also be a critical factor affecting choice of euthanasia method.[30–32] Research needs may require the use of an adjunctive method (eg, bilateral thoracotomy, exsanguination, perfusion with fixatives, injection of potassium chloride). The application of such adjunctive methods is acceptable when the animal is fully anesthetized. Animals used in infectious disease studies may require special handling for animal and human health and safety.

### S2.2 SMALL LABORATORY AND WILD-CAUGHT RODENTS (MICE, RATS, HAMSTERS, GUINEA PIGS, GERBILS, DEGUS, COTTON RATS, ETC)

All activities related to the euthanasia of rodents deserve consideration equivalent to the euthanasia method itself, and may factor into the choice of method. Activities that contribute to distress in rodents include transport, handling (in animals not accustomed to it), disruption of compatible groups, and elimination of established scent marks.[31–45] As eliminating all sources of distress may not be practical or possible, the selected method of euthanizing rodents should minimize these sources of potential distress. Methods of euthanasia likely to elicit distress vocalizations or pheromones that other animals in the room could hear or smell may be best performed in another location, if transportation distress can be minimized. Similarly, wild-caught animals should be handled and euthanized in the manner least stressful to the animals. Death should be confirmed by physical examination, ensured by adjunctive physical method, or obviated by validation of euthanasia chambers and process.[46]

### S2.2.1 Acceptable Methods

*Barbiturates and barbituric acid derivatives*—Injectable barbiturates and barbiturate combinations generally act quickly and smoothly to render rodents unconscious. The euthanasia dose is typically 3 times the anesthetic dose. Pentobarbital is the most commonly used barbiturate for laboratory rodents because of its long shelf life and rapidity of action. There are reports that pain may be associated with barbiturate injections given via the IP route,[10,11,47,48] but the degree of pain and a suitable method for controlling pain have yet to be defined. The concurrent use of local anesthetics and anticonvulsants may aid in prevention of pain,[47] but it should be considered that these compounds may also cause pain with IP injection.

*Dissociative agent combinations*—In conscious rodents, ketamine and similar dissociative agents should be used in combination with an $\alpha_2$-adrenergic receptor agonist such as xylazine or benzodiazepines such as diazepam.[49]

## S2.2.2 Acceptable With Conditions Methods

S2.2.2.1 Inhaled agents

*Halogenated anesthetics*—Halothane, isoflurane, sevoflurane, or desflurane, with or without nitrous oxide, is acceptable with conditions for euthanasia of laboratory rodents.[50] These agents may be useful in cases where physical restraint is difficult or impractical. When used as a sole euthanasia agent delivered via vaporizer for anesthetic induction and overdose, animals may need to be exposed for prolonged time periods to ensure death.[51–57] Death can be rapid when using the open-drop technique, but care must be taken to ensure that the rodent does not come in direct contact with the anesthetic.

*Carbon dioxide*—Carbon dioxide, with or without premedication with halogenated anesthetics, is acceptable with conditions for euthanasia of small rodents.[58] Compressed $CO_2$ gas in cylinders are the recommended source of $CO_2$ because gas inflow to the chamber can be precisely regulated. An optimal flow rate for $CO_2$ euthanasia systems should displace 30% to 70% of the chamber or cage volume/min, with the understanding that there is potential for increased distress due to dyspnea at lower flow rates or mucous membrane pain associated with flow rates at the high ends of this range.[31,32,54,56,59–64] However, as there is no clear evidence of a flow rate that optimally minimizes both pain and distress for all species, sexes, and genetic backgrounds, veterinarians should use their professional judgment to determine which flow rate is appropriate for their circumstances.[31,32,56] Prefilled chambers are not recommended due to the potential for significant pain upon inhalation of the gas.[60,65] If euthanasia is not conducted in the home cage, induction chambers should be emptied and cleaned between uses. Addition of $O_2$ to $CO_2$ will prolong the time to death and may complicate determination of consciousness. There appears to be no advantage to combining $O_2$ with $CO_2$ for euthanasia.[11,60]

*Carbon monoxide*—Although CO administration is not commonly used in laboratory animal settings and has been determined to be as aversive as other inhalants,[52,66] it is acceptable with conditions as a method of rodent euthanasia when the conditions for effective and safe use can be met (see Inhaled Agents).

*Nitrous oxide*—Although nitrous oxide may be less aversive than the other inhaled gases described in this document,[67] it is a weak anesthetic with a long time to loss of consciousness and should not be used alone.[68] Nitrous oxide can be used in conjunction with other gases to shorten the time to loss of consciousness.[50,69]

S2.2.2.2 Noninhaled agents

*Tribromoethanol*—Although unavailable as a commercial or pharmaceutical-grade (USP/National Formulary/British Pharmacopeia) product, tribromoethanol is a commonly used rodent anesthetic. Its use is controversial due to its reported adverse effects (peritonitis and death).[70] However, many IACUCs have approved its use in rodents. Because there are no reports of tribromoethanol being used as a sole agent for euthanasia, it is recommended only as an anesthetic before the application of an approved secondary method.[70,71] Tribromoethanol must be prepared and stored properly and administered at the appropriate dosage.

*Ethanol*—Intraperitoneal injections of 70% to 100% ethanol may be an appropriate method of euthanasia for adult mice when physical methods are not desired or other euthanasia agents are unavailable.[72–75] Mice injected with 0.5 mL of 70% or 100% ethanol demonstrated gradual loss of muscle control, loss of righting reflex, respiratory and cardiac arrest, and death in 2 to 4 minutes.[72,74] The use of ethanol in mice < 35 days of age is questionable due to the long latency to death.[75] Ethanol is not recommended in larger species such as rats due to the large volume required to induce death.[74,75]

S2.2.2.3 Physical methods

*Cervical dislocation*—Cervical dislocation is used in laboratory settings. Cervical dislocation requires neither special equipment nor transport of the animal and yields tissues uncontaminated by chemical agents. Loss of cortical function following cervical dislocation is rapid and occurs within 5 to 10 seconds as measured by a significant reduction in amplitude recordings of visual evoked responses and EEG.[76,77] Cervical dislocation is acceptable with conditions for mice and rats < 200 g. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency.

*Decapitation*—Decapitation is used in laboratory settings because it yields tissues uncontaminated by chemical agents. Loss of cortical function following decapitation is rapid and occurs within 5 to 30 seconds as measured by a significant reduction in amplitude recordings of visual evoked responses and EEG changes.[76–78] Specialized rodent guillotines are available and must be kept clean, in good condition with sharp blades. If handled correctly, rats and mice do not show evidence of hypothalamic-pituitary-adrenal axis activation from decapitation or from being present when other rats or mice are decapitated.[79,80] Decapitation is acceptable with conditions for mice and rats. Personnel should be trained on anesthetized or dead animals to demonstrate proficiency.

*Focused beam microwave irradiation*—Focused beam microwave irradiation, using a machine professionally designed for animal euthanasia (see Physical Methods), is acceptable with conditions for euthanizing mice and rats. It is the preferred method when immediate fixation of brain metabolites is required for research purposes.

## S2.2.3 Unacceptable Methods

S2.2.3.1 Inhaled agents

*Nitrogen and argon*—Administration of $N_2$ or Ar is only acceptable in anesthetized mammals, as a

coexisting $O_2$ concentration of < 2% is necessary to achieve unconsciousness and death. Achieving that condition is difficult. In addition, Ar has been shown to be highly aversive to rats.[81] Although $N_2$ and Ar can kill animals,[82-84] other methods of euthanasia are preferable.

S2.2.3.2 Noninhaled agents

*Potassium chloride*—Intravenous or intracardiac administration of potassium chloride is not acceptable as a sole approach to euthanasia.

*Neuromuscular blocking agents*—Paralytic agents are unacceptable for use as sole euthanasia agents.

*Injectable barbiturates and neuromuscular blocking agents*—Combining injectable barbiturates and neuromuscular blocking agents in the same syringe for administration is unacceptable because the neuromuscular blocking agents may take effect before the animal is anesthetized.

*Opioids*—Opioids are unacceptable for euthanasia of laboratory animals as they are not rapidly acting, require high doses, and are not true anesthetic agents.

*Urethane*—Urethane is a human carcinogen used as a laboratory rodent anesthetic under certain conditions. It has a slow onset of action but a long duration of anesthesia. Because there are no reports of urethane being used as a sole agent for euthanasia, it is recommended only as an anesthetic before the application of an approved adjunctive method.[53,70,71]

*α-Chloralose*—α-Chloralose is unacceptable as a sole agent of euthanasia. α-Chloralose is a hypnotic agent that has poor analgesic properties[53,71] and is recommended only as an anesthetic before the application of an approved adjunctive method.

### S2.2.4 Fetuses and Neonates

Mammalian fetuses are unconscious in utero due to a combination of factors, such as low oxygen tension and hormonal influences in the uterus suppressing consciousness. Rat and mouse pups are born neurologically immature when compared with humans, and their afferent pain pathways are not well developed until after postnatal day 5 to 7, with cortical development occurring later.[85-90] Any laboratory rodents with altricial young, such as mice and rats, must be differentiated from rodents with precocial young, such as guinea pigs. Precocial young should be treated as adults.

S2.2.4.1 Acceptable methods

*Euthanasia of the dam and mammalian fetuses*—Rodent fetuses along with other mammals are unconscious in utero and hypoxia does not evoke a response.[91] Therefore, it is unnecessary to remove fetuses for euthanasia after the dam is euthanized.

S2.2.4.1.1 Noninhaled agents

*Injectable barbiturates alone and in combination with local anesthetics and anticonvulsants; dissociative agents combined with $\alpha_2$-adrenergic receptor agonist or benzodiazepines*—These agents are acceptable for use in fetuses or neonates.[92] See discussion on the use of these agents in adult rodents.

S2.2.4.2 Acceptable with conditions methods

S2.2.4.2.1 Inhaled agents

*Inhaled anesthetics*—Nonflammable volatile anesthetic agents are effective for in utero fetuses. Neonatal mice may take up to 50 minutes to die from $CO_2$ exposure,[93] and neonatal rats may take as long as 35 minutes.[94] Adequate exposure time should be provided, or an adjunctive method (eg, cervical dislocation, or decapitation) should be performed after a neonate is nonresponsive to painful stimuli. An adjunctive method (eg, cervical dislocation, decapitation) must be performed when halogenated anesthetics are used on neonatal rodents to avoid the possibility of recovery.[55]

S2.2.4.2.2 Physical methods

*Hypothermia*—The gradual cooling of fetuses and altricial neonates (mice and rats) is acceptable with conditions. There are no data to support the use of hypothermia as a single method, and it should be followed with a secondary method following loss of movement. As cold surfaces can cause tissue damage and presumably pain, the animals should not come in direct contact with ice or precooled surfaces. Hypothermia for anesthesia is not recommended after approximately 10 days of age.[53,88–90,95–97] Therefore, it is also an unacceptable euthanasia method in animals older than this age.[98]

*Rapid freezing*—Mouse and rat fetuses and neonates < 5 days of age may be quickly killed by rapid freezing in liquid $N_2$.[23,96,97]

*Decapitation*—Decapitation using scissors or sharp blades is acceptable with conditions for altricial neonates.[96,97] Some rodent neonates, whether altricial or precocial, may have a tissue mass that is too large for scissors, so appropriate decapitation tools should be selected.

*Cervical dislocation*—Cervical dislocation by pinching and disrupting the spinal cord in the high cervical region is acceptable with conditions for fetal and neonatal mice and rats.

## S2.3 Laboratory Farm Animals, Dogs, Cats, Ferrets, and Nonhuman Primates

### S2.3.1 General Considerations

The research goals will often influence the choice of method of euthanasia for farm animals, dogs, cats, and ferrets. Generally, sedation (as needed) followed by IV injectable barbiturates will be the preferred method. Tributame administered IV by trained personnel may be an appropriate replacement for dogs if injectable barbiturates are not available. For more information on other methods of euthanasia of farm and companion species, consult the appropriate sections of the guidelines.

For nonhuman primates and other wild-caught or nondomesticated animals used in the laboratory, some general principles apply. Again, the research

being conducted may influence the choice of euthanasia method, and if the institutional animal care and use program staff is unfamiliar with a species, researchers working with the species may provide valuable guidance. Appropriate restraint for the species must always be applied. Distress in animals unfamiliar with handling should be minimized. Venous access should be established or IM agents may be used (delivered via remote injection equipment if necessary) for sedation. These animals are preferentially euthanized with an injectable barbiturate.

### S2.3.2 Special Cases

When animals to be euthanized are fully anesthetized, adjunctive methods such as bilateral thoracotomy, exsanguination, perfusion, and IV or intracardiac injection of potassium chloride are acceptable.

## S2.4 LABORATORY RABBITS

### S2.4.1 General Considerations

Recommended euthanasia methods of rabbits depend on the facilities and conditions in which the animals are housed and maintained. In a controlled setting such as a biomedical research facility where researchers have access to restraint devices and controlled drugs, IV injection of barbiturates is usually used. In large commercial production operations for biomedical research or meat, or to safely euthanize injured wild rabbits, the method selected will depend on the availability of resources and the skill and training of the operator. In all situations, the animals should be handled and euthanized in the manner least distressful to them. Death should always be verified. Lack of breathing and palpable heartbeat as well as a fixed dilated pupil are some of the easiest recognized indicators of death.

### S2.4.2 Acceptable Methods

S2.4.2.1 Noninhaled agents

*Barbiturates and barbituric acid derivatives*—If rabbits are conditioned to handling or restraint devices are available, venous access may be obtained via the ear. In the case of fractious rabbits, sedation may be necessary to gain venous access for administration of an injectable barbiturate or injectable barbiturate combination. Barbiturates may also be administered IP. The concurrent use of local anesthetics and anticonvulsants may aid in prevention of pain,[47] but it should be considered that these compounds may also cause pain with IP injection. These approaches are acceptable for companion rabbits as well.

### S2.4.3 Acceptable With Conditions Methods

S2.4.3.1 Inhaled agents

*Halogenated anesthetics*—Inhaled anesthetics will generally be available only in a controlled setting, such as a biomedical research facility or veterinary care provider. In these situations, it is best to preanesthetize the animal with a sedative before removing it from the home cage and relocating it to access restraint devices and a gas anesthesia machine. This method will reduce their natural tendency to breath-hold when confronted with unpleasant odors.[99–101] Animals already under anesthesia may be euthanized by an overdose of anesthetic.

*Carbon dioxide*—The recommended $CO_2$ displacement rate for rabbits is 50% to 60% of the chamber or cage volume/min. As fossorial animals, rabbits appear to have a higher tolerance for elevated $CO_2$ levels, so its use as a sole euthanasia agent may cause distress.[102,103] In high concentrations (70%, 80%, 90%, 98%), $CO_2$ has been reported[104] to result in aversive behaviors for 15 seconds before the onset of unconsciousness. Walsh[b] reported no distress behaviors observed when $CO_2$ was administered to rabbits at lower flow rates of approximately 28% and 58% chamber volume replacement/min. The fast-flow rate is recommended for $CO_2$ euthanasia, as it resulted in significantly earlier loss of sensibility (40 seconds) and death than gradual fill (99 seconds).[b] Premedication with sedatives may reduce potential aversion responses.

S2.4.3.2 Physical methods

*Cervical dislocation*—Cervical dislocation is acceptable with conditions for rabbits when performed by individuals with a demonstrated high degree of technical proficiency. The need for technical competency is great in heavy or mature rabbits in which the large muscle mass in the cervical region makes manual cervical dislocation more difficult. Mechanical devices designed to firmly hold the rabbit's head and facilitate the operator's applying downward force to the hips and back legs reduce the strength needed by the operator to euthanize rabbits. These devices have been shown to be highly effective (96%) for preweaned kits, growers, and adults. Training with cadavers is recommended.[105]

*PCB*—The use of rabbit-sized PCBs to euthanize rabbits in laboratory or production facilities is acceptable with conditions. The captive bolt must be maintained in clean working order, positioned correctly (by placing the captive bolt slightly paramedian on the frontal bone as close to the ears as possible),[106] and operated safely by trained personnel. It is essential to stabilize the head to prevent misses. Animals should be restrained on nonslip flooring, preferably in an open-top container allowing the rear of the rabbit to be pressed against the container wall. Using the nondominant hand, the operator should restrain the rabbit by pressing on the shoulder blades, and the thumb and forefinger should be placed gently around the neck of the rabbit. These devices are often aesthetically displeasing and often result in environmental contamination, which may be a significant concern when sick animals are being removed from the colony. Operators must be trained, preferably on cadavers.

*NPCB*—The use of rabbit-sized NPCB to eutha-

nize rabbits in laboratory or production facilities is acceptable with conditions. The NPCB has been shown to result in immediate insensibility in 100% of the events.[105] Animals should be restrained on nonslip flooring, preferably in an open-top container allowing the rear of the rabbit to be pressed against the container wall. Using the nondominant hand, the operator should restrain the rabbit by pressing on the shoulder blades, and the thumb and forefinger should be placed gently around the neck of the rabbit. The device must be maintained in clean working order, positioned correctly (center of the forehead, with the barrel placed in front of the ears and behind the eyes), and discharged twice in rapid succession at the pressure recommended for the age and size of the rabbit (55 psi for preweaned kits, 70 psi for growers, 90 psi for adults; **Figure 10**).[105] Operators must be trained, preferably on cadavers.

### S2.4.4 Special Cases

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

Manual application of blunt force trauma to the head has been shown to be difficult and aesthetically displeasing, resulting in undesired tissue damage, as well as being less effective than other means.[105,107,b] Blunt force trauma should be used only in emergency situations under extenuating circumstances, such as an injured rabbit too large for the operator to cervically dislocate and an operator without any supporting resources.

## S2.5 LABORATORY FISH, AMPHIBIANS, AND REPTILES

Recommending euthanasia methods for finfish, aquatic invertebrates, amphibians, and reptiles used in biomedical research is challenging due to the enormous number of species in use and variations in their biological and physiologic characteristics. In this section, only the most commonly used methods will be discussed for several frequently used species. Other methods less often used for euthanizing species used in research are discussed in detail in the relevant sections of the Guidelines. See these sections for additional information.

There are no US FDA-approved drugs for euthanasia of aquatic animals. Tricaine methanesulfate is an FDA-approved drug for temporary immobilization (sedation, anesthesia) of finfish, amphibians, and other aquatic, cold-blooded animals. Immersion of adult zebrafish in MS 222 for 10 minutes following loss of rhythmic opercular movement was previously recommended. However, because recovery of fish after exposure for this time has been shown to occur, 30 minutes is recommended as a precautionary measure until research is available to demonstrate immersion times needed to reliably cause irreversible death in zebrafish. Zebrafish show some signs of stress when immersed in MS 222, and a secondary (physical) method of euthanasia is recommended to ensure death.[108] MS 222 alone is not effective for euthanasia of zebrafish eggs, embryos, or larvae (< 14 days old), and other methods should be used for these life stages.[109]

As described in the aquatics section it is acceptable for zebrafish (*Danio rerio*) to be euthanized by rapid chilling (2° to 4°C) until loss of orientation and cessation of operculur movements. Subsequent additional exposure of the fish to chilled water for times specific to fish size and age[108,110,111] should be used to ensure death. Rapid chilling of adult zebrafish resulted in cessation of vital signs (10.6 ± 3.28 seconds) 20 times as fast as in the case of MS 222 overdose (216.3 ± 62 seconds).[112] Adult zebrafish should be exposed for a minimum of 10 additional minutes following the loss of opercular movements. Zebrafish fry 4 to 14 days after fertilization (dpf) should be exposed for at least 20 additional minutes following loss of opercular movements.[113] Rapid chilling (as well as MS 222) has been shown to be an unreliable euthanasia method for embryos < 3 dpf.[108,112,114] Immersion in diluted sodium or calcium hypochlorite solution is acceptable for embryos up to 7 days of age.[115] If necessary to ensure death of other life stages, rapid chilling may be followed by either an approved adjunctive euthanasia method or a humane killing method. Until further research is conducted, rapid chilling is acceptable with conditions for other small-bodied tropical and subtropical stenothermic species.

Amphibian species commonly used in research include the African and Western clawed frog (*X laevis*, *Xenopus tropicalis*), leopard and bull (*Rana* spp) frogs, and axolotls (*Ambystoma mexicanum*). These species are best euthanized via a physical method while fully anesthetized. While injection of sodium pentobarbital IV, intracelomically, or in the lymph spaces is an acceptable method of euthanasia of these species, high doses are often required and these agents may have an inconsistent time to loss of consciousness (S7.3.4.1).[116]

# S3. Animals Farmed for Food and Fiber

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

## S3.1 GENERAL CONSIDERATIONS

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

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 study[126] 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-

arm of choice is a rifle: 2) a solid-point bullet should be used, 3) it must be fired within close range of the skull (within 30 to 90 cm [1 to 3 feet]), and 4) the bullet must be directed so that proper anatomic placement on the skull is assured.[127]

*Shotguns*—Shotguns loaded with birdshot (lead or steel BBs) or slugs (solid lead projectiles specifically designed for shotguns) are appropriate from a distance of 1 to 2 yards (1 to 2 m). Although all shotguns are lethal at close range, the preferred gauges for euthanasia of cattle are 20, 16, or 12. Number 6 or larger birdshot or shotgun slugs are the best choices for euthanasia of cattle.[124] Birdshot begins to disperse as it leaves the end of the gun barrel; however, if the operator stays within short range of the intended anatomic site, the birdshot will strike the skull as a compact bolus or mass of BBs with ballistic characteristics on entry that are similar to a solid lead bullet. At close range, penetration of the skull is assured with massive destruction of brain tissue from the dispersion of birdshot into the brain that results in immediate loss of consciousness and rapid death.

The Canadian study[126] cited previously evaluated several firearms, including the .410 and 12-gauge shotguns. The .410 loaded with either number 4 or number 6 birdshot fired from a distance of 1 m was very effective and had the advantage of less recoil compared with other firearms used. The 12-gauge shotgun loaded with number 7 1/2 birdshot fired from a distance of 2 m from its target was effective but considered to be more powerful than necessary. Results of a 1-oz rifled slug fired from a 12-gauge shotgun at a distance of 25 m failed to penetrate the brain not because it lacked power, rather because of faulty shot placement. Researchers concluded that the rail sighting system on the shotgun was not sufficient for accurate shot placement if it was necessary to shoot from a distance. They also believed that recoil from this firearm would likely make it unpleasant to use if it were necessary to euthanize a large number of animals.[126]

One advantage of euthanasia using a shotgun is that when properly directed the birdshot will have sufficient energy to penetrate the skull but is unlikely to exit the skull. In the case of a free bullet or shotgun slug there is always the possibility of the bullet or slug exiting the skull, creating an injury risk for operators and observers. For operator and bystander safety, the muzzle of a shotgun (or any other firearm) should never be held directly against the animal's head. Discharge of the firearm results in development of enormous pressure within the barrel that can result in explosion of the barrel if the muzzle end is obstructed or blocked.

*PCB or NPCB*—Penetrating captive bolts are recommended for euthanasia of mature cattle in field situations. The use of NPCBs should be limited to calves. In large cattle NPCB is less effective than PCB.[128,129] Styles include in-line (cylindrical) and pistol grip (resembling a handgun) versions. Pneumatic captive bolt guns (air powered) are limited to use in slaughter plant environments. Models using gunpowder charges are more often used in farm environments. They consist of a steel bolt and piston at 1 end, housed within a barrel. Upon firing, the rapid expansion of gas within the breech and barrel propels the piston forward driving the bolt through the muzzle. A series of cushions are strategically located within the barrel to dissipate excess energy of the bolt. Depending upon model, the bolt may be manually or automatically retracted. Accurate placement over the ideal anatomic site, energy (ie, bolt velocity), and depth of penetration of the bolt determine effectiveness of the device to cause a loss of consciousness and death. Bolt velocity is dependent on maintenance in particular, as well as cleaning and storage of the cartridge charges. Bolt velocities of 55 to 58 m/s are desirable for effective captive bolt use in packing plants.[130–133] Recommended minimum bolt velocities proposed for shooting bulls are as high as 70 m/s. In packing plants where bolt velocity is of particular concern, bolt velocity is routinely monitored to assure proper function of these devices.[134]

In general, captive bolt guns, whether penetrating or nonpenetrating, induce immediate loss of consciousness, but death is not always assured with the use of this device alone. In a study of 1,826 fed steers and heifers, 3 (0.16%) had signs of a return to sensibility or consciousness.[129] Results were similar in observations of 692 bulls and cows where 8 (1.2%) animals had signs consistent with a return to consciousness.[129] Failure to achieve a 100% loss of consciousness with no return to a conscious mental state was attributed to storage of the captive bolt charges in a damp location, poor maintenance of firing pins, inexperienced personnel operating the captive bolt (use of the incorrect anatomic site), misfires associated with a dirty trigger on the captive bolt, and use of the device on cows and bulls with thick, heavy skulls.[129,135]

At the present time, an adjunctive method such as exsanguination, pithing, or the IV injection of a saturated solution of potassium chloride is recommended to ensure death when PCB is used.[127] A newer version of PCB has emerged in recent years.[125] This device is equipped with an extended bolt with sufficient length and cartridge power to increase damage to the brain, including the brainstem. This device is being studied at the present time and may offer a euthanasia option with the PCB that does not require the need for an adjunctive method.

Captive bolt guns are attractive options for euthanasia because they offer a greater degree of safety to the operator and bystanders; but they should only be used by trained people. The muzzle should always be pointed toward the ground and away from the body or bystanders in case of accidental discharge. Protective gear for both ears and eyes is strongly recommended.

Unlike techniques described for gunshot, the

animal must be restrained for accurate placement of the captive bolt. And, unlike use of a firearm, proper use of the captive bolt requires that the muzzle of the device be held firmly against the animal's head. Once the animal is restrained, discharge of the captive bolt should occur with little or no delay so that animal distress is minimized. Adjunctive methods should be implemented as soon as the animal is rendered unconscious to avoid a possible return to sensibility. Thus, when conducting euthanasia by captive bolt, some preplanning and preparation improves the likelihood of a successful outcome.

Visual indicators that an animal has been rendered unconscious from captive bolt or gunshot include the following: immediate collapse; brief tetanic spasms followed by uncoordinated hind limb movements; immediate and sustained cessation of rhythmic breathing; lack of coordinated attempts to rise; absence of vocalization; glazed or glassy appearance to the eyes; and absence of eye reflexes.[117] Nervous system control of the blink or corneal reflex is located in the brainstem; therefore, the presence of a corneal reflex is highly suggestive that an animal is still conscious.

*Anatomic landmarks for use of the PCB and gunshot*—In cattle, the point of entry of the projectile should be at the intersection of 2 imaginary lines, each drawn from the outside corner of the eye to the center of the base of the opposite horn, or an equivalent position in polled animals **(Figure 11A)**.[136] In long-faced cattle or young stock, a point on the midline of the face that is halfway between the top of the poll and an imaginary line connecting the outside corners of the eyes can be used **(Figure 11B)**. Proper positioning of the firearm or PCB is necessary to achieve the desired results.

*Euthanasia of bison*—Proper handling of bison requires an understanding and respect for the wild nature of these animals. Handlers should strive to minimize stress by moving animals slowly utilizing techniques similar to those applied in low-stress handling of cattle. Restraint requires specialized handling equipment designed to accommodate bison body conformation.

The recommended method for the euthanasia of a bison is gunshot. A minimum of 1,356 J (1,000 feet-lb) of muzzle energy is required for euthanasia of yearlings, cows, and mature bulls. This limits the firearm options to higher-caliber centerfire rifles (eg, 30-30, 270, 30-06, and others). The majority of handguns produce muzzle energies well below 1,356 J (1,000 feet-lb) and would not be appropriate for euthanasia of mature bison.[137]

The preferred anatomic site for entry of a bullet is on the forehead approximately 2.5 cm (1 inch) above an imaginary line connecting the bottom of the horns **(Figure 12)**, which places the shot in a similar location to recommendations for mature cattle. Ideally, the angle of entry should be perpendicular to the skull. If it is necessary to shoot the animal from a distance, targets may be the head (frontal or lateral side) or the thorax (heart shot).[137]

*Euthanasia of water buffalo*—There are important anatomic differences that need to be considered when determining the best method of euthanasia for water buffalo, compared with cattle. Skull bones are substantially thicker and the frontal and paranasal sinuses noticeably wider in buffalo, compared with cattle. Moreover, measures of the median distance from the frontal skin surface to the thalamus were 144.8 mm (117.1 to 172.0 mm) vs 102.0 (101.0 to 121.0 mm) in water buffalo and cattle, respectively.[138] Bolt length of conventional captive bolt devices is 9 to 12 cm (3.5 to 4.7 inches), meaning that the ability of the bolt to make direct contact with the thalamus and brainstem is less likely using frontal sites in water buffalo, compared with cattle. For this reason, use of the PCB at frontal sites in water buffalo is generally less effective.

Considering the thicker skin and skull encountered with water buffalo, the use of a high-caliber firearm is the preferred method of euthanasia under field conditions. In the study by Scwhenk et al,[138] the frontal point of entry for captive bolt guns was chosen as the intersection of 2 imaginary lines connecting the lower edge to the upper edge of the contralateral horn **(Figure 13)**. This site would appear to be similar to sites described in the guidelines for domestic cattle.

Depth of concussion was evaluated in 30 water buffalo shot at the poll (described as the back of the head) with a PCB.[139] Prevalence of a shallow depth of concussion was observed in 53% of animals. In 2 of the animals studied, the bolt missed the brain completely and entered the spinal cord. In 1 case, the bolt failed to penetrate into the brain. On postmortem examination of the brain, 79% of animals were shot in the midbrain or brainstem. One animal, an older bull, was shot in the frontal position but failed to collapse. On postmortem examination, researchers confirmed that the bolt never penetrated into the cranial cavity of this animal. Three animals shot in the crown position (ie, the top of the head—sometimes referred to as the poll) immediately collapsed, but 2 resumed breathing shortly before ejection from the stunning pen and had to be reshot. Researchers concluded that poll position stunning could be effective in water buffalo, but accurate placement of the PCB was important to prevent misdirection into the spinal cord, and animals need to be exsanguinated soon after they have lost consciousness.

S3.2.1.3 Adjunctive methods

S3.2.1.3.1 Noninhaled agents

*Potassium chloride and magnesium sulfate*—Bolusing saturated solutions of salt through the heart disrupts electrical conductivity in heart muscle, resulting in fibrillation and heart failure. Animals must be unconscious before administration of potassium chloride or magnesium sulfate. While not acceptable as a sole method of euthanasia, rapid IV injection of

potassium chloride may assist in ensuring death after cattle have been rendered unconscious by PCB, gunshot, or administration of general anesthetics ($\alpha_2$-adrenergic agents such as xylazine alone are insufficient; see comments under Unacceptable methods). Normally, injection of 120 to 250 mL of a saturated solution of potassium chloride is sufficient to cause death; however, the potassium chloride solution should be administered until death is assured. When conducting euthanasia of cattle that may require subsequent administration of potassium chloride, the operator should prepare at least 3 to four 60-mL syringes of solution (equipped with 14- or 16-gauge needles) in advance. This will facilitate rapid administration and ensure the animal does not regain consciousness. Any available vein may be used; however, it is important to position oneself out of the reach of limbs and hooves that may cause injury during periods of involuntary movement. In most cases, it is safest to kneel down near the animal's back and close to the animal's head where one can reach over the neck to administer the injection into the jugular vein. Once the needle is in the vein, the injection should be delivered rapidly.

Magnesium sulfate may be administered similarly to potassium chloride. Death may not occur as rapidly, but similar to administration of potassium chloride, residue risks for predators and scavengers are low (see Noninhaled Agents).

S3.2.1.3.2 Physical methods

*Second shot*—Although 1 well-placed bullet or shot from a PCB usually results in immediate loss of consciousness with little likelihood of return to consciousness, one should always be prepared to deliver a second or even a third shot if necessary. The additional injury to brain tissue along with increased hemorrhage and edema creates substantial intracranial pressure. Compression resulting from this increase in pressure interrupts centers in the brain that control respiratory and cardiac functions and leads to death.

*Exsanguination*—Exsanguination may be performed as an adjunctive measure to ensure death when necessary in an unconscious animal. Exsanguination is usually accomplished via an incision of the ventral aspect of the throat or neck transecting skin, muscle, trachea, esophagus, carotid arteries, jugular veins, and a multitude of sensory and motor nerves and other vessels. This procedure is not recommended as a sole method of euthanasia; rather it is reserved for use as an adjunctive method to ensure death since information in the literature is inconsistent as to length of time between the neck cut and loss of consciousness. Some studies[140,141] demonstrate a rapid loss of brain activity (measured by EEG) with little variation between individual animals. In contrast, direct observation of time to collapse and EEG data indicate that the time from ventral-neck incision to unconsciousness is variable and may be quite prolonged in animals killed by exsanguination.[142–146]

The uncertainty of time from the neck incision to loss of consciousness raises obvious questions: Does the animal feel pain during the neck cut? Does the drop in blood pressure cause discomfort or distress? Opinions on these questions remain divided. Some hold the view that when the knife (*sakin* in Hebrew) is of appropriate size, exceptionally sharp, completely free of blemishes or imperfections, and used in such manner as to create a rapid clean incision (such as performed by a shochet), exsanguination is relatively painless.[147] Others contend that tissues of the neck are well innervated with nociceptive nerve fibers such that transection leads to significant pain and distress sufficient to cause shock at the time of incision.[148–150]

In recognition that this issue remains controversial and that people conducting these procedures for the purposes of euthanasia are not likely to have a sakin or the skills of a shochet, the recommendation is that exsanguination only be used in unconscious animals as an adjunctive method to assure death. It should be performed with a pointed, very sharp knife with a rigid blade at least 15 cm (6 inches) long and conducted as soon as the loss of consciousness is confirmed.

Exsanguination can be disturbing to observe due to the large volume of blood loss; this also raises biosecurity concerns. When only the carotid arteries and jugular veins are cut, bleeding may persist at variable rates for several minutes. Severing these vessels closer to the thoracic inlet where the vessels are larger will increase blood flow rate. Some evidence suggests that restricted blood flow may be caused by the formation of false aneurysms in the severed ends of arteries in cattle.[146]

*Pithing*—Pithing is a technique designed to cause death by increasing destruction of brain and spinal cord tissue. It is performed by inserting a pithing rod through the entry site produced in the skull by a bullet or PCB. The operator manipulates the pithing tool to destroy brainstem and spinal cord tissue to ensure death (see Physical Methods). Muscular activity during the pithing process is often quite violent, but is followed by quiescence that facilitates exsanguination or other procedures.[151]

S3.2.1.4 Special cases and exceptions

In emergency situations, it may be difficult or impossible to safely restrain a dangerous animal for IV injection, and administration of a sedative to allow restraint might not be desirable because of the possibility that the animal could injure itself or bystanders before the sedative could take effect. In such cases, a neuromuscular blocking agent (eg, succinylcholine) may be administered IM, but the bovid must be euthanized via an appropriate method as soon as it can be controlled. Use of succinylcholine alone or without prior anesthesia is not acceptable for euthanasia.

Use of the poll (bony protuberance on the top of the skull) for application of the PCB in abattoirs is not allowed by regulations in the European Union

because the depth of concussion in this region is less than that observed in frontal sites.[152] Conversely, for large bulls and water buffalo, use of the frontal site for administration of a captive bolt is not always effective because of the thickness of the hide and skull in this region. Use of the poll position can be effective if the appropriate captive bolt gun is used and when the muzzle is directed so that the discharged bolt will enter the brain.[152] But in most cases the poll position is not preferred. Research has demonstrated that use of PCB at the poll is prone to operator error and misdirection of the bolt into the spinal cord instead of the brain.[152] More animals were not properly rendered unconscious (ie, depth of concussion was shallow) using the poll position as compared with frontal sites. Additionally, in some emergency situations, when the face is not safely accessible, a shot directed from just behind the poll and aimed toward the base of the tongue may be employed. This positioning should not be routine.

Placement of the captive bolt is critical to ensure that the bolt enters the brain and not the spinal cord. Shots from the poll should be directed toward the base of the tongue unless brainstem tissues are needed for diagnostic reasons. Whether poll shooting is conducted by PCB or gunshot, there is substantial potential for misdirection of the bullet or bolt so that damage to the brain to achieve unconsciousness or death is not assured. This will result in delays in loss of consciousness and a greater likelihood of variable periods of extreme distress.

### S3.2.2 Small Ruminants

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

S3.2.2.1 Acceptable methods

S3.2.2.1.1 Noninhaled agents

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

S3.2.2.2 Acceptable with conditions methods

S3.2.2.1.1 Inhaled agents

Carbon dioxide inhalation as a form of euthanasia has been evaluated in young goat kids (< 3 weeks of age). Aversion testing suggests that concentrations below 70% $CO_2$ are not aversive to goat kids, as they were willing to freely enter a test chamber containing up to 70% concentration to receive a milk meal. All kids entering the chamber of 70% $CO_2$ lost consciousness while consuming the meal. Based on these data and clinical experience it is recommended that once kids are placed in a euthanasia chamber it should be filled at a rate sufficient to achieve a $CO_2$ concentration of > 70% by 5 minutes and to use a dwell time of 10 minutes to assure euthanasia. Euthanasia by $CO_2$ inhalation has not been evaluated in older goats or sheep. Given that aversion to $CO_2$ is variable by species and that physiologic differences in age and weight could affect success, the suitability of this form of euthanasia in other ruminant species warrants further investigation but cannot currently be recommended due to a lack of knowledge.[153,154]

S3.2.2.2.2 Physical methods

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

*PCB and NPCB*—Effective application of the captive bolt in sheep and goats is indicated by immediate loss of consciousness lasting until death by exsanguination or another adjunctive method. While it is presumed that penetration of the bolt causes insensibility, research into the determinants of effective captive bolt use indicates that the impact of the bolt on the cranium is a principal contributor to the loss of consciousness.[131] The use of concussive methods (NPCB) has been determined to be an effective means of inducing insensibility in neonatal goats and kids that will persist until death caused by exsanguination.[132]

*Anatomic landmarks for gunshot and captive bolts*—The location for placement of a captive bolt or entry of a free bullet for euthanasia is similar for both sheep and goats. The optimal site is on the intersection of 2 lines, each of which is drawn from the lateral canthus of one eye to the middle of the base of the opposite ear **(Figures 14AB, 15AB)**.[155,156] Alternative landmarks that provide a very similar placement use the dorsal midline of the head at the level of the

external occipital protuberance aiming downward toward the cranialmost portion of the intermandibular space.[157] Frontal shots, aiming at the foramen magnum, should be reserved for use only with gunshot and provide an alternate approach for heavily horned sheep and goats where the top of the skull may be too hard to access due to the horns. The large and variable sinus cavity of sheep and goats makes frontal shots with a captive bolt more inconsistent.[136]

*Camelids*—The effectiveness of PCB was studied in 96 alpacas. Incorrect placement of the captive bolt resulted in incomplete concussion and the failure of 10 animals to be rendered insensitive. Researchers evaluated 2 anatomic sites: a frontal and a crown site. The frontal site was defined as on the intersection of 2 lines drawn from the medial canthus of each eye to the opposite ear while aiming the PCB at the brainstem. This site proved to be less effective because animals would move their head as the PCB entered their field of view immediately prior to the shot. Placement of the PCB at the crown position (ie, highest point on the head) aiming downward to the base of the jaw **(Figure 16)** was more likely to result in an immediate loss of consciousness and cause damage to the thalamus and brainstem. While both landmarks are considered acceptable for captive bolt placement, if the frontal site is used increased restraint of the animal may be required to prevent movement that would result in incorrect placement.

*Cervids*—For euthanasia of captive farmed cervids, several options exist. For animals accustomed to being worked through drop chutes, the animals can be restrained in a drop chute and shot with a captive bolt. For deer without antlers, the proper site for entry of a bolt from a PCB is the same as that described for goats and sheep previously—on the intersection of 2 lines drawn from the lateral canthus to the base or top of the opposite ear. For antlered deer (bucks), a frontal site may be necessary, but may require a longer bolt length. The frontal site may be determined as on the intersection of 2 lines drawn from the lateral canthus of each eye to the top of the base of the ear or base of the antler **(Figure 17AB)**. When drop chutes are not available or when the animals are not accustomed to being restrained in the chute, it may be beneficial to consider darting the animal with an anesthetic followed by use of a captive bolt or firearm at the sites discussed previously. When immobilization is feasible, it ensures accurate shot placement and minimizes the risk of a prolonged euthanasia. Immobilized animals (either chemically immobilized or immobilized from injury such as being hit by a car) should always be euthanized with a PCB or firearm via the sites discussed above. Shooting in the thorax or so-called center mass should be avoided. In situations where the ability to sufficiently immobilize fractious animals is not available or immobilization poses a significant risk to human health, killing by trained shooters using specialized equipment can be highly effective.

S3.2.2.3 Adjunctive methods

S3.2.2.3.1 Noninhaled agents

*Potassium chloride and magnesium sulfate*—Although not acceptable as a sole method of euthanasia, the rapid IV injection of potassium chloride is an effective method to ensure death in sheep and goats previously rendered unconscious by penetrating or NPCB, gunshot, or administration of anesthetics. When conducting euthanasia of sheep and goats that may require subsequent administration of potassium chloride, the operator should prepare at least 1 or two 30-mL syringes of solution (equipped with an 18-gauge needle) in advance. This will facilitate rapid administration and ensure the animal does not regain consciousness. Any available vein may be used; however, it is important to position oneself out of the reach of limbs and hooves that may cause injury during periods of involuntary movement. Once the needle is in the vein, the injection should be delivered rapidly.

Magnesium sulfate may be administered similarly to potassium chloride. Death may not occur as rapidly, but similar to administration of potassium chloride, residue risks for predators and scavengers are low (see Noninhaled Agents).

S3.2.2.3.2 Physical methods

*Second shot*—Although 1 well-placed bullet or shot from a PCB usually results in immediate loss of consciousness with little likelihood of return to consciousness, one should always be prepared to deliver a second or even a third shot if necessary. The additional injury to brain tissue along with increased hemorrhage and edema creates sufficient intracranial pressure to cause death in most cases, but damage to the brainstem should always be the objective in euthanasia.

*Exsanguination*—Exsanguination may be performed as an adjunctive step to ensure death when necessary in small ruminants. It may be accomplished via an incision of the ventral aspect of the throat or neck transecting skin, muscle, trachea, esophagus, carotid arteries, and jugular veins. Exsanguination should be performed with a pointed, very sharp knife with a rigid blade at least 15 cm (6 inches) long.

Exsanguination can be disturbing for bystanders because of the large volume of blood loss, which also raises biosecurity concerns. When only the carotid arteries and jugular veins are cut, bleeding may persist at variable rates for several minutes. Severing these vessels closer to the thoracic inlet where the vessels are larger will increase blood flow rate.

*Pithing*—Pithing is a technique designed to cause death by increasing destruction of brain and spinal cord tissue. It is performed by inserting a pithing rod through the entry site produced in the skull by a bullet or PCB. The operator manipulates the pithing tool to destroy brainstem and spinal cord tissue to ensure death (see Physical Methods). Muscular activity during the pithing process is often quite violent, but is followed by quiescence that facilitates exsanguination or other procedures.[151]

### S3.2.3 Unacceptable Methods

The following methods are unacceptable for euthanasia of cattle and small ruminants: manually applied blunt trauma to the head; injection of chemical agents into conscious animals (eg, disinfectants, electrolytes such as potassium chloride and magnesium sulfate, nonanesthetic pharmaceutical agents); administration of xylazine or any other $\alpha_2$-adrenergic receptor agonist followed by IV potassium chloride or magnesium sulfate (although large doses of $\alpha_2$-adrenergic receptor agonists can produce a state resembling general anesthesia, they are recognized as being unreliable for that purpose[158]), drowning, or air embolism (ie, injection of air into the vasculature); and electrocution with 120 V, drowning, and exsanguination in conscious animals.

Camelids, specifically alpacas and llamas, are kept primarily for fiber production and as pets. In South America, domestic camelids are maintained for food and as pack animals. Camelids are slaughtered by the method of puntilla, which involves inserting a knife into the back of the neck to sever the spinal cord. This immobilizes the animal, making exsanguination procedures safer[154]; however, research in llamas and cattle indicates that the stab is often ineffective in causing insensibility.[159,160] Observations in 20 llamas killed by puntilla found that repeated stabbing was necessary to penetrate the foramen ovale; all animals exhibited rhythmic breathing and 95% had positive palpebral reflexes.[159] Because of the inconsistency of the puntilla method, it cannot be recommended as an acceptable method of euthanasia of small ruminants or cattle.

### S3.2.4 Neonates

*Neonatal calves, lambs, and kids*—Neonatal calves present special challenges for euthanasia. Methods include barbiturate overdose, gunshot, and captive bolt (penetrating or nonpenetrating) with an adjunctive method applied to ensure death. Manually applied blunt force trauma to the head is not acceptable for calves because their skulls are too hard to achieve immediate destruction of brain tissue leading to unconsciousness and death. In lambs and kids, manually applied blunt force trauma is performed by manual impact of the head by swinging the animal against a hard surface or hitting the animal with a hard object such as a hammer. Both methods are difficult if not impossible to apply consistently and therefore are listed as unacceptable.

Use of a penetrating or purpose-built NPCB (controlled blunt force trauma) is acceptable with conditions for calves, lambs, and kids. Controlled blunt force trauma differs from manually applied blunt force trauma because captive bolts deliver an appropriate and uniform amount of force each time they are fired, and structural brain damage is more consistent. Studies[161] using controlled blunt force trauma methods found that focal as well as diffuse injury caused by penetrating and NPCB pistols was similar and sufficient for both to be considered as effective for euthanasia of lambs. Based on electrophysiologic evidence,[131] researchers determined that the primary determinant of effective shooting is the impact of the bolt and not penetration of the bolt into brain tissues. In contrast, 1 report[162] credits structural changes including focal damage adjacent to the wound track and damage to peripheral tissues of the cerebrum, cerebellum, and brainstem as the predominant factors affecting the loss of respiratory function and consciousness.

Controlled blunt force trauma using an NPCB applies a consistent degree of force and has been determined to cause immediate insensibility in neonatal calves,[154] lambs,[163] and kids.[164,165] The preferred anatomic site in calves is similar to that described for mature cattle. The preferred shooting position for neonatal lambs and kids is to place the muzzle of the NPCB on the midline behind the poll (ie, between the ears) with the chin tucked into the neck **(Figure 18)**.[164,165] In the study by Sutherland et al,[164] a propane-powered NPCB capable of delivering 27.8 J of energy was used as the euthanasia device. In the studies by Grist et al,[163,165] researchers used the Accles and Shelvoke CASH Small Animal Killer (CPK 200) powered by a 1- or 1.25-grain cartridge.

Barbiturate overdose may be used for euthanasia of neonatal calves, lambs, and kids. In noncommercial situations, this method may be preferred over physical methods. Drawbacks include temporary animal distress associated with restraint and needle placement, challenges associated with disposal of remains (residue concerns), and the requirement that only personnel registered with the US DEA may perform the procedure. Assuming these conditions can be met, barbiturate overdose is generally less objectionable to owners and observers than other methods.

### S3.2.5 Dams and Fetuses

Prerequisites for the sensation of pain, distress, or pleasurable experiences are sentience and consciousness. Both are necessary for animals to experience either positive or negative states. Behavioral and EEG evidence indicates that mammalian fetuses are insentient and unconscious throughout the first 75% to 80% of gestation.[23] As neuronal pathways between the cerebral cortex and thalamus become better established, the fetus develops the capacity for sentience. However, while maintained within the protected environment of the animal's uterus it remains in an unconscious state due to the presence of 8 or more neuroinhibitors that act on the cerebral cortex of the fetus to maintain it in the sleep-like state of unconsciousness. At birth, the combined effects of reduced neuroinhibition and onset of neuroactivation contribute to gradual arousal of the mammalian newborn into a state of consciousness that occurs within minutes to several hours after birth.[23]

These observations indicate that the fetus does not suffer as if drowning in amniotic fluid when the

dam is euthanized; nor is it likely to experience pain associated with other types of invasive procedures in utero. These studies also support the rationale for international guidelines on the handling of fetuses suggesting that fetuses should not be removed from the uterus before the EEG is most likely to be isoelectric. For example, when animals are euthanized by physical methods that include exsanguination, delaying removal of the fetus from the uterus for a minimum of 5 minutes after hemorrhaging has ceased generally assures a substantial amount of anoxia-induced damage to the cerebral cortex that will normally prevent progression toward a return to sensibility.[166] If there is any doubt as to the fetus's level of consciousness, it should be euthanized immediately by captive bolt and adjunctive methods as appropriate.

It also addresses the welfare concerns of those who fear that the collection of tissues (in particular, fetal calf blood by intracardiac puncture) from live fetuses in the immediate postslaughter period creates undue suffering. Although the heart may continue to beat (which is necessary for the successful collection of fetal blood), in the absence of breathing there is little likelihood of return to a state of consciousness.[166] Breathing can be prevented by clamping off the fetus's trachea or by delaying its removal from the uterus for 15 to 20 minutes after death of the dam.[166] These are by no means insignificant concerns as there is high demand for fetal tissues to support laboratory research. A 2002 report[167] suggests that world demand for fetal calf serum was 500,000 L/y and growing, a need that would require the harvest of at least 1,000,000 fetuses/y.

The information derived from these observations also has application for fetal rescue situations that may involve euthanasia of late-term pregnant dams by physical methods. The reason one might attempt this is to avoid remains-disposal complications from drug residues as would occur if the fetus were to be delivered by caesarian section using standard surgical methods. Although respiration is interrupted, the heart continues to beat in animals rendered unconscious using physical methods. Therefore, it may be possible to rescue a fetus from an unconscious dam by caesarian section if the procedure can be performed before the fetus suffers irreversible effects of anoxia. Once the fetus is successfully delivered, euthanasia of the dam may be confirmed via any of the previously described adjunctive methods. It is important to understand that there are significant risks to fetal welfare if rescue is attempted. Welfare complications associated with fetal rescue attempts would include impaired brain function caused by anoxia occurring during the rescue attempt, compromised respiratory function and body heat production resulting from fetal immaturity, and greater risk of infection as a consequence of failure of passive transfer of immunity.[23,168,169] When the value of the fetus justifies the effort to secure a successful live delivery, the preferred approach to assure fetal health and welfare is by caesarian section using standard surgical procedures.

*Barbiturates and barbituric acid derivatives*—Pentobarbital readily crosses the placenta resulting in fetal depression in pregnant animals. However, death of the dam normally precedes the death of the fetus. In 1 study[170] cardiac arrest in lambs was delayed for as long as 25 minutes beyond the death of the dam. Similar observations in mice demonstrated that death of the fetuses could only be achieved by the use of doses well in excess of those normally required for euthanasia.[171] Based on these observations, one could offer a similar recommendation to that provided previously for death by exsanguination whereby fetuses should be retained within the uterus for at least 15 to 20 minutes after maternal death has occurred to prevent the delivery of viable fetuses.

## S3.3 SWINE

Methods of euthanasia commonly applied to swine include $CO_2$, gunshot, captive bolt, overdose of an anesthetic administered by a veterinarian, electrocution, and blunt force trauma (suckling piglets only). Gas mixtures of $N_2O$, Ar, $N_2$, and $CO_2$ may also be used but are not currently commonly applied in the field. Selection of the most appropriate method for each situation is dependent upon size and weight of the animal, availability of equipment and facilities, operator skill and experience with the procedure, aesthetic concerns, human safety, and options for disposal of remains. Certain physical methods of euthanasia may require adjunctive methods such as exsanguination or pithing to ensure death. A brief description of each method and appropriate candidates for it are described. Detailed information on inhaled, noninhaled, and physical methods of euthanasia may be found in the respective sections of this document.

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

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

Use of physical methods of euthanasia requires direct contact with the animal, and therefore restraint is necessary. Use of a snare is the most common form of restraint for this class of swine. Studies[172–179] demonstrate varying degrees of stress associated with restraint by snaring techniques. To minimize stress associated with snaring, personnel conducting euthanasia of swine are advised to make advance preparations (eg, prepare the site, load the gun or captive bolt) so that the time during which the animal must be restrained is minimized.

S3.3.1.1 Acceptable methods

S3.3.1.1.1 Noninhaled agents

*Barbiturates and barbituric acid derivatives*—Mature sows, boars, and grower-finisher pigs may be euthanized by IV administration of euthanasia solutions containing barbiturates.[180] A dosage of 1 mL/5

kg (0.45 mL/2.3 lb) up to 30 kg (66 lb), then 1 mL/10 kg (0.45 mL/4.5 lb) thereafter, has been recommended.[181] This method may not cause death if not administered IV. Other anesthetics (gas and/or injectable) may be used to induce unconsciousness, followed by an adjunctive method such as exsanguination. These are not commonly used in field conditions, but may be applicable in some settings. Because these drugs are controlled substances they must be administered by personnel who are registered with the US DEA. Strict record keeping is required of all who use and store these drugs.

Some may find euthanasia by the IV administration of an anesthetic less aesthetically displeasing than gunshot, captive bolt, or electrocution. Options for disposal of animals euthanized with barbiturates are complicated by concerns for residues that create risks for scavengers and other domesticated animals that may consume portions of the animal's remains, and renderers may not accept animal remains contaminated with barbiturate residues.

S3.3.1.2 Acceptable with conditions methods

S3.3.1.2.1 Inhaled agents

*Carbon dioxide, nitrogen, nitrous oxide, and argon*—Studied gas mixtures include $N_2$ with $CO_2$; Ar, alone and with $CO_2$; and CO. Inhaled agents are most commonly used as a method of euthanasia in packing plant venues, and are considered to be acceptable with conditions. However, they are typically not practical in farm situations, and have greater application for pigs weighing 70 lb (31.8 kg) or less, rather than grower-finisher pigs or sows and boars. Gas combinations (eg, Ar and $CO_2$, $N_2O$ and $CO_2$) have been shown to be effective alternatives to $CO_2$ alone. When the concentration of $CO_2$ is high, adequate duration of exposure ensures unconsciousness is followed by death. These methods are described in greater detail for the euthanasia of nursery pigs and in the section on Inhaled Agents.

S3.3.1.2.2 Physical methods

*Gunshot*—Gunshot is commonly used for euthanasia of growing and adult swine. When properly conducted using the appropriate firearm, euthanasia by gunshot produces immediate loss of consciousness and rapid death. There are 3 possible sites for conducting euthanasia in swine: frontal, temporal and from behind the ear toward the opposite eye **(Figure 19AB)**. The frontal site is in the center of the forehead slightly above a line drawn between the eyes. The projectile should be directed toward the spinal canal. The temporal site is slightly anterior and below the ear. Specific sites may vary slightly according to breed.[124,182,183]

Because of the thickness of the pig's skull, muzzle energies of 400 N-m (300 feet-lb) or more are required for euthanasia of adult sows, boars, and growing-finishing pigs. When the alternate site behind the ear is chosen, a .22 caliber firearm loaded with a solid-point bullet may be used. Wadcutters and fragmenting bullets should not be used for euthanasia of swine. Potential for ricochet is reduced when euthanasia by gunshot can be conducted outdoors where bullets that pass through the animal may be captured in an earthen surface. Shotguns may be used at short range and offer the advantage of less potential for bullet ricochet. Twelve-, 16-, or 20-gauge shotguns are recommended for mature pigs. The muzzle should never be held flush to the skull. When performed correctly, the pig drops to the floor immediately, exhibiting varying amounts of tonic and clonic muscle movements. Confirmation that the animal has been rendered insensible includes observing that rhythmic breathing has stopped and that righting reflex is absent, vocalization is absent, and no palpebral reflexes or responses to noxious stimuli are present. All pigs should be observed for evidence of these responses until death has been confirmed.

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

*PCB*—Use of well-maintained PCB guns with ammunition appropriately selected for the size of the animal is acceptable with conditions as a method of euthanasia for growing and adult swine.[184,185] Proper application of the PCB requires restraint of the animal because the device must be held firmly against the forehead over the site described for gunshot (Figure 19A). When performed correctly, the pig drops to the floor immediately, exhibiting varying amounts of tonic and clonic muscle movements. Confirmation that the animal has been rendered insensible includes observation of the following: rhythmic breathing stops, no righting reflex is observed, vocalization is absent, and no palpebral reflexes or responses to noxious stimuli are present. All pigs should be observed for evidence of these responses until death has been confirmed.

Death following use of the PCB is commonly achieved, but is not assured depending upon bolt length and depth of the frontal sinus in mature sows and boars. Therefore, secondary steps to ensure death (eg, a second application of the PCB, exsanguination, pithing) should be applied as necessary. Breed differences result in variable skull shapes making determination of the best anatomic site for conducting euthanasia in mature sows and boars difficult.[182]

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

insufficient charge reduces effectiveness. Personnel must be trained in the proper use of PCBs to ensure effective euthanasia.

*Electrocution*—Electrocution as a sole method of euthanasia can achieve death via 2-step or single-step processes.[186–195] Electrical current must pass through the brain to achieve loss of consciousness, but then must cross the heart to cause fibrillation and cardiac arrest. As a 2-step process, electrode placements are head-head, followed by head to flank, for the appropriate time. For a single-step process for euthanasia, head to opposite flank is an example of appropriate placement.

Head-only electrocution induces a grand mal seizure and immediate unconsciousness, but death does not occur unless followed by head-to-heart electrocution or the application of an adjunctive method to ensure death such as exsanguination.[187,196] The secondary step, whether head-to-heart electrocution or another method, must be performed within 15 seconds of onset of unconsciousness; otherwise, the animal may regain consciousness. Head-only electrocution is performed by placement of the electrodes in 1 of 3 positions: between the eyes and base of the ears on either side of the head; below the base of the ears on either side of the head; or diagonally, below 1 ear to above the opposite eye. Placement of electrodes for head-to-heart electrocution is on the head in front of the brain (some use the base of the ear) with a secondary electrode attached to the body behind the heart on the opposite side. This assures diagonal movement of current through the animal's body. With specific electrode placement, current of 110 V at a minimum frequency of 60 Hz applied for a minimum of 3 seconds is sufficient for euthanasia of pigs up to 125 kg.[197] Systems used for electrocution must be capable of meeting minimum current requirements to ensure insensibility in the head-only method, and insensibility and cardiac fibrillation in the head-heart method.

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

S3.3.1.3 Adjunctive methods

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

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

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

## S3.3.2 Nursery Pigs (70 lb or Lighter)

Nursery pigs may be euthanized by use of inhaled gases (eg CO, $CO_2$, $N_2O$, Ar, $N_2$), gunshot, PCB, NPCB, electrocution, or anesthetic overdose. Descriptions of the use of $CO_2$ and NPCB for euthanasia of young pigs follow. For details on other methods please see the preceding information in this section or the Physical Methods section of the Guidelines.

S3.3.2.1 Acceptable methods

S3.3.2.1.1 Noninhaled agents

*Barbiturates and barbituric acid derivatives*—Nursery pigs may be euthanized by IV administration of euthanasia solutions containing barbiturates. Because these drugs are controlled substances they must be administered by personnel who are registered with the US DEA. Strict record keeping is required of all who use and store these drugs.

Many find euthanasia by the IV administration of an anesthetic less aesthetically displeasing than administration of $CO_2$, captive bolt, or electrocution. Options for disposal of animals euthanized with barbiturates are complicated by concerns for residues that create risks for scavengers and other domesticated animals that may consume portions of the animal's remains.

S3.3.2.2 Acceptable with conditions methods

S3.3.2.2.1 Inhaled agents

*Carbon dioxide*—Carbon dioxide alone or in combination with $N_2O$ or Ar has been used successfully for euthanasia.[198–202,c] Properly applied, inhalation of $CO_2$ is an effective method of euthanasia. On the other hand, if air exchange rates are not carefully controlled and monitored, animals may suffer substantial stress from suffocation prior to loss of consciousness and death (see Inhaled Agents section of Guidelines).

Conducting this procedure on small pigs requires a container large enough for the size and number of pigs to be euthanized. Pigs may be exposed to $CO_2$ by gradually displacing ambient gases (introducing $CO_2$ into the container) or by introducing the animals into a prefilled environment. In the gradual-fill approach, pigs are placed in an enclosed container and $CO_2$ flow is initiated at a rate and for a time to reach a level sufficient to achieve euthanasia. In the prefill approach, a concentrated environment of $CO_2$ is created, pigs are placed in that environment, and $CO_2$ flow is resumed to maintain effective euthanasia concentrations. In both methods, exposure of pigs with normal respiration to a constant supply of 80% to 90% $CO_2$ for a minimum of 5 minutes is necessary for effective euthanasia.[203–212]

Carbon dioxide offers advantages for euthanasia, including that it is relatively inexpensive, nonflammable and nonexplosive, and clean (no blood loss). Drawbacks to the use of $CO_2$ are that it requires spe-

cial equipment and training for efficient and safe application. Systems must be able to achieve a level of anesthesia while not causing hypothermia. An appropriate pressure-reducing regulator, with or without a flow meter, capable of generating the recommended displacement rates for the size container being utilized is necessary. As with other methods, death must be verified for each animal following administration of $CO_2$.[182]

For young pigs, movement during the induction phase has caused some to question the degree of stress that may be induced with this method. Some interpret these movements as indications of aversion. There is evidence that such reactions may be normal for pigs in an unconscious state.[204,209,210] Small or incapacitated piglets have low tidal volumes and will not achieve death as rapidly as larger, more viable pigs. Carbon dioxide euthanasia in chamber settings has not been extensively studied for larger pigs. Meyer and Morrow[213] recommend that chamber volume be exchanged at least 2.5 times to accommodate the wash-in–wash-out principle regardless of the size of swine to be euthanized. Monitoring of equipment and gas must be routine and consistent to ensure there is always sufficient gas to accomplish the objective of euthanasia. Carbon dioxide containers should never be placed in an unventilated area due to risks associated with an overdose of gaseous $CO_2$ for humans.

S3.3.2.2.2 Physical methods

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

Use of a proper functioning NPCB with appropriate charges offers the advantage of delivering a uniform concussive force to the skull (controlled blunt force trauma). This reduces the potential for ineffective stunning and euthanasia that may occur more often with the use of manually applied blunt force trauma. However, this method requires immediate application of an adjunctive method to ensure euthanasia.[214]

*PCB*—Use of well-maintained PCB guns with ammunition appropriately selected for the size of the animal is acceptable with conditions as a method of euthanasia for growing and adult swine.[184,185] Proper application of the PCB requires restraint of the animal because the device must be held firmly against the forehead over the site described for gunshot (Figure 12). When performed correctly, the pig drops to the floor immediately, exhibiting varying amounts of tonic and clonic muscle movements. Confirmation that the animal has been rendered insensible includes observing that rhythmic breathing stops, righting reflex is absent, vocalization is absent, and no palpebral reflexes or responses to noxious stimuli are present. All pigs should be observed for evidence of these responses until death has been confirmed. For more information, see the section on grow-finish swine earlier in the Guidelines.

*Electrocution*—Electrocution is acceptable with conditions for swine over 3 days of age.[214] Details are provided earlier in this section and in the Physical Methods section of the Guidelines.

## S3.3.3 Suckling Pigs

Options for the euthanasia of suckling pigs include $CO_2$; mixtures of $CO_2$ with Ar, $N_2$, or $N_2O$; CO; inhaled anesthetics; purpose-built NPCB; electrocution (for pigs over 3 days of age]); anesthetic overdose; and blunt force trauma. Described in detail are the application of NPCB, manually applied blunt force trauma, and $CO_2$. See previous sections of the Guidelines for more detailed information on the application of other euthanasia techniques.

S3.3.3.1 Acceptable methods

S3.3.3.1.1 Injectable agents

*Barbiturates and barbituric acid derivatives*—Suckling pigs may be euthanized by IV administration of euthanasia solutions containing barbiturates. Because these drugs are controlled substances they must be administered by personnel who are registered with the US DEA. Strict record keeping is required of all who use and store these drugs.

Many find euthanasia by the IV administration of an anesthetic less aesthetically displeasing than administration of $CO_2$, captive bolt, manually applied blunt force trauma, or electrocution. Disposal of animals euthanized with barbiturates is complicated by concerns about residues that create risks for scavengers and other domesticated animals that may consume portions of the animal's remains.

S3.3.3.2 Acceptable with conditions methods

S3.3.3.2.1 Inhaled agents

*Carbon dioxide*—Carbon dioxide may be effective as a method of euthanasia for small groups of neonatal piglets[214]; however, the parameters of the technique need to be optimized and published to ensure consistency and repeatability.

S3.3.3.2.2 Physical methods

*NPCB*—The NPCB can be an effective method of euthanasia for young piglets.[215,216] Loss of consciousness and death are caused by a severe nonpenetrating concussive force applied to the forehead of the piglet. The utility of the NPCB is focused on the unique condition in suckling and young pigs where the frontal bones are not fully developed, leaving the brain susceptible to blunt, high-velocity impact.

When used in appropriately sized and aged pigs a secondary step to ensure death is unnecessary.[217,218] The NPCB can be powered pneumatically or through the use of appropriate ammunition. Some brands of captive bolt guns have been made versatile by providing different heads (varying length of bolt and penetrating or nonpenetrating end), and ammunition for various-size pigs, which allows the same gun to be used in different situations. Current research indi-

cates that euthanasia using an NPCB is effective and repeatable with sufficient power, ammunition, and consistency in the gun design.[215,216]

*Manually applied blunt force trauma*—Manually applied blunt force trauma, when performed correctly, meets the definition of euthanasia, namely causing minimal distress with rapid loss of consciousness leading to death. As with the NPCB, the utility of manually applied blunt force trauma is focused on the unique condition in suckling and young pigs where the frontal bones are not fully developed, leaving the brain susceptible to blunt, high-velocity impact. This method may be less aesthetically acceptable than other alternatives, but when performed with proper training and proper application of technique, death is rapid. Uncertainty of success often causes repeated application or selection of an alternative euthanasia method.[216] The AVMA encourages those using manually applied blunt force trauma to the head as a euthanasia method to actively search for alternatives to ensure that criteria for euthanasia can be consistently met.

## S3.4 POULTRY

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

### S3.4.1 Acceptable Methods

S3.4.1.1 Noninhaled agents

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

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

### S3.4.2 Acceptable With Conditions Methods

S3.4.2.1 Inhaled gases

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

*Carbon dioxide*—The most common gas used for euthanasia of poultry is $CO_2$, and its application has been extensively studied for chickens, turkeys, and ducks with information available about behavioral responses, times to collapse, unconsciousness, death, loss of somatosensory evoked potentials, loss of visually evoked responses, and changes in EEG and ECG (see Inhaled Agents section of the Guidelines). There is no flow rate requirement at this time for the use of carbon dioxide in poultry. Carbon dioxide has successfully been applied for euthanasia of nonhatched eggs (pips), newly hatched poultry in hatcheries, and adult birds (including routine euthanasia of large commercial laying hen flocks[219–221] and on farms keeping birds for research or elite genetics). Because neonatal birds may be more accustomed to high concentrations of $CO_2$ (incubation environments typically include more $CO_2$), concentrations necessary to achieve rapid euthanasia of pipped eggs or newly hatched chicks may be substantially greater (as high as 80% to 90%) than for adults of the same species. One study[222] found that $CO_2$ concentrations needed to be held at 75% or higher for 5 minutes to ensure euthanasia of day-of-hatch chicks.

Carbon dioxide may invoke involuntary (unconscious) motor activity in birds, such as flapping of the wings or other terminal movements, which can damage tissues and be disconcerting for observers.[223,224] Slower induction of euthanasia in hypercapnic atmospheres reduces the severity of convulsions after loss of consciousness.[225,226] Death normally occurs within minutes, depending on the species and the concentration of $CO_2$ present in the closed chamber.

*Carbon monoxide*—Carbon monoxide may also be used for euthanasia of poultry. More convulsions may be observed in the presence of CO than normally occur when $CO_2$ is used for euthanasia.[227] The CO flow rate should be sufficient to rapidly achieve a uniform concentration of at least 6% after birds are placed in the chamber (see Inhaled Agents section). Only pure, commercially available CO should be

used. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and or displacement rate. Appropriate precautions must be taken to ensure human safety because CO has a cumulative effect in binding hemoglobin. Due to danger to human safety, carbon monoxide is not recommended for use in most commercial operations.

*Nitrogen or argon*—Nitrogen or Ar, used alone or mixed with approximately 30% $CO_2$, is acceptable with conditions for euthanasia of poultry provided the residual atmospheric $O_2$ level can be reduced to and held at sufficiently low levels (eg, 2% to 3%).[228–230] These agents tend to cause more convulsions (eg, wing flapping) than $CO_2$ in air (see Inhaled Agents section of the Guidelines).[225,231] It has also been noted that convulsions may start when consciousness, at least to some degree, may still be a possibility.[232,233]

*Reduction of atmospheric pressure*—Reduction of atmospheric pressure by drawing a vacuum in a chamber has been demonstrated to euthanize chicks and mature poultry in a manner comparable to that associated with anoxia-based methods involving inert gases such as $N_2$ or Ar.[222,234] The intensity of convulsions after loss of posture (ie, after loss of consciousness) is similar to that seen with anoxia-based methods. This method is commonly known as LAPS (low-atmospheric-pressure stunning).

S3.4.2.2 Physical methods

The following methods are acceptable with conditions for euthanasia of poultry. Euthanasia methods should be chosen based on the welfare of the bird, human safety, skill and training of personnel, availability of equipment, and the ability to adequately restrain the bird.

*Cervical dislocation*—When performed on conscious poultry, cervical dislocation must result in luxation of the cervical vertebrae without primary crushing of the vertebrae and spinal cord. Manual or mechanical cervical dislocation may be used for poultry of an appropriate size and species when performed by competent personnel who correctly apply the technique. In some classes of poultry there is evidence that cervical dislocation may not cause immediate unconsciousness.[235–238] Cervical dislocation techniques that cause luxation of the vertebrae at or close to the skull may improve kill success and achieve more rapid reduction of reflexes.[239] When performed manually, the shanks of the bird should be grasped (or wings if grasped at the base) and the neck stretched by pulling on the head while applying a ventrodorsal rotational force to the skull until an obvious, typically sudden, reduction of resistance indicates separation of the cervical vertebrae. Separation of the vertebrae should be verified by palpation. Crushing of the cervical vertebrae and spinal cord is not acceptable unless the bird is first rendered unconscious.

*Decapitation*—Decapitation is acceptable with conditions for the euthanasia of poultry when performed by competent personnel. Decapitation should be executed with a sharp instrument, ensuring rapid and unobstructed severing of the head from the neck.

*Manually applied blunt force trauma*—Euthanasia by manually applied blunt force trauma to the head is acceptable with conditions for poultry, particularly birds such as turkeys or broiler breeder chickens that are too large for cervical dislocation.[240,241] Manually applied blunt force trauma must be correctly applied by competent personnel. Blunt force trauma consisting of a single sufficiently strong hit placed in the frontoparietal region of the head resulted in loss of auditory evoked potentials in broilers, broiler breeders, and turkeys of up to 16 kg (35.2 lb).[241] Fatigue can lead to inconsistency in application, creating concern that the technique may be difficult to apply humanely to large numbers of birds. For this reason, the AVMA encourages those using manually applied blunt force trauma to the head as a euthanasia method to search for alternatives.

*Electrocution*—Electrocution is acceptable with conditions for euthanasia of individual birds. Birds subjected to electrocution should be observed to ensure death or an adjunctive method, such as exsanguination or cervical dislocation, should be performed immediately afterwards to ensure death. A small percentage of birds do not develop ventricular fibrillation even when exposed to high amperage current.

*Gunshot*—Gunshot is acceptable with conditions for free-ranging poultry and ratites when capture or restraint would potentially be highly stressful for the animal or dangerous for humans. Gunshot is not recommended for captive poultry where restraint is feasible.

*PCB and NPCB*—Captive bolts (penetrating or nonpenetrating) are acceptable with conditions for euthanasia of poultry (eg, turkeys, broiler breeders, ratites, waterfowl) when performed by competent personnel **(Figures 20–22)**. The captive bolt device must be appropriately designed and configured for the species and bird size, provide sufficient impact energy, and be properly applied. The bird should be appropriately restrained to avoid injury to personnel.[135,239,241–243] Birds should be observed following captive bolt administration to ensure that death occurs. Any bird showing signs of recovery must receive a second shot or be killed by some other means that is acceptable for a conscious bird.

### S3.4.3 Adjunctive Methods

*Potassium chloride or magnesium sulfate*—Although IV or intracardiac administration of potassium chloride or magnesium sulfate to a conscious bird as a sole method of euthanasia is unacceptable, it is acceptable to administer these agents to a bird that is fully anesthetized or otherwise unconscious as a means to ensure death.

*Exsanguination*—Although exsanguination of a conscious bird is an unacceptable method of euthana-

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 $CO_2$ 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 Pathologists[245] 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 $CO_2$ 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 $\alpha_2$-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 $\alpha_2$-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 intrathecally.[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 $O_2$ 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-

tact (eg, birds in zoos, wild birds). Multiple personnel may be required to safely handle larger species, such as ratites, and at least 1 additional person should be available to assist in case of an emergency. Chemical restraint may be useful in some situations, particularly for dangerous birds where human safety may be compromised by efforts at manual restraint. Drugs used for chemical restraint that are administered at high doses may serve as the first step of a 2-step euthanasia process.

## S5.2 METHODS

Individual birds in a clinical or research setting can best be rendered unconscious by use of an inhaled agent (eg, isoflurane, sevoflurane, or halothane), prior to IV administration of an acceptable injectable euthanasia agent (eg, sodium pentobarbital). The following methods are considered to be acceptable or acceptable with conditions for avian species. For more detailed, non–species-specific information on various agents and methods, please refer to the Inhaled Agents, Noninhaled Pharmaceutical Agents, and Physical Methods sections of the Guidelines.

### S5.2.1 Acceptable Methods

S5.2.1.1 Noninhaled agents

Intravenous injection of an injectable euthanasia agent is the quickest and most reliable means of euthanizing birds when it can be performed without causing fear or distress. Wild, fearful, or excited birds may require a sedative or anesthesia before IV injection can be performed. When IV injection is impossible, injectable euthanasia agents can be administered via intracoelomic, intracardiac, or intraosseous routes only if a bird is unconscious or anesthetized. If the intracoelomic route is used for birds, injection into the air sacs must be avoided, because of the potential for respiratory compromise, irritation of the respiratory system, and delayed absorption via the air sacs. Euthanasia agents should also not be administered via the intraosseous route into the humerus or femur because of the potential for drowning or irritation to the respiratory system. Regardless of the route of administration, injectable agents can precipitate in tissues and can induce artifacts at necropsy and on histopathologic examination.[224,254,263] Barbiturates and barbituric acid derivatives can be administered IV for euthanasia of anesthetized or properly restrained unanesthetized birds. Barbiturates commonly used for injection are available as sodium salts that are alkaline and may be irritating and painful when injected directly into tissues, rather than IV. Therefore, when IV injection is impossible, injectable euthanasia agents can be administered via intracoelomic, intracardiac, or intraosseous routes only if a bird is unconscious, or anesthetized. Concepts regarding barbiturate use in mammals generally also apply to birds and more information is available in the Noninhaled Agents section of the Guidelines.

### S5.2.2 Acceptable With Conditions Methods

S5.2.2.1 Inhaled agents

*Inhaled anesthetics*—Inhaled anesthetics may be used at high concentrations as a sole method of euthanasia or may be used to render birds unconscious prior to application of other methods of euthanasia.[244,252] Exposure to high concentrations of inhaled anesthetics (eg, halothane, isoflurane, sevoflurane, with or without $N_2O$) is acceptable with conditions for euthanasia for birds. Birds exposed to high concentrations of inhaled anesthetic gases lose consciousness rapidly. Euthanasia via inhaled gases may be more practical than use of an injectable agent if large numbers of birds, such as in flock or aviary situations, must be euthanized. Euthanasia by exposure to gas anesthetics also induces minimal tissue damage and results in the least amount of tissue artifact for necropsy.[224,254]

*Carbon dioxide*—High (> 40%) concentrations of $CO_2$ induce anesthesia initially followed by loss of consciousness. Euthanasia via exposure to $CO_2$ has been described for individual birds and small groups,[252] and its application to euthanasia of chickens, turkeys, and ducks has been studied extensively, resulting in information regarding times to collapse, unconsciousness, and death; loss of somatosensory evoked potentials; loss of visually evoked responses; and changes in EEG and ECG.[257–259,264] Application rate of $CO_2$ needs to be balanced with situational needs as rapid increases in $CO_2$ concentration decrease the amount of time to loss of posture and consciousness, while slower increases in concentration may cause less aversion or reaction, but increase time of exposure. Field applications of $CO_2$ for broilers have resulted in stress levels similar to that invoked via routine handling[265] or stress and distress similar to the handling or restraint required for other methods of euthanasia.[259] In a recent study, most turkeys would voluntarily enter a feeding chamber filled with Ar (90%), or a mixture of Ar (60%) and $CO_2$ (30%), compared with only 50% of turkeys that would voluntarily enter the chamber when filled with a high concentration of $CO_2$ (72%) alone, suggesting an aversion to 72% $CO_2$.[266] More research is needed to better understand this comparative aversion in turkeys (eg, whether it is dose or species dependent and availability of agent).

Concepts regarding the use of $CO_2$ in mammals as described in the Inhaled Agents section of the Guidelines generally also apply to birds. Exposure to $CO_2$ may cause involuntary (unconscious) motor activity, such as flapping of the wings, which can damage tissues and be disconcerting to, and potentially dangerous for, observers.[223,224]

There are some special considerations for the use of $CO_2$ for euthanasia of birds. Neonatal birds may be more acclimated to high $CO_2$ concentrations, because the unhatched bird's environment typically has a high $CO_2$ concentration (as high as 14% in the embryonic

chicken). Consequently, $CO_2$ concentrations required to achieve euthanasia of newly hatched chicks may be much higher (as much as 80% to 90%) than those for adults of the same species.[244,259] Diving birds also have physiologic adaptations to hypercapnia and may require higher $CO_2$ concentrations for euthanasia.

*Carbon monoxide*—Concepts regarding the use of CO for euthanasia of mammals also apply to birds. See the Inhaled Agents section of the Guidelines for details.

*Nitrogen and argon*—Inert gases such as $N_2$ and Ar, and gas mixtures involving these gases (including mixtures with $CO_2$), have been used for euthanasia of poultry,[267] but are not recommended for euthanasia of companion birds.

Behavioral responses of broiler chickens were examined during short (10 seconds) exposures to 100% Ar, 100% $N_2$, or mixtures (80% Ar:20% $N_2$ and 80% $N_2$:20% Ar). Normal feeding and no aversive behaviors were observed.[268] Birds appear to not have intrapulmonary chemoreceptors for $N_2$ and Ar, and this may account for a lack of aversion during their initial exposure to and hypoxia from these gases.[267] As a euthanasia agent, Ar gas mixed with < 2% $O_2$ was shown to induce rapid loss of posture (average, 11 seconds), convulsions (average, 22 seconds), unconsciousness, and death (isoelectric EEG in 1 minute).[269] Convulsions can occur during euthanasia with these inert gases, but because these signs occurred after collapse and loss of consciousness, these gases are considered to be humane for the birds involved.[267]

S5.2.2.2 Physical methods

Physical methods of euthanasia may be necessary in some field situations if other methods of euthanasia are impractical or impossible to implement. That said, there is little scientific information available regarding the effect of various physical methods on electrical activity in the brain of birds, which makes evaluation of the humaneness of these procedures difficult.

*Cervical dislocation*—Cervical dislocation has generally been used for small birds (< 200 g) when no other method is available, but the procedure has been performed on birds as large as 2.3 kg (5.1 lb). It should only be performed by well-trained personnel who are regularly monitored to ensure proficiency. Skilled individuals have been able to humanely perform cervical dislocation in poultry. There is limited research specific to birds concerning electrical activity in the brain following cervical dislocation. Cervical dislocation of chickens (average weight of 2.3 kg) did not result in loss of visually evoked responses in 90% of cases when compared with use of a percussion bolt pistol, suggesting that fewer than 10% of cervical dislocations resulted in concussion.[238] In 3-week-old turkeys (average weight of 1.6 kg [3.5 lb]) time to insensibility (based on nictitating membrane movement) was longer, but time to death (based on cessation of movement) was shorter after cervical dislocation compared with use of an NPCB and blunt force trauma.[235] Whether pain is perceived is not known. Consciousness and perception of pain are not necessarily concurrent.

*Decapitation*—Based on information currently available, decapitation is considered to be acceptable with conditions for euthanasia of small (< 200 g) birds. The AAZV Guidelines for Euthanasia of Nondomestic Animals[244] also lists decapitation as acceptable with conditions, and suggests the method may be preferred over cervical dislocation under certain field conditions due to clear evidence of a successful procedure. One study[270] indicated that several methods of partial, mechanical decapitation of chickens (weighing 2.1 to 3.5 kg [4.6 to 7.7 lb]) did not result in the loss of visually evoked responses in 90% of cases when compared with use of a percussion bolt pistol and concluded that fewer than 10% of cervical dislocations resulted in concussion. In another study decapitation applied to anesthetized chickens resulted in visually evoked responses up to 30 seconds following decapitation, but because the responses were obtained from anesthetized chickens it is not possible to conclude any association with cognitive processes.[95,270,271] As indicated previously (see discussion of Consciousness and Unconsciousness in the Guidelines), at some level between behavioral unresponsiveness and the induction of a flat EEG, consciousness must vanish; however, EEG data cannot provide definitive answers as to onset of unconsciousness.

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

### S5.2.3 Adjunctive Methods

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

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

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

### S5.2.4 Unacceptable Methods

Thoracic compression (also known as cardiopulmonary or cardiac compression) is a method that has been used by biologists to terminate the lives of wild small mammals and birds, mainly under field conditions.[272] Although this method has been used exten-

sively in the field, data supporting its use, such as degree of distress induced and time to unconsciousness or death, are limited.[273] Given current knowledge of the physiology of small mammals and birds, it cannot be assumed that thoracic compression does not result in pain or distress before animals become unconscious. Consequently, thoracic compression is an unacceptable method of euthanizing animals that are not deeply anesthetized or insentient due to other reasons, but is appropriate as a secondary method for animals that are insentient.

### S5.3 EGGS, EMBRYOS, AND NEONATES

Bird embryos that have attained > 80% incubation demonstrate EEG activity that is sustained, with increases in amplitude suggesting the potential for pain perception in conscious embryos; therefore they should be euthanized by similar methods used in avian neonates such as anesthetic overdose, decapitation, or prolonged (> 20 minutes) exposure to $CO_2$. Eggs at < 80% incubation may be destroyed by prolonged exposure (> 20 minutes) to $CO_2$,[274] cooling (< 4°C for 4 hours), or freezing. Because research is still evolving and there are species-specific differences in development, euthanasia of embryos should be performed based on the best available data and with attention to assuring, as best as possible, that conscious suffering does not occur.

## S6 Fish and Aquatic Invertebrates

### S6.1 GENERAL CONSIDERATIONS

Fish and aquatic invertebrates play important roles as food, pets, research subjects, display animals, sources of recreation, and key components of healthy ecosystems. In each of these situations it may be necessary to cause the death of some animals. Considerable evidence is accumulating that suggests it is appropriate to consider the possibility of pain perception in these species.[275–287] The aim is to accomplish death for these animals rapidly with the minimum amount of pain and distress practicable. Because the environment associated with fish and aquatic invertebrates in each of their roles is different, and because knowledge about the evolutionary and societal status of poikilothermic animals (lower vertebrates and invertebrates) is limited, identifying and applying appropriate criteria for euthanasia can be difficult.

#### S6.1.1 Terms Applicable to Ending Life

Specific to fish, the 3 main terms used to describe the ending of life are euthanasia, slaughter, and humane killing. There is often confusion regarding how these terms and their associated methods differ. The methods described in the Guidelines serve as guidance for veterinarians and others who may need to perform euthanasia. These Guidelines are not intended to address slaughter, depopulation, or other killing methods. The AVMA Guidelines for the Humane Slaughter of Animals[156] and the AVMA Guidelines for the Depopulation of Animals[288] should be referenced in cases of slaughter or depopulation. The term *harvest* specifically refers to the act or process of gathering a crop, as in aquaculture and commercial fishing; however, *harvest* may also be used to describe fish removed from a water body by anglers. Whether harvested fish are slaughtered or humanely killed depends on the context of the activity.[289–292] Addressing euthanasia of invertebrates in some settings is not meant to discount the necessity for and suitability of slaughter and pest control techniques that do not meet the definition of euthanasia.

#### S6.1.2 Human and Animal Considerations

Because of the diversity of physiologic and anatomic characteristics seen among species of fish and aquatic invertebrates, optimal methods for euthanasia will vary. Euthanasia choices for fish and aquatic invertebrates must account for animal stress responses and human safety concerns associated with handling, as well as differences in metabolism, respiration, and tolerance to cerebral hypoxia. Virtually all methods require that personnel be carefully trained and monitored (although some carry more risks of human ineffectiveness than others), some require DEA registration and record keeping, and chemicals regulated by the EPA can only be legally used according to their label directions. Immersion of the fish or aquatic invertebrate in an appropriate euthanasia solution is often an easier method than using injectable forms of euthanasia. Intracoelomic injections carry an inherent risk of organ damage and response time may vary. Intravenous injections require careful handling of fish, as well as trained and experienced personnel. Intramuscular injections with ketamine, $\alpha_2$-adrenergic receptor agonists, or Telazol[d] can be administered via pole syringe or dart gun to larger fish to facilitate handling and reduce handling stress for fish, but rarely achieve surgical planes of anesthesia in teleosts. In all cases, veterinarians and others with expertise relevant to the species of interest should be consulted; professional judgment and relevant expertise should be taken into account when ultimately determining the best method to use. In addition, it is often more difficult to ascertain when a fish or an aquatic invertebrate is dead as compared with birds and mammals. Some unique aspects of euthanasia for fish have been described.[293,294]

#### S6.1.3 Preparation and Environment

As a general principle the preparations for euthanasia of fish should be very similar to the preparations for anesthesia of fish.[295–297] If possible, withholding food for 12 to 24 hours prior to euthanasia will reduce regurgitation, defecation, and nitrogenous waste production. The environment should be as quiet and nonstimulatory as possible given the circumstances. Light intensity should be reduced if

possible, but with adequate lighting for personnel. This can also be achieved through use of a dark or opaque container and lid, or by use of less intense lighting (eg, red light illumination, as red light does not penetrate water well).

Water quality should be similar to that of the environment from which the fish originated, or optimized for that species and situation, for the duration of euthanasia. If the water is of acceptable quality for fish health, the water in which they have been housed or captured should be used, and supplemental aeration and temperature control may be necessary. Either the immersion euthanasia solution is prepared with water from the fish housing system and the fish are transferred into it or a concentrated form of the anesthetic agent as a solution (containing buffering agent if appropriate) is introduced directly into the container of fish to minimize stressors. If euthanizing a large population of fish, it is important to monitor the anesthetic bath water quality (temperature, dissolved $O_2$, ammonia, and organic loading, in particular). The euthanasia agent may need to be supplemented or replaced periodically because it will be removed when absorbed into the fish's bloodstream though the gills. Euthanasia methods should be tested in 1 animal or a small group of animals prior to use in a large population for an unfamiliar species, to ensure effectiveness.[298] If handling the fish is required, appropriate equipment (nets, gloves) should be used to minimize stressors.

### S6.1.4 Indicators of Death in Fish and Aquatic Invertebrates

Because the thousands of species of fish and aquatic invertebrates vary greatly in anatomic and physiologic characteristics, reliable indicators of death may not be available for some. However, there are some standard approaches that can be useful for many of the more commonly encountered species. Loss of movement, loss of reactivity to any stimulus, and initial flaccidity (prior to rigor mortis) may serve as indicators of death for fish and some aquatic invertebrates. More useful indicators for many fish include respiratory arrest (cessation of rhythmic opercular activity) for a minimum of 30 minutes and loss of eye-roll (vestibulo-ocular reflex, the movement of the eye when the fish is rocked from side to side). The latter is no longer present in fish that have been deeply anesthetized or euthanized.[299] The heart can continue to contract even after brain death or removal from the bodies of fish,[300] so the presence of a heartbeat is not a reliable indicator of life, but sustained absence of heartbeat is a strong indicator of death. For more sessile, less active organisms, or those with specific anatomic or physiologic adaptations that prevent use of these indicators, it may be more difficult to assess loss of consciousness and death, and consultation with species experts is recommended. Secondary methods of euthanasia are recommended, when appropriate, after the fish or aquatic invertebrate is anesthetized, to ensure euthanasia.

### S6.1.5 Disposition of Euthanized Animals

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

### S6.1.6 Fish and Aquatic Invertebrates Intended for Human Consumption

As previously indicated, the term *slaughter* is used primarily to refer to the killing of animals intended for human consumption (eg, agricultural harvest, commercial fisheries) and these Guidelines are not intended to address that activity. However, when euthanasia of animals intended for human consumption is desired, tissue residues from the use of drugs and other chemicals will make many methods unacceptable unless they have been approved by the FDA for this purpose. Use of any unapproved chemicals for euthanasia prohibits entry of the fish into the food chain, either by rendering as fish meal or by distributing as directly consumed product.[290] With that said, currently there are no drugs approved by the FDA for euthanasia of fish or aquatic invertebrates. Carbon dioxide is a drug of low regulatory priority[301] that avoids unacceptable residues, but it is not an FDA-approved method for killing aquatic animals used for food. Physical methods that are acceptable with conditions include manually applied blunt force trauma to the head, decapitation, electrocution, and pithing. For more information regarding methods for slaughter, please refer to the AVMA Guidelines for the Humane Slaughter of Animals.[156]

## S6.2 FINFISH

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

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

retail facilities, research laboratories, and fish kept outdoors and in fisheries. An acceptable method reliably meets the requirements of euthanasia. Methods that are acceptable with conditions reliably meet the requirements of euthanasia when specified conditions are met. An unacceptable method does not meet the requirements of euthanasia. Because fishes' anatomic and physiologic characteristics are quite different from those of mammals and birds, classification of techniques may vary from what has been recommended for other species.

### S6.2.1 Noninhaled Agents

*Immersion (1 step)*—Intentional overdose via immersion in anesthetic solutions is a common method of euthanasia for fish.[298,302–304] Some species exhibit aversive responses to particular anesthetic agents, while other species do not.[305,306] Through preference and approach-avoidance testing, many anesthetic agents currently used for euthanasia have been identified as being aversive to varying degrees. Despite some evidence of distress and aversion, immersive anesthetics continue to be administered to fish because the benefits associated with their use outweigh any distress and aversion they may cause, similar to use of inhaled agents for air-breathing animals. Fish should be left in the anesthetic solution for a minimum of 30 minutes after cessation of opercular movement.[251,298,302] A recent study[307] demonstrated that use of buffered MS 222 in a 1-step immersion technique was inadequate for euthanasia of goldfish (*Carassius auratus*), a hypoxia-tolerant species. Results from this study suggest that a 2-step method may be required for euthanasia of hypoxia-tolerant species,: step 1, immersion to render the fish unconscious; step 2, a secondary adjunctive method to complete euthanasia (such as decapitation, pithing, or freezing). Options for immersion agents include the following:

(1) Benzocaine or benzocaine hydrochloride, buffered. Solutions for immersion should be prepared in concentrations ≥ 250 mg/L and should be buffered.[304]

(2) Carbon dioxide. Immersion in $CO_2$-saturated water causes narcosis and loss of consciousness after several minutes.[251,298] Some species may exhibit hyperactivity prior to loss of consciousness.[302] Purity and concentration of $CO_2$ are important for effectiveness. Only $CO_2$ from a source that allows for careful regulation of concentration, such as from cylinders, is acceptable. Care must be taken when using $CO_2$ to prevent exposure to personnel (ie, euthanasia must be conducted in well-ventilated areas).

(3) Ethanol. Ethanol has been suggested as an acceptable alternative method for fish euthanasia.[308] The depressive effects of ethanol on the CNS are well described,[309] and exposure of zebrafish via immersion has become a model for behavioral and molecular responses to alcohol, at concentrations from 10 to 30 mL of 95% ethanol/L.[310–312] At this dose, alcohol induces anesthesia, and prolonged immersion produces death via respiratory depression causing anoxia. This is not equivalent to immersing fish directly into preservative concentrations of ethanol (70%), which is not acceptable as a euthanasia method.

(4) Eugenol, isoeugenol, and clove oil. Whenever possible, products with standardized, known concentrations of essential oils should be used so that accurate dosing is possible. Concentrations required for anesthesia will vary depending on species and other factors, but may be as low as 17 mg/L for some species. Greater concentrations (10 times the upper range for anesthesia) will be required for euthanasia.[298,313–315] These oils are not very water soluble; injecting the solution through a syringe and fine-gauge needle under the water in the container used for euthanizing is helpful in ensuring dispersal in the water. Fish should be left in the anesthetic solution for a minimum of 10 minutes after cessation of opercular movement. These compounds are equivocal or known carcinogens according to the National Toxicology Program.[316] Some studies in rodents indicate this group of anesthetics may cause paralysis in addition to having anesthetic effects, and analgesic properties are unknown.[70,317–319] The FDA strictly prohibits the use of clove oil and eugenol as anesthetics in fish having the potential to enter the food chain, except under Investigational New Animal Drug exemptions.[320] Isoeugenol is a potential carcinogen[316] so human safety in the application of that agent is of concern.

(5) Isoflurane, sevoflurane. These concentrated liquid anesthetics can be added to water, although they are generally not very water soluble.[302] Injecting the solution through a syringe and fine gauge needle under the water in the container used for euthanizing is helpful in ensuring dispersal in the water. Doses of > 5 to 20 mL/L can be used (10 times the upper range for anesthesia). However, because both anesthetics are highly volatile, human safety is of concern and use in a well-ventilated area is imperative.

(6) Quinaldine sulfate. Solutions for immersion should be prepared in concentrations ≥ 100 mg/L.[321] Quinaldine sulfate will acidify water; therefore, buffering is required to prevent distress from acute drop in pH.

(7) Tricaine methanesulfonate, buffered (MS 222, TMS). An aversive response to MS 222 has been demonstrated for zebrafish and medaka, while carp, fathead minnow, and rainbow trout showed no aversion.[305,306] Despite evidence of distress and aversion, immersive anesthetics continue to be administered to fish because the benefits associated with their use outweigh any distress and aversion they may cause. Solutions must be buff-

ered, and concentrations required for euthanasia may vary depending upon the species, life stage, and water chemistry parameters. A concentration of 250 to 500 mg/L, or 5 to 10 times the anesthetic dosage, is effective for most species.[298,304] MS 222 at a dose of 400 mg/L has been shown to be ineffective for a few species (eg, Gulf of Mexico sturgeon).[298] A recent study[307] demonstrated that use of buffered MS 222 in a 1-step immersion technique was inadequate for euthanasia of goldfish (*C auratus*), a hypoxia-tolerant species. Results from this study support the recommendation for use of a 2-step method for euthanasia of goldfish and some other hypoxia-tolerant species, including cichlids, with the first step of involving immersion to render the fish unconscious and the second involving application of an adjunctive method (such as decapitation, pithing, or freezing) to complete euthanasia. Fish that are too large for practical or cost-effective immersion in lethal doses of buffered MS 222 can be euthanized by applying the concentrated, buffered solution directly to the gills.[298,302]

(8) 2-phenoxyethanol. Solutions for immersion should be prepared in concentrations ≥ 0.5 to 0.6 mL/L or 0.3 to 0.4 mg/L.[321]

(9) Lidocaine. A buffered solution at 400 mg/L is effective for euthanasia of adult zebrafish,[109] but response to lidocaine by immersion varies considerably across species.

*Injection*—Injectable agents have been administered for euthanasia via IV, intracoelomic, IM, and intracardiac routes.[298,308]

(1) Pentobarbital (1 step). Sodium pentobarbital (60 to 100 mg/kg [27.3 to 45.5 mg/lb]) can be administered by IV, intracardiac, or intracoelomic routes for euthanasia.[251] Pentobarbital may also be administered via intracardiac injection for anesthetized animals as the second step of a 2-step euthanasia procedure. Death usually occurs within 30 minutes.

(2) Ketamine (2 step). Ketamine may be administered at dosages from 66 to 88 mg/kg[315] (30 to 40 mg/lb) via an IM injection followed by a lethal dose of pentobarbital. Observers should be advised about the possibility of ketamine-induced muscle spasms during induction.[298]

(3) Ketamine-medetomidine (2 step). A combination of ketamine, at dosages of 1 to 2 mg/kg, with medetomidine, at dosages of 0.05 to 0.1 mg/kg (0.02 to 0.05 mg/lb), may be administered via IM injection followed by a lethal dose of pentobarbital.[315](4) Propofol (2 step). A dose of 1.5 to 2.5 mg/kg (0.7 to 1.1 mg/lb) can be administered IV followed by an injection of a lethal dose of pentobarbital.[315]

## S6.2.2 Physical Methods

The following methods can be applied for euthanasia, providing they are performed with the proper equipment by trained personnel who are regularly monitored for proficiency.

(1) Decapitation followed by pithing (2 step). Rapid severance of the head and brain from the spinal cord, followed by pithing of the brain, will cause rapid death and unconsciousness. Decapitation alone is not considered a humane approach to euthanasia, especially for species that may be particularly tolerant of low $O_2$ concentrations. Pithing helps ensure rapid loss of brain function and death for those species.[322]

(2) Cervical transection using a knife or other sharp instrument inserted caudal to the skull to sever the spinal cord and cervical vertebrae, followed by pithing (2 step). The rationale for this approach is similar to that for decapitation (destruction of connections between brain and spinal cord) and pithing (destruction of brain tissue), except that the head is still physically attached by musculature to the body.

(3) Manually applied blunt force trauma (cranial concussion; **Figure 24**) followed by pithing or exsanguination (2 step). Manually applied blunt force trauma (a rapid, accurately placed blow of sufficient energy to the cranium with an appropriate-sized club) can cause immediate unconsciousness and potentially death, but should be followed by pithing or exsanguination to ensure death. The fish's size, species, and anatomy and characteristics of the blow (including its accuracy, speed, and club mass) will determine the efficacy of manually applied blunt force trauma. This procedure requires training and monitoring for proficiency. Anatomic features, such as the location of the eyes, can help serve as a guide to the location of the brain.[322,323]

(4) Penetrating captive bolt or NPCB **(Figure 25)**. These methods are usually applied to large fish species.[322]

(5) Maceration (1 step). When applied correctly, using a well-maintained macerator specifically designed for the size of fish being euthanized, death is nearly instantaneous.[324] The process is aesthetically unpleasant for some operators and observers.

(6) Rapid chilling (hypothermic shock; 1 step or 2 step). It is acceptable for zebrafish (*D rerio*) to be euthanized by rapid chilling (2° to 4°C) until loss of orientation and operculum movements[108,110,111] and subsequent holding times in ice-chilled water, specific to fish size and age. Zebrafish adults (approx 3.8 cm long) can be rapidly killed (10 to 20 seconds) by immersion in 2° to 4°C (36° to 39°F) water. Adult zebrafish should be exposed for a minimum of 10 minutes and fry 4 to 7 dpf for at least 20 minutes following loss of operculum movement to ensure death. Use of rapid chilling and use of buffered MS 222 alone have been shown to be unreliable euthanasia methods for zebrafish embryos < 3 dpf. To ensure embry-

onic lethality these methods should be followed with an adjunctive method such as use of dilute sodium or calcium hypochlorite solution at 500 mg/L.[111,115] If necessary to ensure death of other life stages, rapid chilling may be followed by either an approved adjunctive euthanasia method or a humane killing method. Until further research is conducted, rapid chilling is acceptable with conditions for other small-bodied, similarly sized tropical and subtropical stenothermic species. Species-specific thermal tolerance and body size will determine the appropriateness and effectiveness of rapid chilling for euthanasia of fish. Fish size is important because the rate of heat loss via thermal conduction from a body is proportional to its surface area. Based on these 2 factors, it has been suggested that rapid chilling in water associated with an ice slurry is a suitable killing method for small tropical and subtropical fish species 3.8 cm in length (tip of the snout to the posterior end of the last vertebra) or smaller, having lower lethal temperatures above 4°C.

To ensure optimal hypothermal shock (ie, rapid killing), transfer of fish into ice water must be completed as quickly as possible. This means rapid transitions from acclimatization temperature to 2° to 4°C must be achieved. This can be accomplished by using minimal water volume to transfer fish (ie, using a net to place fish in chilled water). In addition, fish should not be in direct contact with the ice in the water; rather a depression should be formed in the ice slurry to expose the entire surface of the fish to the chilled water. Full contact with cold water ensures optimal exposure and rapid chilling of the fish. Water temperature must not exceed 2° to 4°C. Well-insulated containers, such as coolers, will assist in maintaining the ice slurry and a probe thermometer can be used to confirm water temperature.

This method of euthanasia is not appropriate for temperate, cool, or cold-water–tolerant fish, such as carp, koi, goldfish, or other species that can survive at 4°C and below. It is appropriate for zebrafish and other small-bodied (3.8-cm-long or smaller) tropical and subtropical stenothermic fish, for which the lower lethal temperature range is above 4°C.[108,110,111] This method can also be acceptable for small to medium-sized (2.8- to 13.5-cm-long) Australian river gizzard shad, as long as secondary euthanasia methods are applied after fish are rendered nonresponsive.[110] However, because of surface-to-volume considerations, use of this method is not appropriate in other medium- to large-bodied fish until data regarding its applicability to euthanasia for those species become available.

### S6.2.3 Adjunctive Methods

Decapitation, pithing, exsanguination, freezing, and other physical or chemical methods for destroying brain function may be used as the second step of a 2-step procedure when fish have been rendered unconscious prior to their application by an acceptable or acceptable-with-conditions, first-step method. If necessary to ensure death, rapid chilling for specified groups may be followed by an approved adjunctive euthanasia method. Use of a dilute sodium hypochlorite or calcium hypochlorite solution may be an adjunctive method for early life stages of fish, including embryos and larvae.[108,115]

### S6.2.4 Unacceptable Methods

The following are unacceptable methods of euthanasia in any situation. Flushing of fish into sewer, septic, or other types of outflow systems is unacceptable for many reasons. Water chemistry and quality may delay time to death and result in exposure to noxious compounds. For systems in close proximity to and/or connected to natural waterways, pathogen release or transmission may occur from diseased or carrier animals. Slow chilling or freezing of unanesthetized animals, including placing fish into a freezer without prior anesthesia, is also an unacceptable method. Similarly death by anoxia and desiccation after removal from the water or by anoxia in water; any death due to exposure to caustic chemicals; and death including prolonged traumatic injury prior to unconsciousness are unacceptable.

While metomidate has been used for euthanasia of some finfish species, its listing in the Index of Legally Marketed Unapproved New Animal Drugs for Minor Species by the FDA (with a specified use for sedation and anesthesia) means that its extralabel use for euthanasia is currently illegal.

### S6.2.5 Life Stage Considerations

The effectiveness of euthanasia methods described in these guidelines may vary by life stage, as well as by species. Early stages in the lives of fish, including embryos and larvae, may require higher concentrations of immersion anesthetics or a longer duration of exposure.[303] As an example, immersion in a buffered MS 222 solution having a concentration > 1 g/L is not a reliable method for killing some fish in younger life stages.[108,111,303] For some species and in some situations, adjunctive methods to guarantee death may need to be applied for these animals after anesthesia with buffered MS 222. Rapid chilling followed by an adjunctive method such as immersion in a dilute sodium hypochlorite or calcium hypochlorite solution is acceptable for zebrafish embryos and larvae as a 2-step method and is also acceptable with conditions as a 2-step method for destruction of other (nonzebrafish) species' embryos and larvae.[111,115]

### S6.2.6 Finfish in Particular Environments

S6.2.6.1 Veterinary private practice—companion and ornamental (display) fish

Clients with pet or display fish of any species often value them as companion animals and share a

human-animal bond similar to that seen between clients and other pets, such as dogs and cats. Therefore, it is important to consider the perception of the client when euthanasia methods are chosen. Clients should be offered the opportunity to be present during euthanasia whenever feasible; however, clients also should be educated as to what method will be used and what they may observe during euthanasia. For example, clients may believe the excitement phase of anesthesia, which can result in increased motor activity or the appearance of agitation,[302] is unduly painful or stressful for the fish even when it is not.

The following methods are acceptable for use in this environment:

(1) Immersion in solutions of buffered MS 222, buffered benzocaine, isoflurane and sevoflurane, quinaldine sulfate, and 2-phenoxyethanol.
(2) Injections of pentobarbital, ketamine followed by pentobarbital, a combination of ketamine and medetomidine followed by pentobarbital, and propofol followed by pentobarbital. Owners should be advised about the possibility of ketamine-induced muscle spasms during induction when using that agent.

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

(1) Immersion in eugenol, isoeugenol, or clove oil. Fish should be left in the solution for a minimum of 10 minutes after cessation of opercular movement.[298,302]

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

(1) Immersion in $CO_2$-saturated water is not recommended because some fish exposed to this method may become hyperactive, which can be disconcerting for staff and owners.
(2) Manually applied blunt force trauma to the head, decapitation, and pithing are not recommended because their application can be distressing for owners and staff.

Early stages in the lives of fish, including embryos and larvae, may require higher concentrations of immersion anesthetics or a longer duration of exposure.[303] As an example, immersion in a buffered MS 222 solution having a concentration > 1 g/L is not a reliable method for killing some fish in early life stages.[108,111,303] For some species and in some situations, adjunctive methods to guarantee death may need to be applied for these animals after anesthesia with buffered MS 222.

Rapid chilling followed by immersion in a dilute sodium hypochlorite or calcium hypochlorite solution is acceptable for zebrafish embryos and larvae as a 2-step method and is also acceptable with conditions as a 2-step method for destruction of other (non-zebrafish) species' embryos and larvae.[111,115]

S6.2.6.2 Aquarium fish wholesale and retail facilities

Freshwater and marine aquarium fish are commercially collected from the wild, and are also bred in captivity. Tropical aquarium fish are sold at retail pet shops and fish stores from systems housing 1 or more species of fish per tank. Individual fish or populations of fish may become injured or diseased and require euthanasia. Methods of euthanasia used in this environment need to be applicable to individual fish, to all fish in an aquarium, to fish held in multiple aquariums on a central filtration system, or for fish ponds. In certain situations euthanasia may not be feasible and depopulation methods may be required.

The following methods are acceptable for use in this environment:

Immersion in solutions of buffered MS 222, buffered benzocaine, ethanol, and quinaldine sulfate. Fish should be left in the anesthetic solution for 30 minutes after cessation of opercular movement.[251,298,302]

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

(1) Immersion in $CO_2$-saturated water (as long as observers are advised and can accept that some fish exposed to this method may exhibit hyperactivity and appear to be in distress); immersion in a eugenol, isoeugenol, or clove oil solution; or immersion in an ethanol solution.
(2) Decapitation, cervical transection, or manually applied blunt force trauma as step 1 of a 2-step method, followed by pithing.
(3) Freezing may be used as an adjunctive method of euthanasia following anesthesia.
(4) Rapid chilling (hypothermic shock) for small-bodied (3.8-cm-long or smaller) tropical and subtropical stenothermic fish, for which the lower lethal temperature range is above 4°C.[108,110,111]

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

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

Early stages in the lives of fish, including embryos and larvae, may require higher concentrations of immersion anesthetics or a longer duration of exposure.[303] As an example, immersion in a buffered MS 222 solution having a concentration > 1 g/L is not a reliable method for killing some fish in early life stages.[108,111,303] For some species and in some situations, adjunctive methods to guarantee death may need to be applied for these animals after anesthesia with buffered MS 222.

Rapid chilling followed by immersion in a dilute sodium hypochlorite or calcium hypochlorite solution is acceptable for zebrafish embryos and larvae as a 2-step method and is also acceptable with conditions as a 2-step method for destruction of other (non-zebrafish) species' embryos and larvae.[111,115]

S6.2.6.3 Research facilities

Researchers working in laboratories should have

materials readily available to provide appropriate euthanasia for their research subjects when required, and should be trained and monitored for proficiency in the use of chosen techniques. Many facilities using fish as research subjects are engaged in biomedical research. Zebrafish are the most common species used for research and are usually kept in small-scale tank systems; however, some research facilities may also have large-scale housing and production systems or keep other larger species of fish and, consequently, may need to consider additional options for euthanasia.[272] The expertise of those knowledgeable about these settings and species should be sought as necessary.

The following methods are acceptable for use in this environment:

(1) Immersion in solutions of buffered MS 222, buffered benzocaine, lidocaine, quinaldine sulfate, and 2-phenoxyethanol. Fish euthanized with these methods are not approved for use as human food.
(2) Rapid chilling (hypothermic shock) is acceptable for zebrafish (*D rerio*) and Australian river gizzard shad (*N erebi*) as long as transfer from acclimatized temperatures to water associated with a 2° to 4°C ice slurry occurs rapidly with as little transfer of warmer water as possible.

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

(1) Immersion in $CO_2$-saturated water (as long as observers are advised and can accept that some fish exposed to this method may exhibit hyperactivity and appear to be in distress) or immersion in a eugenol, isoeugenol, or clove oil solution.
(2) Rapid chilling (hypothermic shock) to 2° to 4°C is acceptable with conditions for small-bodied (3.8-cm-long or smaller) tropical and subtropical stenothermic fish, for which the lower lethal temperature range is above 4°C. Because of surface-to-volume considerations, use of this method is not appropriate for other medium- to large-bodied fish until additional data for those species become available.
(3) Maceration is acceptable with conditions when death is instantaneous from using a well-maintained macerator designed for the size of fish being euthanized. The process is likely to be aesthetically unpleasant for those observing it.
(4) Decapitation followed by pithing. Rapid severance of the head and brain from the spinal cord, followed by pithing of the brain, will cause rapid death and unconsciousness.[272]
(5) Manually applied blunt force trauma (cranial concussion) followed by pithing or exsanguination.

Early stages in the lives of fish, including embryos and larvae, may require higher concentrations of immersion anesthetics or a longer duration of exposure.[303] As an example, immersion in a buffered MS 222 solution having a concentration > 1 g/L is not a reliable method for killing some fish in earlier life stages.[108,111,303] For some species and in some situations, adjunctive methods to guarantee death may need to be applied for these animals after anesthesia with buffered MS 222.

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

S6.2.6.4 Fish kept outdoors and in fisheries

Field research on fish takes place in a complex environment that must be understood by both researchers and their respective IACUC.[272] Field research is frequently conducted on a scale comparable to commercial fishing, often with the same equipment, boats, and personnel. The large number of fish, limited boat space, adverse environmental conditions, and personnel safety concerns may justify use of harvest techniques that may not meet the criteria for euthanasia, but in all situations, pain and distress should be minimized to the greatest extent possible. Similarly, fisheries biologists may be faced with situations involving numerous fish requiring depopulation (eg, invasive species) rather than euthanasia.

Fieldwork on fish may also be conducted on a smaller scale under conditions that make euthanasia feasible. In such cases, the following methods should be applied and convenience for the researcher should not be a primary consideration.

The following methods are acceptable for use in this environment:

(1) Immersion in solutions of buffered MS 222, buffered benzocaine, quinaldine sulfate, isoflurane or sevoflurane, ethanol, quinaldine sulfate, and 2-phenoxyethanol. Although a general concern for all environments and situations, the potential effects of drug residues and proper disposal of animal remains should be considered when using any of these drugs.
(2) An injection of pentobarbital (60 to 100 mg/kg) can be administered IV or intracoelomically.[321] Pentobarbital may also be administered intracardially in anesthetized animals. Two-step injection procedures may also be used, including ketamine (IM) followed by a lethal dose of pentobarbital; a combination of ketamine and medetomidine (IM) followed by a lethal dose of pentobarbital; and propofol (IV) followed by a lethal dose of pentobarbital. Although a general concern for all environments and situations, the potential effects of drug residues and proper disposal of animal remains should be considered when using any of these drugs.

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

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

(2) Manually applied blunt force trauma to the head followed by pithing or exsanguination.
(3) Decapitation followed by pithing. Decapitation alone is not considered a humane form of euthanasia, especially for species that may be particularly tolerant of low $O_2$ concentrations. Pithing helps ensure rapid death for those species.
(4) Cervical transection followed by pithing or exsanguination. The rationale for this approach is similar to that for decapitation and pithing, except that the head is still physically attached by musculature to the body.
(5) Captive bolt. This method is usually applied to large fish species.
(6) Rapid chilling (hypothermic shock) in water of 2° to 4°C for small-bodied (3.8-cm-long or smaller) tropical and subtropical stenothermic species (as previously described for zebrafish). Because of surface-to-volume considerations, use of this method is not appropriate in medium- to large-bodied fish until pertinent data for those species become available.

Early stages in the lives of fish, including embryos and larvae, may require higher concentrations of immersion anesthetics or a longer duration of exposure.[303] As an example, immersion in a buffered MS 222 solution having a concentration > 1 g/L is not a reliable method for killing some fish in early life stages.[108,111,303] For some species and in some situations, adjunctive methods to guarantee death may need to be applied for these animals after anesthesia with buffered MS 222. Rapid chilling followed by immersion in a dilute sodium hypochlorite or calcium hypochlorite solution is acceptable for zebrafish embryos and larvae as a 2-step method and is also acceptable with conditions as a 2-step method for destruction of other (nonzebrafish) species' embryos and larvae.[111,115]

## S6.3 AQUATIC INVERTEBRATES

Overdose of a general anesthetic is as appropriate a euthanasia strategy for aquatic invertebrates as it is for fish. And, immersion is an effective route of administration of anesthetic and euthanasia agents.[325,326]

Because confirming the death of many invertebrates is difficult, 2-step euthanasia procedures are often recommended in which chemical induction of anesthesia, nonresponsiveness, or presumptive death is followed by an adjunctive method that destroys the brain or major ganglia physically (eg, pithing, freezing, boiling) or chemically (eg, alcohol, formalin). Application of the latter methods by themselves is generally not considered to meet the criteria established for euthanasia.[325,326]

### S6.3.1 Acceptable First Steps of 2-Step Methods

S6.3.1.1 Noninhaled agents for immersion

*Magnesium salts*—Magnesium salts are a near-universal anesthetic agent, relaxing agent, and euthanasia agent for aquatic invertebrates, although they are ineffective for crustaceans. Research indicates the magnesium ion acts centrally and also blocks both afferent and efferent nerve transmission in suppressing neural activity of cephalopods.[327,328] A range of concentrations has been recommended for various phyla. A stock solution of $MgCl_2.6H_2O$ at a concentration of 7.5% (75 g/L, about 370mM in deionized water) is nearly isosmotic with seawater and can be added to water in increasing ratios up to 1:1 stock solution volume to water volume (3.75%, 37.5 g/L, about 185 mM) or higher to effect euthanasia.[329,330] A direct addition of magnesium salts to seawater results in a hyperosmotic solution.[331] Magnesium salts may be combined with ethanol for euthanasia of cephalopods.[331] Immersion for at least 15 minutes is recommended for cephalopods, as is an adjunct method like decerebration at least 5 minutes after respiratory arrest or after the animal is insensible.[328,330] At least 30 minutes' immersion is recommended if brain destruction is not an option.[330] Species susceptibility to the effects of magnesium salts varies.[329,330]

*Clove oil or eugenol*—Clove oil or eugenol has been used effectively as an immersion agent for the euthanasia of some crustaceans (0.125 mL/L).[325,332] Isoeugenol is a potential carcinogen[316] so human safety in the application of that agent is of concern.

*Ethanol*—Ethanol has been used for euthanasia of some phyla, acting by inhibiting neuronal sodium and calcium channels in molluscs.[309] It inhibits both afferent and efferent nerve transmission in cephalopods.[328] Initial aversion and excitement have been reported as occurring in some but not all cephalopods.[327,333,334] It is used at a concentration of 1% to 5% (10 to 50 mL/L)[328,335] up to 10%,[329] in contrast with concentrations of > 70% used for preservation, and may be less effective at cooler temperatures.[334] It is recommended that ethanol be added slowly with mixing.[329] Ethanol may be combined with a magnesium salt solution for euthanasia of cephalopods.[331] Immersion of at least 10 minutes followed by an adjunctive method such as decerebration is recommended.[330] Species susceptibility to the effects of ethanol varies.[330]

Other agents for euthanasia, while less common, have been described and may be useful for specific applications.[325,329]

### S6.3.2 Acceptable Second Steps of 2-Step Methods

S6.3.2.1 Noninhaled agents for immersion

Noninhaled agents that can be administered via immersion as the second step of a 2-step euthanasia approach include 70% alcohol and neutral-buffered 10% formalin. These agents are not acceptable, however, for immersion as a single-step procedure, nor as the first step of a 2-step procedure.

S6.3.2.2 Physical methods

Pithing, freezing, and boiling are acceptable as the second step (adjunctive methods) of a 2-step eu-

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-

ences should be consulted for appropriate doses of anesthetics and anxiolytics and preferred routes of administration.[8,340–342] Animals can be premedicated via IM injection and/or orally. Intravenous administration of drugs is generally difficult without physical or chemical restraint. Chamber delivery of inhaled agents having little odor, such as sevoflurane, allows for induction of anesthesia in smaller species with minimal stress. Injectable anesthesia can be momentarily painful or discomforting during or immediately after administration due to a combination of volume, formulation, and route of administration, as well as the distress associated with physical restraint. The advantages and disadvantages of administering anxiolytics, anesthetics, or other drugs and applying physical restraint should be balanced against the benefit of providing a swift death to end suffering. Research is needed to improve the euthanasia options available for some taxonomic groups and circumstances.

## S7.2 CAPTIVE INVERTEBRATES

Invertebrates comprise more than 95% of the animal kingdom's species and include unrelated taxonomic groups: spiders (Araneae),[343] centipedes and millipedes (Myriapoda), insects (Hexapoda),[344] and many others. Terrestrial invertebrates play important roles in laboratory research, as display animals, and as companions in the home. Despite their varied roles, limited guidance is available on appropriate methods by which invertebrates may be euthanized.[251,336,345–347] This is due, in part, to a lack of coverage under animal welfare regulations applicable to animals used for research and other purposes in the United States and other countries.[333,348] Diversity in anatomic, physiologic, and other characteristics limits generalizations across taxa.[349] Of particular relevance are differences in innervation and circulatory systems, some of which do not have close corollaries in familiar vertebrate systems. This creates challenges for developing humane means of terminating invertebrates' lives.

While there is ongoing debate about invertebrates' abilities to perceive pain or otherwise experience compromised welfare, the Guidelines assume that a conservative and humane approach to the care of any creature is warranted and expected by society. Consequently, euthanasia methods should be used that minimize the potential for pain or distress. Most commonly used methods involve terminal anesthesia, followed by physical destruction of the nervous system, to assure lack of sensory perception and death of the animal. The diversity of invertebrate taxa may require equally diverse approaches to euthanasia.

### S7.2.1 Acceptable Methods

S7.2.1.1 Noninhaled agents

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

### S7.2.2 Acceptable With Conditions Methods

S7.2.2.1 Inhaled agents

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

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

S7.2.2.2 Acceptable first steps of 2-step methods

Two-step euthanasia procedures, as described for aquatic invertebrates, are acceptable for some or many species of invertebrates. Recent research documented the efficacy of immersion in 5% laboratory-grade ethanol or an undiluted, uncarbonated beer (5% ethanol content) served to anesthetize land snails (*Succinea putris*) without signs of distress as a first-step procedure.[335] This was followed by immersion in solutions of 70% to 95% ethanol or neutral-buffered 10% formalin that served to euthanize snails and preserve tissue. Further research is needed to establish the general validity of applying this 2-step method and other methods to terrestrial invertebrate species.

S7.2.2.3 Physical and chemical methods

Physical (eg, boiling, freezing, pithing) and chemical (eg, alcohol, formalin) methods act by destroying the brain or major ganglia. Physical and chemical methods should be applied adjunctively, following pharmaceutical or other chemical induction of anesthesia, nonresponsiveness, or presumptive death. These methods are not considered to be humane as sole methods of euthanasia.[345,346,350,351]

*Pithing*—This method requires detailed anatomic knowledge of the species in question.

### S7.2.3 Unacceptable Methods

Because information on the physiologic responses of invertebrates to many methods of euthanasia is not available at this time, comments regarding unacceptable methods of euthanasia are limited to those that should not be applied as sole methods of euthanasia (see comments under Acceptable With Conditions Methods).

### S7.2.4 Developmental Stages of Invertebrates

Recommendations for euthanasia of invertebrates in developmental stages are currently not available.

## S7.3 CAPTIVE AMPHIBIANS AND REPTILES

### S7.3.1 Anatomy and Physiology

Amphibians and reptiles include caecilians (order Gymnophiona), frogs (order Anura), salamanders (order Caudata), snakes (suborder Serpentes), lizards (suborder Lacertilia), crocodilians (order Crocodilia), and turtles and tortoises (superorder Chelonia). Once again, these taxonomic groups differ substantially anatomically and physiologically from each other, as well as from mammals. Of particular concern for amphibians and reptiles are differences in metabolism and high tolerances to hypoxia, as compared with mammals, that limit the effectiveness of methods based on anoxia. In addition, consistent access to the vasculature can be challenging and, therefore, many conventional methods of euthanasia are less efficacious for these species. Because it is often difficult to confirm that an amphibian or reptile is dead, the application of 2 or more euthanasia procedures is usually recommended.[293,352–354]

Our understanding of amphibians' and reptiles' nociception and responses to stimuli is incomplete; therefore, many recommendations for minimizing pain and distress are extrapolated from information available about mammals. Where uncertainty exists, erring to proactively alleviate potential pain and suffering is recommended as an appropriate approach to euthanizing amphibians and reptiles. Consulting multiple references on amphibian and reptile euthanasia is advised as a means of identifying methods that are most appropriate for a given species and set of circumstances.[116,293,294,352–356]

### S7.3.2 Restraint

*Physical restraint*—Manual restraint is possible for many species. Equipment may be required for restraint of some species in some situations (eg, venomous species). Multiple people may be required for larger species, and at least 1 additional person should be available for emergencies. Large animals may represent a proportionately greater risk for personnel.

*Chemical restraint*—Chemical restraint may be useful in some situations, particularly for venomous or large animals where human safety would be compromised by manual restraint. Chemical restraint at high doses may serve as a first or preparatory step of euthanasia in some situations.

### S7.3.3 Verification of Death

Methods used to verify death in mammalian species, such as auscultation, ECG, Doppler ultrasound, or pulse oximetry, can be used for amphibians and reptiles, but it is important to remember that amphibian and reptilian hearts can beat even after brain death. Death should always be confirmed by physical intervention.

### S7.3.4 Acceptable Methods

S7.3.4.1 Noninhaled agents

*Injectable agents*—Venous access for administration of euthanasia agents can be challenging for some species. Intracoelomic, subcutaneous lymph spaces, and lymph sacs are acceptable routes of administration. Direct injection into the brain through the parietal eye, while under anesthesia, has been described for some lizard species.[357]

Sodium pentobarbital (60 to 100 mg/kg of body weight) can be administered IV, intracoelomically, in the subcutaneous lymph spaces, or in the lymph sacs, although doses vary by species.[358] Doses as high as 1,100 mg/kg (500 mg/lb) of sodium pentobarbital with sodium phenytoin administered intracoelomically may be required for euthanasia of some species such as *X laevis*.[116] Time to effect may vary, with death occurring instantaneously or up to 30 minutes later.[293,352–354,359,360] Barbiturates are best administered intravascularly to minimize the discomfort upon injection.[361] However, where intravascular administration is not possible or its benefits are outweighed by distress imposed by additional restraint, pain from alternate methods, risk to personnel, or other similar reasons, intracoelomic administration is an acceptable route for administration of barbiturates.

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

*External or topical agents*—Buffered MS 222 may be administered via water baths (amphibians), or injected directly into the lymph sacs (amphibians) or the coelomic cavity (small amphibians and reptiles).[362–365] Prolonged immersion (as long as 1 hour) may be required for 5- to 10-g/L water baths.[116,358] Tricaine methanesulfonate does not create histopathologic artifacts.[362] See the Noninhaled Agents section of the Guidelines for additional information.

Benzocaine hydrochloride, a compound similar to MS 222, may be used as a bath or in a recirculation system at concentrations ≥ 250 mg/L or applied topically to the ventrum as a 7.5% or 20% gel for euthanasia of amphibians.[366] A dose of 182 mg/kg of benzocaine gel (20% concentration, 2.0-cm × 1.0-mm application) has been reported as effective for euthanasia of adult *X laevis*.[116] Pure benzocaine is not water soluble and should be avoided for anesthesia or euthanasia because it requires the use of acetone or ethanol solvents, which may be irritating to tissues.[367]

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

plicable across a range of species and sizes of animals. However, general anesthesia may be required prior to administration, some require IV administration for vessels that may be difficult to access, they may produce undesirable tissue artifacts, a controlled substance license is required for barbiturates and some other products, and there may be environmental pollution and toxicity concerns depending on method of disposal of the remains.

## S7.3.5 Acceptable With Conditions Methods

S7.3.5.1 Inhaled agents

*Inhaled anesthetics*—Inhaled anesthetics are acceptable with conditions when they are more practical than the previously mentioned acceptable methods, and where the limitations of this method are understood and addressed. Many reptiles and amphibians are capable of breath holding and shunting of their blood, which permits conversion to anaerobic metabolism for survival during prolonged periods of anoxia (up to 27 hours for some species).[368–373] Because of this, induction of anesthesia and time to loss of consciousness may be greatly prolonged when inhaled agents are used. Death may not occur even with prolonged exposure.[293,352–354] Lizards and most snakes do not hold their breath to the same extent as some of the chelonians, and are therefore more likely to have a clinical response to inhaled agents. Regardless of the species or taxonomic group, death must be verified prior to terminating the use of the inhaled agent, or a second, guaranteed lethal procedure (eg, decapitation) should be performed to ensure death.

Inhaled anesthetics are effective, have a moderately rapid onset, appear to induce a painless death, can maximize use of the euthanized animal for analytical studies, and can minimize the need for animal handling. Caveats include that inhaled anesthetics are most suitable for smaller species, animals may experience an excitation phase prior to becoming anesthetized, they present environmental pollution and occupational hazard concerns, some are irritants or are perceived as noxious, and amphibians and reptiles may be resistant to their action because of breath holding.

*Carbon dioxide*—Carbon dioxide may be considered for euthanasia of amphibians and reptiles if alternate methods are not practical and where the limitations of this method are understood and addressed.[293,294,352–354,356] Due to the potential lack of response to this method by many species and the requirement for a prolonged exposure time, other methods are preferable. Death by $CO_2$ must be verified, and preferably, assured by application of a secondary lethal procedure.

S7.3.5.2 Physical methods

*PCB or firearm*—Crocodilians and other large reptiles can be euthanized by a PCB or gunshot (free bullet) delivered to the brain.[355,374] Nonpenetrating captive bolt can be effective for euthanasia of American alligators[374] that are 5 to 15 kg (11 to 33 lb), and further research is needed to establish this method's general applicability to large reptilian and amphibian species. Line drawings of the head of various amphibians and reptiles, with recommended locations for captive bolt or firearm projectile penetration, are available **(Figure 26)**.[356] Refer to ballistics details in the Physical Methods section and experts for more information on selection and use of firearms.

These methods are moderately rapid (allowing for restraint), are applicable across a wide range of species and sizes, and leave no environmental residues other than lead (in the case of free bullet), which can be sequestered. However, size-appropriate equipment and appropriately trained personnel are required, violent muscle contractions can occur following their application, and they may be aesthetically unpleasant for onlookers.

*Manually applied blunt force trauma to the head*—This method is acceptable with conditions, when other options are unavailable, as long as it is performed by well-trained and skilled personnel and if an adjunctive method, such as decapitation or pithing, is promptly applied to ensure death.[95,336,352,366] Further research is needed to clarify methods, taxa, and size ranges where this method is effective and humane.

*Rapid freezing*—Reptiles and amphibians can be euthanized by rapid freezing when it results in immediate death. Based on rodent models, it is likely that this can be achieved by placing animals < 4 g (0.1 oz) in liquid $N_2$.[95] However, due to a dearth of empirical evidence supporting this method, operators should consider a secondary method to ensure that recovery does not occur. The technique should not be used for species that have adapted freeze tolerance strategies, as this method may not result in instant death.[375] Placement of animals ≥ 4 g in liquid $N_2$ or other uses of hypothermia are not acceptable.

*Spinal cord severance followed by destruction of brain tissue*—Death can be humanely and effectively induced in 5- to 15-kg American alligators by spinal cord severance promptly followed by pithing of the brain when operators are trained and skilled in the procedure.[374] This method may be appropriate for some sizes of other reptile and amphibian species, but further research is needed to confirm this. Destruction of brain tissue after spinal cord severance can also be achieved by use of PCB or NPCB, and this approach may be less prone to operator error when equipment is in good working order.

## S7.3.6 Adjunctive Methods

*Decapitation*—After animals have been anesthetized, decapitation using heavy shears or a guillotine is effective for some species. It has been assumed that stopping blood supply to the brain by decapitation causes rapid loss of consciousness. However, because the CNS of reptiles and amphibians is tolerant to hypoxic and hypotensive conditions,[356] decapitation

must be followed by pithing or another method of destroying brain tissue.[352,354,361,374] Decapitation should only be performed as part of a 3-step euthanasia protocol (injectable anesthetic, decapitation, pithing).

*Pithing*—Pithing can be used as a second-step euthanasia method in unconscious animals when performed by properly trained individuals.[352,354] The pithing site in frogs is the foramen magnum, and it is identified by a slight midline skin depression posterior to the skull, midline between the eyes, with the neck flexed.[293,353]

### S7.3.7 Unacceptable Methods

*Hypothermia*—Hypothermia is an inappropriate method of restraint or euthanasia for amphibians and reptiles unless animals are sufficiently small (< 4 g)[95] to permit immediate and irreversible death if placed in liquid $N_2$ (rapid freezing).[352,354,361] Hypothermia reduces amphibians' tolerance for noxious stimuli[376,377] and there is no evidence that it is clinically efficacious for euthanasia.[378] In addition, it is believed that freezing can result in the formation of ice crystals in tissues that may cause pain.[95,356] Consequently, because amphibians and reptiles lack behavioral or physiologic means of demonstrating pain or distress while hypothermic, generalized prohibitions on hypothermia for restraint or euthanasia are appropriate. Localized cooling in frogs may reduce nociception, but this localized effect is not appropriately applied to the whole body as a part of euthanasia procedures.[379] Freezing of deeply anesthetized animals may be justified under circumstances where human safety could be compromised.[380]

### S7.3.8 Special Cases and Exceptions

Intracardiac administration of euthanasia agents is acceptable for captive amphibians and reptiles that are unresponsive to stimuli because of disease or the application of other euthanasia methods, or in cases where other routes are not possible.

Neuromuscular blocking agents may be used for routine anesthetic procedures of crocodilians and some other taxa and are, therefore, considered acceptable with conditions for restraint of reptiles if given immediately prior to administration of a lethal agent. These agents are not acceptable as a sole means of euthanasia.

Injectable agents such as lidocaine hydrochloride, potassium salts, or magnesium salts may be useful as an adjunctive method to prevent recovery.[354]

Perfusion with fixative of a deeply anesthetized animal can be used to euthanize amphibians and reptiles when scientifically justified.

### S7.3.9 Destruction of Viable Eggs

Little information is available on the sensory capacity of amphibians and reptiles at the egg stage of development.[95] Freezing is likely appropriate for newly oviposited eggs, as would be methods of maceration that result in instantaneous death. Later stages may be destroyed using methods that are acceptable for adult animals. More research needs to be done to determine the most appropriate methods for disposing of live eggs.

## S7.4 CAPTIVE NONMARINE MAMMALS

### S7.4.1 General Considerations

The anatomic, physiologic, behavioral, and size variations of nondomestic mammals far exceed those of their domestic counterparts. This presents challenges for the application of conventional methods of euthanasia and the recognition of anxiety and pain. Differences from similar domestic species must be recognized and addressed as thoroughly as practical when preparing for and performing euthanasia.

In zoos or other captive settings, euthanasia of wildlife is typically performed in the presence of staff members who are responsible for caring for these animals. Consequently, sensitivity to the meaning and value to caregivers of animals in this kind of setting is important. This can be addressed, in part, with attention to stewardship, and relief from pain and anxiety prior to administration of a euthanasia method. Most euthanasia procedures should include the use of inhaled or injectable anesthetics to achieve unconsciousness, followed by use of an approved method to end life.

In some cases animals may experience intolerable suffering, or the situation may not allow for ideal stewardship as a prelude to the act of euthanasia. These situations typically require a more direct approach to limit how much an animal is allowed to suffer. Such situations also require a brief explanation to personnel, where possible, as well as a more complete explanation of the choice of method subsequent to completion of the procedure. Preparing staff ahead of time to be cognizant of the possibility of these kinds of situations will likewise help to better prepare for situations where a more ideal procedure is not feasible.

Alternate approved methods of euthanasia might be applicable if an animal is anesthetized prior to euthanasia. Any candidate method not specifically mentioned in the text that follows should be evaluated conceptually to address good stewardship principles prior to its use.

Following euthanasia, verification of death is important. Methods that can be used for verification of cessation of cardiac function include, but may not be limited to, palpation for a pulse in an appropriate anatomic location based on species, auscultation with a stethoscope, and use of Doppler ultrasound.

### S7.4.2 Restraint

*Physical restraint*—Manual restraint is possible for many species. Nets or other equipment may be appropriate for smaller species that do not pose an excessive risk for personnel. For the largest species (hoofstock and megavertebrates), chutes or other

equipment may provide sufficient restraint for IM or IV administration of anesthetics and/or anxiolytics. Brief restraint followed by IV administration of a euthanasia agent may be possible as an approach to euthanasia in some situations. However, administration of a preanesthetic or sedatives before administration of a euthanasia agent should be the default in most cases.

*Chemical restraint*—Chemical restraint may be useful in some situations, particularly for dangerous animals where human safety would be compromised with manual restraint, as well as to reduce unnecessary stress and discomfort for the animal(s). Chemical restraint at high doses may serve as the first step of euthanasia in some situations.[8,340–342]

## S7.4.3 Acceptable Methods

S7.4.3.1 Noninhaled agents

*Barbiturates*—Barbiturates may be administered IV or IP. Intracardiac administration must be limited to animals that are unconscious due to disease or the effects of anesthetics. Onset of action is slower with IP administration and premedication with anesthetics may reduce discomfort due to tissue irritation. Barbiturates are best administered intravascularly to minimize discomfort upon injection.[361] However, where intravascular administration is not possible or its benefits are outweighed by distress imposed by additional restraint, pain from alternate methods, risk to personnel, or other similar reasons, IP administration is an acceptable route for administration of barbiturates.

Barbiturates are highly effective as euthanasia agents, have a rapid onset of action, and are applicable across a wide range of species and sizes of animals. However, they do have drawbacks, including that individuals must be trained to correctly administer injections, general anesthesia or sedation with injectable or inhaled agents may be required prior to their administration (depending on the animal and the situation), they can produce undesirable tissue artifacts, a controlled substance license is required for their acquisition, and environmental pollution and toxicity may be of concern depending on the method used to dispose of animal remains.

*Nonbarbiturate anesthetic overdose*—Opioids and other anesthetics may be administered IV or IM for euthanasia when animal size, restraint requirements, or other circumstances indicate these drugs are the best option for euthanasia.

Intramuscular administration of opioids is advantageous when other routes of administration are not available. Opioids tend to have a rapid onset of action, and the volume of drug to be administered may be smaller than for other agents. There are also disadvantages associated with administering an overdose of opioids, including requirements for DEA licensing, risks to human safety if exposure to drugs occurs, and the potential for secondary toxicity if tissues are consumed.

## S7.4.4 Acceptable With Conditions Methods

S7.4.4.1 Inhaled agents

*Inhaled anesthetics*—Inhaled anesthetics are acceptable with conditions when they are more practical than acceptable methods, and where the limitations of this method are understood and addressed. Inhaled anesthetics may be administered via face mask or chambers. Placing an animal's entire crate into a chamber will allow anesthesia to be induced with the least amount of distress. As discussed in the Inhaled Agents section of the Guidelines, agents with minimal odor are preferred.

Inhaled anesthetics have a moderately rapid onset of action, do not appear to cause pain on administration, maximize the availability of the animal's remains for analytical studies, and can be applied with minimal handling of the animal. They also, however, have some disadvantages in that they are most suitable for smaller species, some are irritants or are perceived as noxious, animals can experience an excitation phase prior to induction of anesthesia, and they may present environmental pollution and occupational safety concerns.

*Carbon monoxide, carbon dioxide, and inert gases*—These agents are acceptable with conditions for application where animal welfare and pragmatic concerns warrant their use and risks to personnel safety can be addressed. For more information, please consult the Guidelines section on Inhaled Agents.

S7.4.4.2 Physical methods

*PCB or firearm*—Use of a PCB or firearm (free bullet) may be appropriate for some species as a first step or adjunct method of euthanasia, when there is species-specific knowledge of target sites and safety considerations can be met.

Advantages of these methods are that they are moderately rapid (considering application of any needed restraint), they may be relatively easily implemented under various conditions, they are applicable across a wide range of species and sizes, and they leave no environmental residues (other than lead, which may be sequestered). There are some disadvantages in that they require appropriate, well-maintained equipment and well-trained personnel, they are potentially aesthetically displeasing for observers, and they present safety risks for personnel associated with the keeping and use of firearms. Refer to ballistics details in the section on Physical Methods and experts for more information on selection and use of firearms.

## S7.4.5 Adjunctive Methods

*Potassium chloride*—Potassium chloride can be administered IV or intracardially to stop the heart of animals that are deeply anesthetized or unconscious. Potassium chloride does not create artifacts that can interfere with histopathologic examination and, therefore, its application may be appropriate when accurate postmortem diagnostic or research results

are important. Potassium chloride may also be used adjunctively for large animals that are first anesthetized with barbiturates, particularly where volume of administration is a limitation. In many cases significant agonal reflex activity can be avoided where barbiturates are administered prior to administration of potassium chloride.

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

*Cervical dislocation or decapitation*—Applied to small mammals and birds, this method may be useful as an adjunct or as a first-step method of euthanasia. A paucity of data for wildlife and the potential for interspecies variation creates challenges for establishing specific size recommendations. However, based on domestic animals, manual cervical dislocation may be appropriate for birds < 3 kg (6.6 lb), rodents < 200 g (0.44 lb), and rabbits < 1 kg (2.2 lb).[365] A secondary method such as decapitation or exsanguination should be employed to ensure death when feasible.

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

### S7.4.6 Unacceptable Methods

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

### S7.4.7 Embryos, Fetuses, and Neonates

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

## S7.5 CAPTIVE MARINE MAMMALS

Due to their unique anatomic and physiologic adaptations for aquatic environments, the large size of some species, and the challenges associated with performing euthanasia under typical circumstances, marine mammals are considered separately from other mammals. To facilitate making appropriate recommendations regarding euthanasia, marine mammals have been divided into physiologically and anatomically distinct groups. These groups follow taxonomic lines to some extent, though it is appropriate to consider the sea otter (a large mustelid) with small pinnipeds: 1) pinnipeds, 2) odontocetes, 3) mysticetes, and 4) sirinids. Methods addressed under S7.4 Captive Nonmarine Mammals are applicable to polar bears and therefore will not be addressed in this section. Sizes of the animals vary dramatically among and within these groups and each group should minimally be divided into subgroups by size (large and small). Recommendations for euthanasia of marine mammals in managed care facilities differ from those used for free-ranging marine mammals, because of differences in environment and facilities, restraint capabilities, and personnel and observers.

### S7.5.1 Acceptable Methods

S7.5.1.1 Noninhaled agents

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

The advantage of using barbiturates is that death is usually rapid. Unfortunately, voluntary peripheral vasoconstriction by cetaceans or hypovolemic shock may limit access to peripheral veins. There is also a risk of injury for personnel attempting venipuncture if animals are not restrained. Furthermore tissue residues can present challenges for disposal of the animal's remains and personnel are responsible for ensuring that secondary toxicity does not occur.

Intramuscular administration of sedatives or anesthetics may be required to immobilize large, anxious, or fractious animals to ensure animal and personnel safety prior to administration of IV euthanasia agents. Agents that have successfully been used alone or in combination for this purpose include tiletamine-zolazepam, ketamine, xylazine, meperidine, fentanyl, midazolam, diazepam, acepromazine, and etorphine.[381] Veterinarians should be aware that administration of anesthetics or sedatives in fat layers can result in prolonged time to effect and diminished depth of sedation and anesthesia. In addition, tissue residues, particularly when ultrapotent opioids are administered, need to be considered when disposing of the animal's remains.[382]

### S7.5.2 Acceptable With Conditions Methods

S7.5.2.1 Inhaled agents

Inhaled anesthetics (eg, halothane, isoflurane, sevoflurane, methoxyflurane, enflurane) are uncommonly used to euthanize marine mammals because these animals' ability to breath-hold means that extended periods of physical restraint are necessary for their administration. Extended restraint generally poses unacceptable risks and stress for the animal and for personnel unless the animal is substantially

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 collaboration 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 nondomestic 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.

Care must be taken to prevent secondary intoxication of animals or people during disposal of the remains of free-ranging wildlife that contain residues of euthanasia agents. This is a legal requirement that often requires deep burial, incineration, or rendering. In other situations, however, natural decomposition may be desirable. Use of gunshot can minimize concerns for secondary toxicity, with the exception of lead ballistics. Alternatives to lead ballistics are recommended where possible.

Although not typically a part of wildlife management programs, disease outbreaks or overpopulation may require culling or large-scale killing of animals. In addition to selecting the most appropriate methods for minimizing spread of infectious agent, protecting animal welfare, and protecting the environment, such situations must consider the concerns and perceptions of the general public, as well as impacts upon personnel who are directly involved in culling, killing, or euthanasia. Detailed information about depopulation methods is beyond the scope of this document, but is available in the AVMA Guidelines for the Depopulation of Animals.[288]

Research objectives may limit the use of some euthanasia agents or methods for wildlife species. Nevertheless, termination of life still dictates that the most humane, rather than the most convenient, methods be used to meet the study's objectives.

Within the context of wildlife rehabilitation, euthanasia of individual animals must be considered if a fully functional animal cannot be returned to the wild, if the release of such animals would pose a threat to the health of the free-ranging wildlife population, or if no alternatives for care or housing exist. While there are a limited number of nonreleasable animals that can be used for educational or display purposes, most animals that are determined to be unfit for release should be euthanized as soon as possible. Because most animals in rehabilitation facilities are confined, adequate control through physical or chemical restraint can usually be achieved that will allow administration of euthanasia agents as described in the taxonomically based sections for nondomestic animals.

## S7.6.3 Methods

Little published information is available regarding appropriate methods for euthanasia of specific species of free-ranging wildlife. Schwartz et al[385] evaluated immobilization and euthanasia for white-tailed deer, Hyman[386] and Needham[387] described euthanasia methods for captive or stranded marine mammals, and the euthanasia of waterfowl was described by Gullett[388] and Franson.[252] Methods for euthanasia of wildlife in rehabilitation facilities have also been described.[283]

While multiple publications describe euthanasia methods for domestic and nondomestic animals,[95,251,271,336,337] as well as for wildlife under free-ranging conditions,[389-392] their recommendations are inconsistent. Many conventional euthanasia techniques and methods can be applied to free-ranging wildlife, if the animals are sufficiently under the control of personnel. However, because of the variety of conditions under which euthanasia of free-ranging wildlife may need to be conducted, choice of the most humane method will vary by species, situation, and individual animal. Conditions specified for use of various methods in previous sections will generally apply to free-ranging wildlife, but may be modified according to circumstances to minimize animal distress and pain, as well as emotional impact and physical risks to personnel.

S7.6.3.1 Acceptable methods

S7.6.3.1.1 Noninhaled agents

Chemical methods of euthanasia applicable to free-ranging wildlife include overdoses of injectable anesthetic agents (including barbiturates), T-61, or other agents that are listed as acceptable for domestic animals or captive wildlife. Premedication with an injectable or inhaled agent may reduce animal distress and/or human safety risks, under some circumstances.

S7.6.3.2 Acceptable with conditions methods

S7.6.3.2.1 Inhaled agents

*Inhaled anesthetics*—Inhaled anesthetics are acceptable with conditions for euthanasia of avian and mammalian wildlife species when these methods are more practical than acceptable methods, and where the limitations of this method are understood and addressed.. Smaller species that can be confined in enclosed containers can be euthanized using open-drop methods of administration.[393] Larger species may be restrained for face-mask administration, when animal distress associated with restraint can be minimized. Portable equipment is available that can make these methods practical. Preference should be given to the use of alternate methods for taxa that can breath-hold for extended periods of time.

*Carbon dioxide, carbon monoxide, and other inert gases*—These agents, which are classified as being acceptable with conditions for domestic animals, are also acceptable with conditions for euthanasia of free-ranging wildlife. Conditions that must be met for using these agents are similar to those for domestic animals.

S7.6.3.2.2 Physical methods

Gunshot is acceptable with conditions for euthanasia of free-ranging, captured, or confined wildlife, provided that bullet placement is to the head (targeted to destroy the brain).[337] Gunshot targeted to the heart (chest) or to the neck (vertebrae, with the intent of severing the spinal cord) presents challenges for accurate placement, but may be the best option for free-ranging or other settings where close approach is not possible or where the head must be preserved for disease testing (rabies, chronic wasting, or other suspected neurologic diseases). Based on domestic animal models (see section of the Guidelines addressing Farmed Animals Used for Food and

Fiber), gunshot to the chest or neck may not result in rapid death and may be considered humane killing, rather than euthanasia. In some environments (eg, urban and suburban areas), discharge of a firearm may present a serious threat to human safety and may be inappropriate. Refer to ballistics details in the Physical Methods section and experts for more information on selection and use of firearms.

S7.6.3.3 Adjunctive methods

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

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

*Cervical dislocation or decapitation*—Applied to small mammals and birds, this method may be useful as an adjunct or as a first-step method of euthanasia. A paucity of data for wildlife and the potential for interspecies variation create challenges for establishing specific size recommendations. However, on the basis of data for domestic animals, manual cervical dislocation without the use of tools may be appropriate for birds < 3 kg, rodents < 200 g, and rabbits < 1 kg.[365] A secondary method such as decapitation or exsanguination should be employed to ensure death when feasible.

*Thoracic compression*—Thoracic compression may be useful in rare circumstances in animals that are deeply anesthetized or otherwise unconscious, or as a final, confirmatory method to ensure death when the animal's status is uncertain.[273]

S7.6.3.4 Unacceptable methods

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

### S7.6.4 Embryos, Fetuses, and Neonates

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

## S7.7 FREE-RANGING MARINE MAMMALS

Selecting a method of euthanasia for free-ranging marine mammals can be a substantial challenge because of large body size, environmental constraints, and concerns for the safety of personnel. It can also be difficult to determine when stranded marine mammals are unconscious or dead.[394] Currently available euthanasia methods generally have significant limitations that fail to meet aesthetic or other conventional standards for euthanasia of marine mammals under field conditions, particularly for large animals. Nevertheless, the options available must be evaluated to identify the best option under a given set of circumstances. Further research is warranted to identify improved methods of euthanasia.

### S7.7.1 Acceptable Methods

S7.7.1.1 Noninhaled agents

*Injectable agents*—Overdoses of injectable anesthetics can be used to euthanize marine mammals under field conditions. Anesthetics that can be used alone or in combination include tiletamine-zolazepam, ketamine, xylazine, meperidine, fentanyl, midazolam, diazepam, butorphanol, acepromazine, barbiturates, and etorphine.[381,382,395,396] Intramuscular administration of anesthetics may be required to achieve restraint of conscious animals before personnel can safely perform euthanasia using injectable agents by an intravascular route. A clear understanding of species anatomy and use of sufficiently long needles are required to ensure that muscle, rather than fat, is the site of injection.

Injectable anesthetics may be administered by multiple routes. Mucocutaneous administration, via the blowhole, can be an effective method that maximizes personnel safety.[396] Intravenous administration can be rapid and reliable for small pinnipeds, small odontocetes, and sirinids. For larger animals, safe IV administration is generally limited to animals that are anesthetized or unconscious. In addition, drug dilution in large blood volumes of large odontocetes and mysticetes may limit the effectiveness of IV administered agents. Intraperitoneal administration can be effective for small marine mammals if sufficiently long needles are available to access the peritoneal cavity. However, delayed absorption may limit the efficacy of drugs administered via this route. Intracardiac administration is acceptable only in anesthetized, moribund, or unconscious animals. This approach requires special, strong, and long needles to ensure that the heart can be accessed.

Advantages of injectable anesthetics are that they act rapidly and personnel experienced with these methods are readily available. Their administration is logistically simple and aesthetically acceptable in small to medium-sized animals, and public safety is relatively easy to secure. However, voluntary peripheral vasoconstriction by cetaceans or hypovolemic shock may limit access to peripheral veins and fat layers must be bypassed for effective administration. Large quantities of drug may be required to effectively euthanize large animals, and administration of single types of agents, such as $\alpha_2$-adrenergic receptor agonists, can result in animals passing through aesthetically displeasing and potentially unsafe excitation phases of anesthesia. There is a risk of injury for personnel attempting to access veins if animals are not appropriately restrained, and personnel may also

face self-administration risks (especially for ultrapotent opioids). Environmental contamination and scavenger exposure are possible due to residues in the animal's remains.

### S7.7.2 Acceptable With Conditions Methods

S7.7.2.1 Physical methods

*Gunshot*—Gunshot is acceptable with conditions for euthanizing small marine mammals when injectable methods are not practical; conventional projectile ballistics are not recommended for use in large odontocetes or large mysticetes. References are available to assist in identifying appropriate anatomic landmarks and caliber of ballistics.[397–403]

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

*Manually applied blunt force trauma*—In situations where other options are not available, a concussive blow to the head may be an effective method of euthanasia for small juvenile marine mammals.[404] The advantages of properly applied manual blunt force trauma are that it results in rapid death, no special equipment is required, and there is limited potential for secondary toxicity for scavengers. However, the efficacy of manually applied blunt force trauma is highly dependent on knowledge and experience of the operator and it is aesthetically displeasing for personnel and observers.

*Implosive decerebration*—Decerebration of large mysticetes and odontocetes can be effectively accomplished through the detonation of properly placed, shaped, and dimensioned explosive charges.[405,406] Advantages of this technique include a rapid death, limited potential for exposure of scavengers to toxic residues, and protection of personnel from injury by tail flukes. Its efficacy, however, is highly dependent on the knowledge, skills, and experience of the operator; it is aesthetically displeasing; and personnel and bystanders must be sufficiently distant from the resulting explosion to avoid injury. If these conditions can be met, implosive decerebration is an acceptable method of euthanasia.

### S7.7.3 Adjunctive Methods

*Potassium chloride or succinylcholine*—While unacceptable as sole agents of euthanasia in awake animals, potassium chloride or succinylcholine may be used to ensure the death of animals that are anesthetized or unconscious. Saturated potassium chloride solutions can be mixed inexpensively in large volumes and can be administered IV or intracardially, with a low risk of secondary toxicity for scavengers when preferred methods of disposal of the remains (eg, deep burial, rendering) are not available.[381,382,407]

### S7.7.4 Unacceptable Methods

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

*Exsanguination*—Exsanguination is inappropriate as a sole method of euthanasia because it requires an excessively long time to death, is believed to produce anxiety associated with extreme hypovolemia, and is aesthetically displeasing to bystanders. It can, however, be used as an adjunctive method to ensure the death of unconscious animals.[402]

## S8 Footnotes

a. Mays J. Euthanasia certification (slide presentation). Natl Anim Control Assoc Euthanasia Certification Workshop, Dayton, Ohio, September 2011.

b. Walsh JL. *Evaluation of methods for on-farm euthanasia of commercial meat rabbits*. MS thesis, Department of Pathobiology, University of Guelph, Guelph, ON, Canada, 2016.

c. Channon HA, Walker PJ, Kerr MG, et al. Using a gas mixture of nitrous oxide and carbon dioxide during stunning provides only small improvements to pig welfare (abstr), in *Proceedings*. 10th Bienn Conf Australas Pig Sci Assoc 2005;13.

d. Telazol, Fort Dodge Animal Health, Overland Park, Kan.

## S9 References


1. Hart LA, Hart BL, Mader B. Humane euthanasia and companion animal death: caring for the animal, the client, and the veterinarian. *J Am Vet Med Assoc* 1990;197:1292–1299.
2. Nogueira Borden LJ, Adams CL, Bonnett BN, et al. Use of the measure of patient-centered communication to analyze euthanasia discussions in companion animal practice. *J Am Vet Med Assoc* 2010;237:1275–1287.
3. Lagoni L, Butler C. Facilitating companion animal death. *Compend Contin Educ Pract Vet* 1994;16:70–76.
4. Martin F, Ruby KL, Deking TM, et al. Factors associated with client, staff, and student satisfaction regarding small animal euthanasia procedures at a veterinary teaching hospital. *J Am Vet Med Assoc* 2004;224:1774–1779.
5. Reeve CL, Rogelberg SG, Spitzmuller C, et al. The caring-killing paradox: euthanasia-related strain among animal shelter workers. *J Appl Soc Psychol* 2005;35:119–143.
6. Rogelberg SG, Reeve CL, Spitzmüller C, et al. Impact of euthanasia rates, euthanasia practices, and human resource practices on employee turnover in animal shelters. *J Am Vet Med Assoc* 2007;230:713–719.
7. Rhoades RH. The euthanasia area. In: *The Humane Society of the United States euthanasia training manual*. Washington, DC: The Humane Society of the United States, 2002;21–30.
8. Carpenter JW. *Exotic animal formulary*. 3rd ed. St Louis: WB Saunders Co, 2005.
9. Wadham JJB, Townsend P, Morton DB. Intraperitoneal injection of sodium pentobarbitone as a method of euthanasia for rodents. *ANZCCART News* 1997;10:8.
10. Svendsen O, Kok L, Lauritzen B. Nociception after intra-

peritoneal injection of a sodium pentobarbitone formulation with and without lidocaine in rats quantified by expression of neuronal c-fos in the spinal cord—a preliminary study. *Lab Anim* 2007;41:197–203.
11. Ambrose N, Wadham J, Morton D. Refinement of euthanasia. In: Balls M, Zeller A-M, Halder ME, eds. *Progress in the reduction, refinement and replacement of animal experimentation*. Amsterdam: Elsevier, 2000;1159–1170.
12. Grier RL, Schaffer CB. Evaluation of intraperitoneal and intrahepatic administration of a euthanasia agent in animal shelter cats. *J Am Vet Med Assoc* 1990;197:1611–1615.
13. Hellebrekers LJ, Baumans V, Bertens APMG, et al. On the use of T61 for euthanasia of domestic and laboratory animals; an ethical evaluation. *Lab Anim* 1990;24:200–204.
14. Fakkema D. *Operational guide for animal care and control agencies: euthanasia by injection*. Denver: American Humane Association, 2010.
15. Rhoades RH. Selecting the injection site. In: *The Humane Society of the United States euthanasia training manual*. Washington, DC: The Humane Society of the United States, 2002;41–50.
16. Cooney KA. *In-home pet euthanasia techniques: the veterinarian's guide to helping pets and their families say goodbye in the comfort of home*. Loveland, Colo: Home to Heaven PC, 2011.
17. Longair JA, Finley GG, Laniel MA, et al. Guidelines for the euthanasia of domestic animals by firearms. *Can Vet J* 1991;32:724–726.
18. Hanyok PM. Guidelines for police officers when responding to emergency animal incidents. *Anim Welf Inf Center Bull* winter 2001–spring 2002;11(3–4). Available at: www.nal.usda.gov/awic/newsletters/v11n3/11n3hany.htm. Accessed Sep 12, 2011.
19. Dennis MB, Jr., Dong WK, Weisbrod KA, et al. Use of captive bolt as a method of euthanasia in larger laboratory animal species. *Lab Anim Sci* 1988;38:459–462.
20. Ramsay EC, Wetzel RW. Comparison of five regimens for oral administration of medication to induce sedation in dogs prior to euthanasia. *J Am Vet Med Assoc* 1998;213:240–242.
21. Wetzel RW, Ramsay EC. Comparison of four regimens for intraoral administration of medication to induce sedation in cats prior to euthanasia. *J Am Vet Med Assoc* 1998;213:243–245.
22. Rhoades RH. Pre-euthanasia anesthetic. In: *The Humane Society of the United States euthanasia training manual*. Washington, DC: The Humane Society of the United States, 2002;67–80.
23. Mellor DJ. Galloping colts, fetal feelings, and reassuring regulations: putting animal welfare science into practice. *J Vet Med Educ* 2010;37:94–100.
24. Leist KH, Grauwiler J. Fetal pathology in rats following uterine-vessel clamping on day 14 of gestation. *Teratology* 1974;10:55–67.
25. Rhoades RH. Understanding euthanasia. In: *The Humane Society of the United States euthanasia training manual*. Washington, DC: The Humane Society of the United States, 2002;1–10.
26. Rhoades RH. Physical restraint. In: *The Humane Society of the United States euthanasia training manual*. Washington, DC: The Humane Society of the United States, 2002;51–66.
27. Arnold M, Langhans W. Effects of anesthesia and blood sampling techniques on plasma metabolites and corticosterone in the rat. *Physiol Behav* 2010;99:592–598.
28. Grieves JL, Dick EJ, Schlabritz-Loutsevich NE, et al. Barbiturate euthanasia solution-induced tissue artifact in nonhuman primates. *J Med Primatol* 2008;37:154–161.
29. Traslavina RP, King EJ, Loar AS, et al. Euthanasia by $CO_2$ inhalation affects potassium levels in mice. *J Am Assoc Lab Anim Sci* 2010;49:316–322.
30. Faupel RP, Seitz HJ, Tarnowski W, et al. The problem of tissue sampling from experimental animals with respect to freezing technique, anoxia, stress and narcosis. A new method for sampling rat liver tissue and the physiological values of glycolytic intermediates and related compounds. *Arch Biochem Biophys* 1972;148:509–522.
31. Boivin GP, Bottomley MA, Schiml PA, et al. Physiologic, behavioral, and histologic responses to various euthanasia methods in C57BL/6NTac male mice. *J Am Assoc Lab Anim Sci* 2017;56:69–78.
32. Boivin GP, Hickman DL, Creamer-Hente MA, et al. Review of $CO_2$ as a euthanasia agent for laboratory rats and mice. *J Am Assoc Lab Anim Sci* 2017;56:491–499.
33. Castelhano-Carlos MJ, Baumans V. The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Lab Anim* 2009;43:311–327.
34. Crawley J. Aggression. In: *What's wrong with my mouse?* 2nd ed. New York: Wiley & Sons, 2007;213–217.
35. Balcombe JP, Barnard ND, Sandusky C, et al. Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci* 2004;43:42–51.
36. Sharp J, Zammit T, Azar T, et al. Are "by-stander" female Sprague-Dawley rats affected by experimental procedures? *Contemp Top Lab Anim Sci* 2003;42:19–27.
37. Sharp J, Zammit T, Azar T, et al. Stress-like responses to common procedures in individually and group-housed female rats. *Contemp Top Lab Anim Sci* 2003;42:9–18.
38. Sharp J, Zammit T, Azar T, et al. Does witnessing experimental procedures produce stress in male rats? *Contemp Top Lab Anim Sci* 2002;41(5):8–12.
39. Sharp JL, Zammit TG, Azar T, et al. Stress-like responses to common procedures in male rats housed alone or with other rats. *Contemp Top Lab Anim Sci* 2002;41(4):8–14.
40. Daev EV, Vorob'ev KV, Zimina SA. Olfactory stress and modification of phagocytosis in peripheral blood cells of adult male mice [in Russian]. *Tsitologiia* 2001;43:954–960.
41. Moynihan JA, Karp JD, Cohen N, et al. Immune deviation following stress odor exposure: role of endogenous opioids. *J Neuroimmunol* 2000;102:145–153.
42. Baines MG, Haddad EK, Pomerantz DK, et al. Effects of sensory stimuli on the incidence of fetal resorption in a murine model of spontaneous abortion: the presence of an alien male and postimplantation embryo survival. *J Reprod Fertil* 1994;102:221–228.
43. Moynihan JA, Karp JD, Cohen N, et al. Alterations in interleukin-4 and antibody production following pheromone exposure: role of glucocorticoids. *J Neuroimmunol* 1994;54:51–58.
44. Stevens DA, Gerzog-Thomas DA. Fright reactions in rats to conspecific tissue. *Physiol Behav* 1977;18:47–51.
45. Stevens DA, Saplikoski NJ. Rats' reactions to conspecific muscle and blood—evidence for an alarm substance. *Behav Biol* 1973;8:75–82.
46. Garnett N. PHS policy on humane care and use of laboratory animals clarification regarding use of carbon dioxide for euthanasia of small laboratory animals. Release date: July 17, 2002. Available at: grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html. Accessed Dec 14, 2010.
47. Khoo SY, Lay BPP, Joya J, et al. Local anaesthetic refinement of pentobarbital euthanasia reduces abdominal writhing without affecting immunohistochemical endpoints in rats. *Lab Anim* 2018;52:152–162.
48. Dutton JW, Artwohl JE, Huang X, et al. Assessment of pain associated with the injection of sodium pentobarbital in laboratory mice (*Mus musculus*). *J Am Assoc Lab Anim Sci* 2019;58:373–379.
49. Vaupel DB, McCoun D, Cone EJ. Phencyclidine analogs and precursors: rotarod and lethal dose studies in the mouse. *J Pharmacol Exp Ther* 1984;230:20–27.
50. Constantinides C, Mean R, Janssen BJ. Effects of isoflurane anesthesia on the cardiovascular function of the C57BL/6 mouse. *ILAR J* 2011;52:e21–e31.
51. Brunson DB. Pharmacology of inhalation anesthetics. In: Kohn DF, Wixson SK, White WJ, et al, eds. *Anesthesia and analgesia in laboratory animals*. San Diego: Academic Press, 1997;32–33.

52. Makowska LJ, Weary DM. Rat aversion to induction with inhaled anaesthetics. *Appl Anim Behav Sci* 2009;119:229–235.
53. Gaertner DJ, Hallman TM, Hankenson FC, et al. Anesthesia and analgesia for laboratory rodents. In: Fish RE, Brown MJ, Danneman PJ, et al, eds. *Anesthesia and analgesia in laboratory animals*. New York: Elsevier, 2008;278.
54. Marquardt N, Feja M, Hunigen H, et al. Euthanasia of laboratory mice: are isoflurane and sevoflurane real alternatives to carbon dioxide? *PLoS One* 2018;13:e0203793.
55. Seymour TL, Nagamine CM. Evaluation of isoflurane overdose for euthanasia of neonatal mice. *J Am Assoc Lab Anim Sci* 2016;55:321–323.
56. Valentim AM, Guedes SR, Pereira AM, et al. Euthanasia using gaseous agents in laboratory rodents. *Lab Anim* 2016;50:241–253.
57. Hickman DL, Johnson SW. Evaluation of the aesthetics of physical methods of euthanasia of anesthetized rats. *J Am Assoc Lab Anim Sci* 2011;50:695–701.
58. Valentine H, Williams WO, Maurer KJ. Sedation or inhalant anesthesia before euthanasia with $CO_2$ does not reduce behavioral or physiologic signs of pain and stress in mice. *J Am Assoc Lab Anim Sci* 2012;51:50–57.
59. Hornett TD, Haynes AP. Comparison of carbon dioxide/air mixture and nitrogen/air mixture for the euthanasia of rodents: design of a system for inhalation euthanasia. *Anim Technol* 1984;35:93–99.
60. Hewett TA, Kovacs MS, Artwohl JE, et al. A comparison of euthanasia methods in rats, using carbon dioxide in prefilled and fixed flow-rate filled chambers. *Lab Anim Sci* 1993;43:579–582.
61. Powell K, Ethun K, Taylor DK. The effect of light level, $CO_2$ flow rate, and anesthesia on the stress response of mice during $CO_2$ euthanasia. *Lab Anim (NY)* 2016;45:386–395.
62. Boivin GP, Bottomley MA, Dudley ES, et al. Physiological, behavioral, and histological responses of male C57BL/6N mice to different $CO_2$ chamber replacement rates. *J Am Assoc Lab Anim Sci* 2016;55:451–461.
63. Hickman DL, Fitz SD, Bernabe CS, et al. Evaluation of low versus high volume per minute displacement $CO_2$ methods of euthanasia in the induction and duration of panic-associated behavior and physiology. *Animals (Basel)* 2016;6:45.
64. Chisholm JM, Pang DS. Assessment of carbon dioxide, carbon dioxide/oxygen, isoflurane and pentobarbital killing methods in adult female Sprague-Dawley rats. *PLoS One* 2016;11:e0162639.
65. Danneman PJ, Stein S, Walshaw SO. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. *Lab Anim Sci* 1997;47:376–385.
66. Weary DM, Makowska IJ. Rat aversion to carbon monoxide. *Appl Anim Behav Sci* 2009;121:148–151.
67. Ramsay DS, Watson CH, Leroux BG, et al. Conditioned place aversion and self-administration of nitrous oxide in rats. *Pharmacol Biochem Behav* 2003;74:623–633.
68. Duke T, Caulkett NA, Tataryn JM. The effect of nitrous oxide on halothane, isoflurane and sevoflurane requirements in ventilated dogs undergoing ovariohysterectomy. *Vet Anaesth Analg* 2006;33:343–350.
69. Thomas AA, Flecknell PA, Golledge HD. Combining nitrous oxide with carbon dioxide decreases the time to loss of consciousness during euthanasia in mice—refinement of animal welfare? *PLoS One* 2012;7:e32290.
70. Meyer RE, Fish R. Pharmacology of injectable anesthetics, sedatives, and tranquilizers. In: Fish RE, Danneman PJ, Brown M, et al, eds. *Anesthesia and analgesia of laboratory animals*. 2nd ed. San Diego: Academic Press, 2008;27–82.
71. Wixson SK, Smiler KL. Anesthesia and analgesia in rodents. In: Kohn SJ, Wixson SK, White WJ, et al, eds. *Anesthesia and analgesia in laboratory animals*. San Diego: Academic Press Inc, 1997;165–200.
72. Lord R. Use of ethanol for euthanasia of mice. *Aust Vet J* 1989;66:268.
73. Lord R. Humane killing. *Nature* 1991;350:456.
74. Allen-Worthington KH, Brice AK, Marx JO, et al. Intraperitoneal injection of ethanol for the euthanasia of laboratory mice (*Mus musculus*) and rats (*Rattus norvegicus*). *J Am Assoc Lab Anim Sci* 2015;54:769–778.
75. de Souza Dyer C, Brice AK, Marx JO. Intraperitoneal administration of ethanol as a means of euthanasia for neonatal mice (*Mus musculus*). *J Am Assoc Lab Anim Sci* 2017;56:299–306.
76. Cartner SC, Barlow SC, Ness TJ. Loss of cortical function in mice after decapitation, cervical dislocation, potassium chloride injection, and $CO_2$ inhalation. *Comp Med* 2007;57:570–573.
77. Vanderwolf CH, Buzak DP, Cain RK, et al. Neocortical and hippocampal electrical activity following decapitation in the rat. *Brain Res* 1988;451:340–344.
78. Mikeska JA, Klemm WR. EEG evaluation of humaneness of asphyxia and decapitation euthanasia of the laboratory rat. *Lab Anim Sci* 1975;25:175–179.
79. Fagin KD, Shinsako J, Dallman MF. Effects of housing and chronic cannulation on plasma ACTH and corticosterone in the rat. *Am J Physiol* 1983;245:E515–E520.
80. Boivin GP, Bottomley MA, Grobe N. Responses of male C57BL/6N mice to observing the euthanasia of other mice. *J Am Assoc Lab Anim Sci* 2016;55:406–411.
81. Burkholder TH, Niel L, Weed JL, et al. Comparison of carbon dioxide and argon euthanasia: effects on behavior, heart rate, and respiratory lesions in rats. *J Am Assoc Lab Anim Sci* 2010;49:448–453.
82. Sharp J, Azar T, Lawson D. Comparison of carbon dioxide, argon, and nitrogen for inducing unconsciousness or euthanasia of rats. *J Am Assoc Lab Anim Sci* 2006;45:21–25.
83. Gent TC, Detotto C, Vyssotski AL, et al. Epileptiform activity during inert gas euthanasia of mice. *PLoS One* 2018;13:e0195872.
84. Detotto C, Isler S, Wehrle M, et al. Nitrogen gas produces less behavioural and neurophysiological excitation than carbon dioxide in mice undergoing euthanasia. *PLoS One* 2019;14:e0210818.
85. Fitzgerald M. The development of nociceptive circuits. *Nat Rev Neurosci* 2005;6:507.
86. Biran V, Verney C, Ferriero DM. Perinatal cerebellar injury in human and animal models. *Neurol Res Int* 2012;2012:858929.
87. Silverman J, Hendricks G. Sensory neuron development in mouse coccygeal vertebrae and its relationship to tail biopsies for genotyping. *PLoS One* 2014;9:e88158.
88. Cunningham MG, McKay RD. A hypothermic miniaturized stereotaxic instrument for surgery in newborn rats. *J Neurosci Methods* 1993;47:105–114.
89. Danneman PJ, Mandrell TD. Evaluation of five agents/methods for anesthesia of neonatal rats. *Lab Anim Sci* 1997;47:386–395.
90. Phifer CB, Terry LM. Use of hypothermia for general anesthesia in preweanling rodents. *Physiol Behav* 1986;38:887–890.
91. Mellor DJ, Diesch TJ, Gunn AJ, et al. The importance of 'awareness' for understanding fetal pain. *Brain Res Brain Res Rev* 2005;49:455–471.
92. Muñoz-Mediavilla C, Cámara JA, Salazar S, et al. Evaluation of the foetal time to death in mice after application of direct and indirect euthanasia methods. *Lab Anim* 2016;50:100–107.
93. Pritchett K, Corrow D, Stockwell J, et al. Euthanasia of neonatal mice with carbon dioxide. *Comp Med* 2005;55:275–281.
94. Pritchett-Corning KR. Euthanasia of neonatal rats with carbon dioxide. *J Am Assoc Lab Anim Sci* 2009;48:23–27.
95. Close B, Banister K, Baumans V, et al. Recommendations for euthanasia of experimental animals: part 2. DGXT of the European Commission. *Lab Anim* 1997;31:1–32.
96. Diesch TJ, Mellor DJ, Johnson CB, et al. Electroencephalographic responses to tail clamping in anaesthetised rat pups. *Lab Anim* 2009;43:224–231.
97. Mellor DJ, Diesch TJ, Johnson CB. When do mammalian young become sentient? *ALTEX* 2010;27(suppl 1):281–286.

98. Vogler G. Anesthesia and analgesia. In: Suckow MA, Weisbroth SH, Franklin CL, eds. *The laboratory rat*. 2nd ed. San Diego: Academic Press, 2006;658.
99. Flecknell PA, Roughan JV, Hedenqvist P. Induction of anaesthesia with sevoflurane and isoflurane in the rabbit. *Lab Anim* 1999;33:41–46.
100. Flecknell PA. *Laboratory animal anaesthesia*. 2nd ed. San Diego: Elsevier Academic Press, 1996;168–171.
101. Hedenqvist P, Roughan JV, Antunes L, et al. Induction of anaesthesia with desflurane and isoflurane in the rabbit. *Lab Anim* 2001;35:172–179.
102. Hayward JS, Lisson PA. Carbon dioxide tolerance of rabbits and its relation to burrow fumigation. *Aust Wildl Res* 1978;5:253–261.
103. Hayward JS. Abnormal concentrations of respiratory gases in rabbit burrows. *J Mammal* 1966;47:723–724.
104. Dalmau A, Pallisera J, Pedernera C, et al. Use of high concentrations of carbon dioxide for stunning rabbits reared for meat production. *World Rabbit Sci* 2016;24:25–37.
105. Walsh JL, Percival A, Turner PV. Efficacy of blunt force trauma, a novel mechanical cervical dislocation device, and a non-penetrating captive bolt device for on-farm euthanasia of pre-weaned kits, growers, and adult commercial meat rabbits. *Animals (Basel)* 2017;7:100.
106. Schütt-Abraham I, Knauer-Kraetzl B, Wormuth HJ. Observations during captive bolt stunning of rabbits. *Berl Munch Tierarztl Wochenschr* 1992;105:10–15.
107. Walsh J, Percival A, Tapscott B, et al. On-farm euthanasia practices and attitudes of commercial meat rabbit producers. *Vet Rec* 2017;181:292.
108. Wilson JM, Bunte RM, Carty AJ. Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). *J Am Assoc Lab Anim Sci* 2009;48:785–789.
109. Collymore C, Banks KE, Turner PV. Lidocaine hydrochloride compared with MS222 for the euthanasia of zebrafish (*Danio rerio*). *J Am Assoc Lab Anim Sci* 2016;55:816–820.
110. Blessing JJ, Marshal JC, Balcombe SR. Humane killing of fishes for scientific research: a comparison of two methods. *J Fish Biol* 2010;76:2571–2577.
111. Varga ZM, Matthews M, Trevarrow B, et al. *Hypothermic shock is a reliable and rapid euthanasia method for zebrafish*. Final report to OLAW on euthanasia of zebrafish. Bethesda, Md: Office of Laboratory Animal Welfare, National Institutes of Health, 2008.
112. Köhler A, Collymore C, Finger-Baier K, et al. Report of Workshop on Euthanasia for Zebrafish—a matter of welfare and science. *Zebrafish* 2017;14:547–551.
113. Strykowski JL, Schech JM. Effectiveness of recommended euthanasia methods in larval zebrafish (*Danio rerio*). *J Am Assoc Lab Anim Sci* 2015;54:81–84.
114. Wallace CK, Bright LA, Marx JO, et al. Effectiveness of rapid cooling as a method of euthanasia for young zebrafish (*Danio rerio*). *J Am Assoc Lab Anim Sci* 2018;57:58–63.
115. National Institutes of Health. *Guidelines for use of zebrafish in the NIH intramural research program*. Bethesda, Md: National Institutes of Health, 2009. Available at: oacu.od.nih.gov/arac/documents/Zebrafish.pdf. Accessed Nov 25, 2010.
116. Torreilles SL, McClure DE, Green SL. Evaluation and refinement of euthanasia methods for *Xenopus laevis*. *J Am Assoc Lab Anim Sci* 2009;48:512–516.
117. Grandin T. Objective scoring of animal handling and stunning practices at slaughter plants. *J Am Vet Med Assoc* 1998;212:36–39.
118. Grandin T. Effect of animal welfare audits of slaughter plants by a major fast food company on cattle handling and stunning practices. *J Am Vet Med Assoc* 2000;216:848–851.
119. Grandin T. Euthanasia and slaughter of livestock. *J Am Vet Med Assoc* 1994;204:1354–1360.
120. Grandin T. Pig behavior studies applied to slaughter-plant design. *Appl Anim Ethol* 1982;9:141–151.
121. Grandin T. Observations of cattle behavior applied to design of cattle handling facilities. *Appl Anim Ethol* 1980;6:19–31.
122. Thurmon JC. Euthanasia of food animals. *Vet Clin North Am Food Anim Pract* 1986;2:743–756.
123. Fulwider WK, Grandin T, Rollin BE, et al. Survey of management practices on one hundred and thirteen north central and northeastern United States dairies. *J Dairy Sci* 2008;91:1686–1692.
124. Humane Slaughter Association. *Humane killing of livestock using firearms: guidance notes #3*. 2nd ed. Wheathampstead, England: Humane Slaughter Association, 2005.
125. Woods J, Shearer JK, Hill J. Recommended on-farm euthanasia practices. In: Grandin T, ed. *Improving animal welfare: a practical approach*. Wallingford, England: CABI Publishing, 2010.
126. Baker HJ, Scrimgeour HJ. Evaluation of methods for the euthanasia of cattle in a foreign animal disease outbreak. *Can Vet J* 1995;36:160–165.
127. Finnie IW. Traumatic head injury in ruminant livestock. *Aust Vet J* 1997;75:204–208.
128. Finnie JW, Manavis J, Summersides GE, et al. Brain damage in pigs produced by impact with a non-penetrating captive bolt pistol. *Aust Vet J* 2003;81:153–155.
129. Grandin T. Return-to-sensibility problems after penetrating captive bolt stunning of cattle in commercial beef slaughter plants. *J Am Vet Med Assoc* 2002;221:1258–1261.
130. Blackmore DK. Energy requirements for the penetration of heads of domestic stock and the development of a multiple projectile. *Vet Rec* 1985;116:36–40.
131. Daly CC, Whittington PE. Investigation into the principal determinants of effective captive bolt stunning of sheep. *Res Vet Sci* 1989;46:406–408.
132. Daly CC. Recent developments in captive bolt stunning. In: *Humane slaughter of animals for food*. Potters Bar, England: Universities Federation for Animal Welfare, 1986;15–20.
133. Gregory N, Shaw F. Penetrating captive bolt stunning and exsanguination of cattle in abattoirs. *J Appl Anim Welf Sci* 2000;3:215–230.
134. Grandin T. Maintenance of good animal welfare standards in beef slaughter plants by use of auditing programs. *J Am Vet Med Assoc* 2005;226:370–373.
135. Gibson TJ, Rebelo CB, Gowers TA, et al. Electroencephalographic assessment of concussive non-penetrative captive bolt stunning of turkeys. *Br Poult Sci* 2018;59:13–20.
136. World Organisation for Animal Health (OIE). Chapter 7.6: killing of animals for disease control purposes. In: *Terrestrial animal health code*. 20th ed. Paris: OIE, 2011. Available at: www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.7.6.htm. Accessed May 16, 2011.
137. National Farm Animal Care Council. Code of Practice for the Care and Handling of Bison. 2017. Available at: www.nfacc.ca/codes-of-practice/bison. Accessed Nov 11, 2019.
138. Schwenk BK, Lechner I, Ross SG, et al. Magnetic resonance imaging and computer tomography of brain lesions in water buffaloes and cattle stunned with handguns or captive bolts. *Meat Sci* 2016;113:35–40.
139. Gregory NG, Spence JY, Mason CW, et al. Effectiveness of poll stunning water buffalo with captive bolt guns. *Meat Sci* 2009;81:178–182.
140. Gregory NG, Wotton SB. Time to loss of brain responsiveness following exsanguination in calves. *Res Vet Sci* 1984;37:141–143.
141. Schulze W, Schultze-Petzold H, Hazem AS, et al. Experiments on the objective assessment of pain and consciousness in slaughtering sheep and calves by the conventional method (humane killer stunning) and by ritual slaughtering laws (shechita). *Dtsch Tierarztl Wochenschr* 1978;85:62–66.
142. Blackmore DK. Differences in behavior between sheep and cattle during slaughter. *Res Vet Sci* 1984;37:223–226.
143. Bager F, Devine CE, Gilbert KV. Jugular blood flow in calves after head-only electrical stunning and throat-cutting. *Meat Sci* 1988;22:237–243.
144. Daly CC, Kallweit E, Ellendorf F. Cortical function in cattle during slaughter: conventional captive bolt stunning fol-

lowed by exsanguination compared with shechita slaughter. *Vet Rec* 1988;122:325–329.
145. Newhook JC, Blackmore DK. Electroencephalographic studies of stunning and slaughter of sheep and calves: part 1—the onset of permanent insensibility in sheep during slaughter. *Meat Sci* 1982;6:221–233.
146. Gregory NG, Fielding HR, von Wenzlawowicz M, et al. Time to collapse following slaughter without stunning in cattle. *Meat Sci* 2010;85:66–69.
147. Rosen SD. Physiological insights into shechita. *Vet Rec* 2004;154:759–765.
148. Gregory NG. Physiology of stress, distress, stunning and slaughter. In: *Animal welfare and meat science*. Wallingford, England: CABI Publishing, 1998;64–92.
149. Gibson TJ, Johnson CB, Murrell JC, et al. Components of electroencephalographic responses to slaughter in halothane-anesthetized calves: effects of cutting neck tissues compared with major blood vessels. *N Z Vet J* 2009;57:84–89.
150. Mellor DJ, Gibson TJ, Johnson CB. A re-evaluation of the need to stun calves prior to slaughter by ventral-neck incision: an introductory review. *N Z Vet J* 2009;57:74–76.
151. Leach TM, Wilkins LJ. Observations on the physiological effects of pithing cattle at slaughter. *Meat Sci* 1985;15:101–106.
152. Daly CC, Whittington PE. Concussive methods of pre-slaughter stunning in sheep: effects of captive bolt stunning in the poll position on brain function. *Res Vet Sci* 1986;41:353–355.
153. Withrock IC. The use of carbon dioxide (CO2) as an alternative euthanasia method for goat kids. Available at: search.proquest.com/docview/1733971790/abstract/C0605E819BD543A6PQ/1. Accessed Nov 11, 2019.
154. Gibson TJ, Whitehead C, Taylor R, et al. Pathophysiology of penetrating captive bolt stunning in Alpacas (*Vicugna pacos*). *Meat Sci* 2015;100:227–231.
155. Plummer PJ, Shearer JK, Kleinhenz KE, et al. Determination of anatomic landmarks for optimal placement in captive-bolt euthanasia of goats. *Am J Vet Res* 2018;79:276–281.
156. AVMA. AVMA guidelines for the humane slaughter of animals: 2016 edition. Available at: www.avma.org/KB/Resources/Reference/AnimalWelfare/Documents/Humane-Slaughter-Guidelines.pdf.
157. Collins SL, Caldwell M, Hecht S, et al. Comparison of penetrating and nonpenetrating captive bolt methods in horned goats. *Am J Vet Res* 2017;78:151–157.
158. Evers AS, Crowder CM, Balser JR. General anesthetics. In: Brunton LL, Lazo JS, Parker KL, eds. *Goodman and Gillman's the pharmacological basis of therapeutics*. 11th ed. New York: McGraw-Hill Medical Publishing Division, 2006;362.
159. Limon G, Guitian J, Gregory NG. A note on the slaughter of llamas in Bolivia by the puntilla method. *Meat Sci* 2009;82:405–406.
160. Limon G, Guitian J, Gregory NG. An evaluation of the humaneness of puntilla in cattle. *Meat Sci* 2010;84:352–355.
161. Finnie JW, Blumbergs PC, Manavis J, et al. Evaluation of brain damage resulting from penetrating and non-penetrating captive bolt stunning using lambs. *Aust Vet J* 2000;78:775–778.
162. Finnie JW, Manavis J, Blumberg PC, et al. Brain damage in sheep from penetrating captive bolt stunning. *Aust Vet J* 2002;80:67–69.
163. Grist A, Lines JA, Knowles TG, et al. The use of a mechanical non-penetrating captive bolt device for the euthanasia of neonate lambs. *Animals (Basel)* 2018;8:49.
164. Sutherland MA, Watson TJ, Johnson CB, et al. Evaluation of the efficacy of a non-penetrating captive bolt to euthanase neonatal goats up to 48 hours of age. *Anim Welf* 2016;25:471–479.
165. Grist A, Lines JA, Knowles TG, et al. Use of a non-penetrating captive bolt for euthanasia of neonate goats. *Animals (Basel)* 2018;8:58.
166. Chapter OIE. 7.5.5: management of fetuses during slaughter of pregnant animals. In: *Terrestrial animal health code*. 17th ed. Paris: OIE, 2008;284.
167. Jochems CE, van der Valk JB, Stafleu FR, et al. The use of fetal bovine serum: ethical or scientific problem? *Altern Lab Anim* 2002;30:219–227.
168. Yan EB, Barburamani AA, Walker AM, et al. Changes in cerebral blood flow, cerebral metabolites, and breathing movements in the sheep fetus following asphyxia produced by occlusion of the umbilical cord. *Am J Physiol Regul Integr Comp Physiol* 2009;297:R60–R69.
169. Mellor DJ. Integration of perinatal events, pathophysiological changes and consequences for the newborn lamb. *Br Vet J* 1988;144:552–569.
170. Peisker N, Preissel AK, Rechenbach HD, et al. Foetal stress responses to euthanasia of pregnant sheep. *Berl Munch Tierarztl Wochenschr* 2010;123:2–10.
171. Klaunberg BA, O'Malley J, Clark T, et al. Euthanasia of mouse fetuses and neonates. *Contemp Top Lab Anim Sci* 2004;43:29–34.
172. Küchenmeister U, Kuhn G, Ender K. Preslaughter handling of pigs and the effect on heart rate, meat quality, including tenderness, and sarcoplasmic reticulum Ca2+ transport. *Meat Sci* 2005;71:690–695.
173. Küchenmeister U, Kuhn G, Stabenow B, et al. The effect of experimental stress on sarcoplasmic reticulum Ca2+ transport and meat quality in pig muscle. *Meat Sci* 2002;61:375–380.
174. Geverink NA, Schouten WGP, Gort G, et al. Individual differences in behavioral and physiological responses to restraint stress in pigs. *Physiol Behav* 2002;77:451–457.
175. Magnusson U, Wattrang E, Tsuma V, et al. Effects of stress resulting from short-term restraint on in vitro functional capacity of leukocytes obtained from pigs. *Am J Vet Res* 1998;59:421–425.
176. Neubert E, Gurtler H, Vallentin G. Effect of restraining growth pigs with snare restraints on plasma levels of catecholamines, cortisol, insulin and metabolic parameters. *Berl Munch Tierarztl Wochenschr* 1996;109:409–413.
177. Roozen AW, Magnusson U. Effects of short-term restraint stress on leukocyte counts, lymphocyte proliferation and lysis of erythrocytes in gilts. *Zentralbl Veterinarmed B* 1996;43:505–511.
178. Roozen AWM, Tsuma VT, Magnusson U. Effects of short-term restraint stress on plasma concentrations of catecholamines, β-endorphin, and cortisol in gilts. *Am J Vet Res* 1995;56:1225–1227.
179. Farmer C, Dubreuil P, Couture Y, et al. Hormonal changes following an acute stress in control and somatostatin-immunized pigs. *Domest Anim Endocrinol* 1991;8:527–536.
180. Muir W. *Handbook of veterinary anesthesia*. 3rd ed. St Louis: Mosby, 2000.
181. Maisch A, Ritzmann M, Heinritzi K. The humane euthanasia of pigs with pentobarbital. *Tierarztl Umsch* 2005;60:679–683.
182. National Pork Board, American Association of Swine Practitioners. *On-farm euthanasia of swine*. 2nd edition. Des Moines, Iowa: National Pork Board, 2009.
183. Althen TG, Ono K, Topel DG. Effect of stress susceptibility or stunning method on catecholamine levels in swine. *J Anim Sci* 1977;44:985–989.
184. Humane Slaughter Association. *Captive bolt stunning of livestock: guidance notes No. 2*. 4th ed. Wheathampstead, England: Humane Slaughter Association, 2006.
185. Van der Wal PP. Stunning, sticking and exsanguination as stress factors in pigs, in *Proceedings*. 2nd Int Symp Cond Meat Qual Pigs 1971;153–158.
186. Anil MH, McKinstry JL. Reflexes and loss of sensibility following head-to-back electrical stunning in sheep. *Vet Rec* 1991;128:106–107.
187. Blackmore DK, Newhook JC. Insensibility during slaughter of pigs in comparison to other domestic stock. *N Z Vet J* 1981;29:219–222.
188. McKinstry JL, Anil MH. The effect of repeat application of electrical stunning on the welfare of pigs. *Meat Sci* 2004;67:121–128.
189. Humane Slaughter Association. *Electrical stunning of red*

*meat animals*: *guidance notes No. 4*. Wheathampstead, England: Humane Slaughter Association, 2000;1–22.
190. Anil MH, McKinstry JL. Variations in electrical stunning tong placements and relative consequences in slaughter pigs. *Vet J* 1998;155:85–90.
191. Anil MH, McKinstry JL. The effectiveness of high frequency electrical stunning in pigs. *Meat Sci* 1992;31:481–491.
192. Lambooij B, Merkus GSM, VonVoorst N, et al. Effect of a low voltage with a high frequency electrical stunning on unconsciousness in slaughter pigs. *Fleischwirtschaft (Frankf)* 1996;76:1327–1328.
193. Lambooij E. Stunning of animals on the farm. *Tijdschr Diergeneeskd* 1994;119:264–266.
194. Troeger K, Woltersdorf W. Electrical stunning and meat quality in the pig. *Fleischwirtschaft (Frankf)* 1990;70:901–904.
195. Wotton SB, Gregory NG. Pig slaughtering procedures: time to loss of brain responsiveness after exsanguination of cardiac arrest. *Res Vet Sci* 1986;40:148–151.
196. Hoenderken R. Electrical stunning of pigs. In: Fabiansson S, ed. *Hearing on pre-slaughter stunning (report No. 52)*. Kavlinge, Sweden: Swedish Meat Research Centre, 1978;29–38.
197. Denicourt M, Klopfenstein C, Dufour C, et al. Using an electrical approach to euthanize pigs on-farm: fundamental principles to know, in *Proceedings*. 41st Annu Meet Am Assoc Swine Vet 2010;451–468.
198. Raj ABM, Gregory NG. Welfare implications of the gas stunning of pigs: 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. *Anim Welf* 1995;4:273–280.
199. Raj AB. Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. *Vet Rec* 1999;144:165–168.
200. Raj AB, Johnson SP, Wotton SB, et al. Welfare implications of gas stunning pigs: 3. the time to loss of somatosensory evoked potentials and spontaneous electrocorticogram of pigs during exposure to gases. *Vet J* 1997;153:329–339.
201. Raj ABM, Gregory NG. Welfare implications of the gas stunning of pigs: 2. Stress of induction of anaesthesia. *Anim Welf* 1996;5:71–78.
202. Troeger K, Woltersdorf W. Gas anesthesia of slaughter pigs. 1. Stunning experiments under laboratory conditions with fat pigs of known halothane reaction type—meat quality, animal protection. *Fleischwirtschaft (Frankf)* 1991;72:1063–1068.
203. Martoft L, Lomholt L, Kolthoff C, et al. Effects of $CO_2$ anaesthesia on central nervous system activity in swine. *Lab Anim* 2002;36:115–126.
204. Forslid A. Transient neocortical, hippocampal, and amygdaloid EEG silence induced by one minute inhalation of high $CO_2$ concentration in swine. *Acta Physiol Scand* 1987;130:1–10.
205. Jongman EC, Barnett JL, Hemsworth PH. The aversiveness of carbon dioxide stunning in pigs and a comparison of the $CO_2$ stunner crate vs the V-restrainer. *Appl Anim Behav Sci* 2000;67:67–76.
206. Velarde A, Cruz J, Gispert M, et al. Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. *Anim Welf* 2007;16:513–522.
207. Nowak B, Mueffling TV, Caspari K, et al. Validation of a method for the detection of virulent *Yersinia enterocolitica* and their distribution in slaughter pigs from conventional and alternative housing systems. *Vet Microbiol* 2006;117:219–228.
208. Channon HA, Payne AM, Warner RD. Halothane genotype, pre-slaughter handling and stunning method all influence pork quality. *Meat Sci* 2000;56:291–299.
209. Forslid A. Muscle spasms during pre-slaughter CO2 anaesthesia in pigs. Ethical considerations. *Fleischwirtschaft (Frankf)* 1992;72:167–168.
210. Forslid A, Augustinsson O. Acidosis, hypoxia and stress hormone release in response to one-minute inhalation of 80% $CO_2$ in swine. *Acta Physiol Scand* 1988;132:223–231.
211. Gregory NG, Moss BW, Leeson RH. An assessment of carbon dioxide stunning in pigs. *Vet Rec* 1987;121:517–518.
212. Overstreet JW, Marple DN, Huffman DL, et al. Effect of stunning methods on porcine muscle glycolysis. *J Anim Sci* 1975;41:1014–1020.
213. Meyer RE, Morrow WEM. Carbon dioxide for emergency on-farm euthanasia of swine. *J Swine Health Prod* 2005;13:210–217.
214. Sadler LJ, Karriker LA, Schwartz KJ, et al. Are severely depressed suckling pigs resistant to gas euthanasia? *Anim Welf* 2014;23:145–155.
215. Widowski T. *Effectiveness of a non-penetrating captive bolt for on-farm euthanasia of low viability piglets*. Des Moines, Iowa: National Pork Board, 2008.
216. Whiting TL, Steele GG, Wamnes S, et al. Evaluation of methods of rapid mass killing of segregated early weaned piglets. *Can Vet J* 2011;52:753–758.
217. Grist A, Lines JA, Knowles T, et al. The use of a non-penetrating captive bolt for the euthanasia of neonate piglets. *Animals (Basel)* 2018;8:48.
218. Casey-Trott TM, Millman ST, Turner PV, et al. Effectiveness of a nonpenetrating captive bolt for euthanasia of 3 kg to 9 kg pigs. *J Anim Sci* 2014;92:5166–5174.
219. Webster AB, Fletcher DL, Savage SI. Humane on-farm killing of spent hens. *J Appl Poult Res* 1996;5:191–200.
220. United Egg Producers. Animal husbandry guidelines for US egg-laying flocks. 2010 edition. Alpharetta, Ga: United Egg Producers, 2010. Available at: www.unitedegg.org/information/pdf/UEP_2010_Animal_Welfare_Guidelines.pdf. Accessed Aug 13, 2012.
221. Jaksch W. Euthanasia of day-old male chicks in the poultry industry. *Int J Study Anim Probl* 1981;2:203–213.
222. Gurung S, White D, Archer G, et al. Evaluation of alternative euthanasia methods of neonatal chickens. *Animals (Basel)* 2018;8:37.
223. Blackshaw JK, Fenwick DC, Beattie AW, et al. The behavior of chickens, mice and rats during euthanasia with chloroform, carbon dioxide and ether. *Lab Anim* 1988;22:67–75.
224. Latimer KS, Rakich PM. Necropsy examination. In: Ritchie BW, Harrison GJ, Harrison LR, eds. *Avian medicine: principles and application*. Lake Worth, Fla: Wingers Publishing Inc, 1994;355–379.
225. Webster AB, Fletcher DL. Reactions of laying hens and broilers to different gases used for stunning poultry. *Poult Sci* 2001;80:1371–1377.
226. Lambooij E, Gerritzen MA, Engel B, et al. Behavioural responses during exposure of broiler chickens to different gas mixtures. *Appl Anim Behav Sci* 1999;62:255–265.
227. Gerritzen MA, Lambooij E, Stegeman JA, et al. Slaughter of poultry during the epidemic of avian influenza in the Netherlands in 2003. *Vet Rec* 2006;159:39–42.
228. Mohan Raj AB, Wotton SB, Gregory NG. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of hens during stunning with a carbon dioxide and argon mixture. *Br Vet J* 1992;148:147–156.
229. Webster AB, Collett SR. A mobile modified-atmosphere killing system for small-flock depopulation. *J Appl Poult Res* 2012;21:131–144.
230. Raj ABM, Whittington PE. Euthanasia of day-old chicks with carbon dioxide and argon. *Vet Rec* 1995;136:292–294.
231. Poole GH, Fletcher DL. A comparison of argon, carbon dioxide, and nitrogen in a broiler killing system. *Poult Sci* 1995;74:1218–1223.
232. McKeegan DEF, McIntyre JA, Demmers TGM, et al. Physiological and behavioural responses of broilers to controlled atmosphere stunning: implications for welfare. *Anim Welf* 2007;16:409–426.
233. Coenen AML, Lankhaar J, Lowe JC, et al. Remote monitoring of electroencephalogram, electrocardiogram, and behavior during controlled atmosphere stunning in broilers: implications for welfare. *Poult Sci* 2009;88:10–19.
234. McKeegan DEF, Sandercock DA, Gerritzen MA. Physiological responses to low atmospheric pressure stunning and the implications for welfare. *Poult Sci* 2013;92:858–868.
235. Erasmus MA, Lawlis P, Duncan IJ, et al. Using time to insensi-

bility and estimated time of death to evaluate a nonpenetrating captive bolt, cervical dislocation, and blunt trauma for on-farm killing of turkeys. *Poult Sci* 2010;89:1345–1354.
236. Erasmus MA, Turner PV, Niekamp SG, et al. Brain and skull lesions resulting from use of percussive bolt, cervical dislocation by stretching, cervical dislocation by crushing and blunt trauma in turkeys. *Vet Rec* 2010;167:850–858.
237. Erasmus MA, Turner PV, Widowski TM. Measures of insensibility used to determine effective stunning and killing of poultry. *J Appl Poult Res* 2010;19:288–298.
238. Gregory NG, Wotton SB. Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. *Vet Rec* 1990;126:570–572.
239. Martin JE, Sandercock DA, Sandilands V, et al. Welfare risk of repeated application of on-farm killing methods for poultry. *Animals (Basel)* 2018;8:E39.
240. Bader S, Meyer-Kühling B, Güntheret R, et al. Anatomical and histologic pathology induced by cervical dislocation following blunt head trauma for on-farm euthanasia of poultry. *J Appl Poult Res* 2014;23:546–556.
241. Cors JC, Gruber AD, Günther R, et al. Electroencephalographic evaluation of the effectiveness of blunt trauma to induce loss of consciousness for on-farm killing of chickens and turkeys. *Poult Sci* 2015;94:147–155.
242. Martin JE, McKeegan DEG, Sparrey J, et al. Comparison of novel mechanical cervical dislocation and a modified captive bolt for on-farm killing of poultry on behavioural reflex responses and anatomical pathology. *Anim Welf* 2016;25:227–241.
243. Woolcott CR, Torrey S, Turner PV, et al. Evaluation of two models of non-penetrating captive bolt devices for on-farm euthanasia of turkeys. *Animals (Basel)* 2018;8:E42.
244. Orosz S. Birds. In: *Guidelines for euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;46–49.
245. American Association of Avian Pathologists (AAAP) Animal Welfare and Management Practices Committee. *Review of mechanical euthanasia of day-old poultry*. Athens, Ga: American Association of Avian Pathologists, 2005.
246. Federation of Animal Science Societies (FASS). *Guide for the care and use of agricultural animals in agricultural research and teaching*. Champaign, Ill: Federation of Animal Science Societies, 2010.
247. Agriculture Canada. *Recommended code of practice for the care and handling of poultry from hatchery to processing plant*. Publication 1757/E.1989. Ottawa: Agriculture Canada, 1989.
248. European Council. *European Council Directive 93/119/EC of 22 December 1993 on the protection of animals at the time of slaughter or killing. Annex G: killing of surplus chicks and embryos in hatchery waste*. Brussels: European Council, 1993.
249. Shearer JK, Nicoletti P. Anatomical landmarks. Available at: www.vetmed.iastate.edu/vdpam/extension/dairy/programs/humane-euthanasia/anatomical-landmarks. Accessed Jun 24, 2011.
250. Aleman M, Davis E, Williams DC, et al. Electrophysiologic study of a method of euthanasia using intrathecal lidocaine hydrochloride administered during intravenous anesthesia in horses. *J Vet Intern Med* 2015;29:1676–1682.
251. AVMA. AVMA guidelines on euthanasia. June 2007. Available at: www.avma.org/issues/animal_welfare/euthanasia.pdf. Accessed May 7, 2011.
252. Franson JC. Euthanasia. In: Friend M, Franson JC, eds. *Field manual of wildlife diseases. General field procedures and diseases of birds*. Biological Resources Division information and technology report 1999–001. Washington, DC: US Department of the Interior and US Geological Survey, 1999;49–53.
253. Mason C, Spence J, Bilbe L, et al. Methods for dispatching backyard poultry. *Vet Rec* 2009;164:220.
254. Rae M. Necropsy. In: *Clinical avian medicine*. Vol 2. Palm Beach, Fla: Spix Publishing Inc, 2006;661–678.
255. Hess L. Euthanasia techniques in birds—roundtable discussion. *J Avian Med Surg* 2005;19:242–245.
256. Gaunt AS, Oring LW. *Guidelines to the use of wild birds in research*. Washington, DC: The Ornithological Council, 1997.
257. Dawson MD, Johnson KJ, Benson ER, et al. Determining cessation of brain activity during depopulation or euthanasia of broilers using accelerometers. *J Appl Poult Res* 2009;18:135–142.
258. Raj M, O'Callaghan M, Thompson K, et al. Large scale killing of poultry species on farm during outbreaks of diseases: evaluation and development of a humane containerised gas killing system. *Worlds Poult Sci J* 2008;64:227–244.
259. Raj M. Humane killing of nonhuman animals for disease control purposes. *J Appl Anim Welf Sci* 2008;11:112–124.
260. Powell FL. Respiration. In: Whittow GC, ed. *Sturkie's avian physiology*. 5th ed. San Diego: Academic Press, 2000;233–264.
261. King AS, McLelland J. Respiratory system. In: King AS, McLelland J, eds. *Birds: their structure and function*. 2nd ed. Eastbourne, England: Bailliere Tindall, 1984;110–144.
262. Dumonceaux G, Harrison GJ. Toxins. In: Ritchie BW, Harrison GJ, Harrison LR, eds. *Avian medicine: principles and application*. Lake Worth, Fla: Wingers Publishing, 1994;1030–1052.
263. Scott KE, Bracchi LA, Lieberman MT, et al. Evaluation of best practices for the euthanasia of zebra finches (*Taeniopygia guttata*). *J Am Assoc Lab Anim Sci* 2017;56:802–806.
264. Gerritzen M, Lambooij B, Reimert H, et al. A note on behaviour of poultry exposed to increasing carbon dioxide concentrations. *Appl Anim Behav Sci* 2007;108:179–185.
265. Benson E, Malone GW, Alphin RL, et al. Foam-based mass emergency depopulation of floor-reared meat-type poultry operations. *Poult Sci* 2007;86:219–224.
266. Raj ABM. Aversive reactions to argon, carbon dioxide and a mixture of carbon dioxide and argon. *Vet Rec* 1996;138:592–593.
267. Raj ABM. Recent developments in stunning and slaughter of poultry. *Worlds Poult Sci J* 2006;62:462–484.
268. McKeegan DEF, McIntyre J, Demmers TGM, et al. Behavioural responses of broiler chickens during acute exposure to gaseous stimulation. *Appl Anim Behav Sci* 2006;99:271–286.
269. Raj ABM, Gregory NG, Wotton SB. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of hens during stunning in argon-induced anoxia. *Br Vet J* 1991;147:322–330.
270. Close B, Banister K, Baumans V, et al. Recommendations for euthanasia of experimental animals: part 1. DGXI of the European Commission. *Lab Anim* 1996;30:293–316.
271. Gregory NG, Wotton SB. Effect of slaughter on the spontaneous and evoked activity of the brain. *Br Poult Sci* 1986;27:195–205.
272. Borski RJ, Hodson RG. Fish research and the institutional animal care and use committee. *ILAR J* 2003;44:286–294.
273. Paul-Murphy JR, Engilis A Jr, Pascoe PJ, et al. Comparison of intraosseous pentobarbital administration and thoracic compression for euthanasia of anesthetized sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*). *Am J Vet Res* 2017;78:887–899.
274. Clifford DH. Preanesthesia, anesthesia, analgesia, and euthanasia. In: Fox JG, Cohen BJ, Loew FM, eds. *Laboratory animal medicine*. New York: Academic Press Inc, 1984;528–563.
275. Dyakonova VE. Role of opioid peptides in behavior of invertebrates. *J Evol Biochem Physiol* 2001;37:335–347.
276. Rose JD. The neurobehavioral nature of fishes and the question of awareness and pain. *Rev Fish Sci* 2002;10:1–38.
277. Nordgreen J, Horsberg TE, Ranheim B, et al. Somatosensory evoked potentials in the telencephalon of Atlantic salmon (*Salmo salar*) following galvanic stimulation of the tail. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2007;193:1235–1242.

278. Dunlop R, Laming P. Mechanoreceptive and nociceptive responses in the central nervous system of goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*). *J Pain* 2005;6:561–568.
279. Elwood RW, Appel M. Pain experience in hermit crabs? *Anim Behav* 2009;77:1243–1246.
280. Barr S, Laming PR, Dick JTA, et al. Nociception or pain in a decapod crustacean? *Anim Behav* 2008;75:745–751.
281. Ashley PJ, Sneddon LU, McCrohan CR. Nociception in fish: stimulus response properties of receptors on the head of trout *Oncorhynchus mykiss*. *Brain Res* 2007;1166:47–54.
282. Braithwaite VA, Boulcott P. Pain perception, aversion and fear in fish. *Dis Aquat Organ* 2007;75:131–138.
283. Alvarez FA, Rodriguez-Martin I, Gonzalez-Nuñez V, et al. New kappa opioid receptor from zebrafish *Danio rerio*. *Neurosci Lett* 2006;405:94–99.
284. Sneddon LU. Trigeminal somatosensory innervation of the head of a teleost fish with particular reference to nociception. *Brain Res* 2003;972:44–52.
285. Buatti MC, Pasternak GW. Multiple opiate receptors: phylogenetic differences. *Brain Res* 1981;218:400–405.
286. Finger TE. Fish that taste with their feet: spinal sensory pathways in the sea robin, *Prionotus carolinus*. *Biol Bull* 1981;161:154–161.
287. Schulman JA, Finger TE, Brecha NC, et al. Enkephalin immunoreactivity in Golgi cells and mossy fibres of mammalian, avian and teleost cerebellum. *Neuroscience* 1981;6:2407–2416.
288. AVMA. AVMA guidelines for the depopulation of animals: 2019 edition. Available at: www.avma.org/KB/Policies/documents/AVMA-Guidelines-for-the-Depopulation-of-Animals.pdf. Accessed Nov 11, 2019.
289. Jepson J. A linguistic analysis of discourse on the killing of nonhuman animals. *Soc Anim* 2008;16:127–148.
290. Yanong RPE, Hartman KH, Watson CA, et al. *Fish slaughter, killing, and euthanasia: a review of major published US guidance documents and general considerations of methods*. Publication #CIR1525. Gainesville, Fla: Fisheries and Aquatic Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, 2007. Available at: edis.ifas.ufl.edu/fa150. Accessed May 16, 2011.
291. Hartman KH. Fish. In: *Guidelines for euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;28–38.
292. Håstein T, Scarfe AD, Lund VL. Science-based assessment of welfare: aquatic animals. *Rev Sci Tech* 2005;24:529–547.
293. Burns R. Considerations in the euthanasia of reptiles, fish and amphibians, in *Proceedings*. Am Assoc Zoo Vet Wildl Dis Assoc Am Assoc Wildl Vet Joint Conf 1995;243–249.
294. Zwart P, de Vries HR, Cooper JE. The humane killing of fishes, amphibia, reptiles and birds. *Tijdschr Diergeneeskd* 1989;114:557–565 [[in Dutch]].
295. Brown LA. Anesthesia and restraint. In: Stoskopf MK, ed. *Fish medicine*. Philadelphia: WB Saunders, 1993;79–90.
296. Roberts HE. Anesthesia, analgesia and euthanasia. In: Roberts HE, ed. *Fundamentals of ornamental fish health*. Ames, Iowa: Blackwell, 2010;166–171.
297. Saint-Erne N. Anesthesia. In: *Advanced koi care*. 2nd ed. Glendale, Ariz: Erne Enterprises, 2010;50–52.
298. Neiffer DL, Stamper MA. Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. *ILAR J* 2009;50:343–360.
299. Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes. *Recommendations concerning farmed fish*. Strasbourg, France: European Convention for the Protection of Animals Kept for Farming Purposes, 2006.
300. Stetter MD. Fish and amphibian anesthesia. *Vet Clin North Am Exot Anim Pract* 2001;4:69–82.
301. US FDA Center for Veterinary Medicine. *Enforcement priorities for drug use in aquaculture*. Silver Spring, Md: US FDA, 2011. Available at: www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/PoliciesProceduresManual/UC M046931.pdf. Accessed Jan 10, 2011.
302. Ross LG, Ross B. *Anaesthetic and sedative techniques for aquatic animals*. 3rd ed. Oxford, England: Blackwell, 2008.
303. Rombough PJ. Ontogenetic changes in the toxicity and efficacy of the anaesthetic MS222 (tricaine methanesulfonate) in zebrafish (*Danio rerio*) larvae. *Comp Biochem Physiol A Mol Integr Physiol* 2007;148:463–469.
304. Canadian Council on Animal Care. *Guidelines on: the care and use of fish in research, teaching and testing*. Ottawa: Canadian Council on Animal Care, 2005. Available at: www.ccac.ca/Documents/Standards/Guidelines/Fish.pdf. Accessed Dec 19, 2010.
305. Readman GD, Owen SF, Knowles TG. Species specific anaesthetics for fish anaesthesia and euthanasia. *Sci Rep* 2017;7:7102.
306. Wong D, von Keyserlingk MAG, Richards JG, et al. Conditioned place avoidance of zebrafish (*Danio rerio*) to three chemicals used for euthanasia and anaesthesia. *PLoS One* 2014;9:e88030.
307. Balko JA, Oda A, Posner LP. Use of tricaine methanesulfonate or propofol for immersion euthanasia of goldfish (*Carassius auratus*). *J Am Vet Med Assoc* 2018;252:1555–1561.
308. Harms C. Anesthesia in fish. In: Fowler ME, Miller RE, eds. *Zoo and wild animal medicine: current therapy 4*. Philadelphia: WB Saunders Co, 1999;158–163.
309. Deitrich RA, Dunwiddie TV, Harris RA, et al. Mechanism of action of ethanol: initial central nervous system actions. *Pharmacol Rev* 1989;41:489–537.
310. Peng J, Wagle M, Mueller T, et al. Ethanol-modulated camouflage response screen in zebrafish uncovers a novel role for camp and extracellular signal-regulated kinase signaling in behavioral sensitivity to ethanol. *J Neurosci* 2009;29:8408–8418.
311. Dlugos CA, Rabin RA. Ethanol effects on three strains of zebrafish: model system for genetic investigations. *Pharmacol Biochem Behav* 2003;74:471–480.
312. Gerlai R, Lahav M, Guo S, et al. Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacol Biochem Behav* 2000;67:773–782.
313. Gladden JN, Brainard BM, Shelton JL, et al. Evaluation of isoeugenol for anesthesia in koi carp (*Cyprinus carpio*). *Am J Vet Res* 2010;71:859–866.
314. Holloway A, Keene JL, Noakes DG, et al. Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Aquacult Res* 2004;35:1025–1030.
315. Lewbart GA. Fish. In: Carpenter JW, ed. *Exotic animal formulary*. 3rd ed. St Louis: Elsevier Saunders, 2005;5–29.
316. National Toxicology Program. *NTP technical report on the toxicology and carcinogenesis studies of isoeugenol (CAS No. 97–54–1) in F344/N rats and B6C3F1 mice (gavage studies)*. NTP TR 551. NIH publication No. 08–5892. Washington, DC: US Department of Health and Human Services, 2008. Available at: ntp.niehs.nih.gov/files/TR551board_web.pdf. Accessed May 16, 2011.
317. Sladky KK, Swanson CR, Stoskopf MK, et al. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). *Am J Vet Res* 2001;62:337–342.
318. Brodin P, Roed A. Effects of eugenol on rat phrenic nerve and phrenic-diaphragm preparations. *Arch Oral Biol* 1984;29:611–615.
319. Ingvast-Larsson JC, Axén VC, Kiessling AK. Effects of isoeugenol on in vitro neuromuscular blockade of rat phrenic nerve-diaphragm preparations. *Am J Vet Res* 2003;64:690–693.
320. FDA. *Concerns related to the use of clove oil as an anesthetic for fish*. Guidance for industry 150. Washington, DC: Department of Health and Human Services, 2007. Available at: www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052520.pdf. Accessed Jan 20, 2011.

321. Noga EJ. Pharmacopoeia. In: *Fish disease: diagnosis and treatment*. 2nd ed. Ames, Iowa: Wiley-Blackwell, 2010;375–420.
322. Davie PS, Kopf RK. Physiology, behaviour and welfare of fish during recreational fishing and after release. *N Z Vet J* 2006;54:161–172.
323. Van De Vis H, Kestin S, Robb D, et al. Is humane slaughter of fish possible for industry? *Aquacult Res* 2003;34:211–220.
324. Animal Procedures Committee. *Report of the Animal Procedures Committee for 2009*. London: The Stationery Office, 2010;27.
325. Murray MJ. Euthanasia. In: Lewbart GA, ed. *Invertebrate medicine*. Ames, Iowa: Blackwell, 2006;303–304.
326. Murray MJ. Invertebrates. In: *Guidelines for the euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;25–27.
327. Messenger JB, Nixon M, Ryan KP. Magnesium chloride as an anaesthetic for cephalopods. *Comp Biochem Physiol C* 1985;82:203–205.
328. Butler-Struben HM, Brophy SM, Johnson NA, et al. *In vivo* recording of neural and behavioral correlates of anesthesia induction, reversal, and euthanasia in cephalopod molluscs. *Front Physiol* 2018;9:109.
329. Ross LG, Ross B. Anaesthesia of aquatic invertebrates. In: *Anaesthetic and sedative techniques for aquatic animals*. 3rd ed. Oxford, England: Wiley Blackwell, 2008;167–176.
330. Andrews PLR, Darmaillacq AS, Dennison N, et al. The identification and management of pain, suffering and distress in cephalopods, including anesthesia, analgesia and humane killing. *J Exp Mar Biol Ecol* 2013;447:46–64.
331. Pugliese C, Mazza R, Andrews PLR, et al. Effect of different formulations of magnesium chloride used as anesthetic agents on the performance of the isolated heart of *Octopus vulgaris*. *Front Physiol* 2016;7:610.
332. Waterstrat PR, Pinkham L. Evaluation of eugenol as an anesthetic for the American lobster *Homarus americanus*. *J World Aquacult Soc* 2005;36:420–424.
333. Gunkel C, Lewbart GA. Invertebrates. In: West G, Heard D, Caulkett N, eds. *Zoo animal and wildlife immobilization and anesthesia*. Ames, Iowa: Blackwell, 2007;147–158.
334. Gleadall IG. The effects of prospective anaesthetic substances on cephalopods: summary of original data and a brief review of studies over the last two decades. *J Exp Mar Biol Ecol* 2013;447:23–30.
335. Gilbertson CR, Wyatt JD. Evaluation of euthanasia techniques for an invertebrate species, land snails (*Succinea putris*). *J Am Assoc Lab Anim Sci* 2016;55:577–581.
336. Reilly JS, ed. *Euthanasia of animals used for scientific purposes*. Adelaide, SA, Australia: Australia and New Zealand Council for the Care of Animals in Research and Teaching, Department of Environmental Biology, Adelaide University, 2001.
337. American Association of Zoo Veterinarians (AAZV). *Guidelines for the euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006.
338. Canadian Council on Animal Care. Guidelines on: the care and use of wildlife. Ottawa: Canadian Council on Animal Care, 2003. Available at: ccac.ca/Documents/Standards/Guidelines/Wildlife.pdf. Accessed Jul 2, 2011.
339. Fowler M. *Restraint and handling of wild and domestic animals*. 3rd ed. Ames, Iowa: Wiley-Blackwell, 2008.
340. West G, Heard D, Caulkett N. *Zoo Animal & wildlife immobilization and anesthesia*. Ames, Iowa: Blackwell, 2007.
341. Kreeger TJ, Arnemo J. *Handbook of wildlife chemical immobilization*. International ed. Fort Collins, Colo: Wildlife Pharmaceuticals Inc, 2002.
342. Clark RK, Jessup DA. *Wildlife restraint series*. Fort Collins, Colo: International Wildlife Veterinary Services, 1992.
343. Platnick NI. American Museum of Natural History research sites. The world spider catalog, version 13.0. Available at: research.amnh.org/entomology/spiders/catalog/index.html. Accessed Aug 14, 2012.
344. Ruppert E, Fox R, Barnes R. *Invertebrate zoology: a functional evolutionary approach*. 7th ed. Thomson Learning, 2007.
345. Murray MJ. Euthanasia. In: Lewbart GA, ed. *Invertebrate medicine*. 2nd ed. Ames, Iowa: Wiley-Blackwell, 2011;441–444.
346. Braun ME, Heatley JJ, Chitty J. Clinical techniques of invertebrates. *Vet Clin North Am Exot Anim Pract* 2006;9:205–221.
347. Cooper JE. Anesthesia, analgesia and euthanasia of invertebrates. *ILAR J* 2011;52:196–204.
348. Gunkel C, Lewbart GA. Anesthesia and analgesia of invertebrates. In: Fish R, Danneman P, Brown M, et al, eds. *Anesthesia and analgesia in laboratory animals*. 2nd ed. San Diego: Academic Press, 2008;535–546.
349. Lewbart GA, ed. *Invertebrate medicine*. Oxford, England: Blackwell, 2006.
350. Pizzi R. Spiders. In: Lewbart GA, ed. *Invertebrate medicine*. Ames, Iowa: Blackwell, 2006;143–168.
351. Pizzi R, Cooper JE, George S. Spider health, husbandry, and welfare in zoological collections, in *Proceedings*. Br Vet Zool Soc Conf Stand Welf Conserv Zoo Exot Pract 2002;54–59.
352. Baier J. Amphibians. In: *Guidelines for the euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;39–41.
353. Burns R, McMahan B. Euthanasia methods for ectothermic vertebrates. In: Bonagura JD, ed. *Continuing veterinary therapy XII*. Philadelphia: WB Saunders Co, 1995;1379–1381.
354. Baier J. Reptiles. In: *Guidelines for the euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;42–45.
355. Universities Federation for Animal Welfare. *Humane killing of animals*. 4th ed. South Mimms, Potters Bar, England: Universities Federation for Animal Welfare, 1988;16–22.
356. Cooper JE, Ewbank R, Platt C, et al. *Euthanasia of amphibians and reptiles*. London: Universities Federation for Animal Welfare and World Society for the Protection of Animals, 1989.
357. Mader DR. Euthanasia. In: Mader DR, ed. *Reptile medicine and surgery*. St Louis: Saunders/Elsevier, 2006;564–568.
358. Gentz EJ. Medicine and surgery of amphibians. *ILAR J* 2007;48:255–259.
359. National Research Committee on Pain and Distress in Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. *Recognition and alleviation of pain and distress in laboratory animals*. Washington, DC: National Academy Press, 1992.
360. Andrews EJ, Bennet BT, Clark JD, et al. 1993 report of the AVMA Panel on Euthanasia. *J Am Vet Med Assoc* 1993;202:229–249.
361. Heard DJ. Principles and techniques of anesthesia and analgesia for exotic practice. *Vet Clin North Am Small Anim Pract* 1993;23:1301–1327.
362. Conroy CJ, Papenfuss T, Parker J, et al. Use of tricaine methanesulfonate (MS-222) for euthanasia of reptiles. *J Am Assoc Lab Anim Sci* 2009;48:28–32.
363. Harrell L. Handling euthanasia in production facilities. In: Schaeffer DO, Kleinow KM, Krulisch L, eds. *The care and use of amphibians, reptiles and fish in research*. Bethesda, Md: Scientists Center for Animal Welfare, 1992;129.
364. Letcher J. Intracelomic use of tricaine methane sulfonate for anesthesia of bullfrogs (*Rana catesbeiana*) and leopard frogs (*Rana pipiens*). *Zoo Biol* 1992;11:243–251.
365. Canadian Council on Animal Care. *CCAC guidelines on: euthanasia of animals used in science*. Ottawa: Canadian Council on Animal Care, 2010. Available at: www.ccac.ca/Documents/Standards/Guidelines/Euthanasia.pdf. Accessed Jul 2, 2011.
366. Breazile JE, Kitchell RL. Euthanasia for laboratory animals. *Fed Proc* 1969;28:1577–1579.
367. Stoskopf MK. Anesthesia. In: Brown LA, ed. *Aquaculture for veterinarians: fish husbandry and medicine*. Oxford, England: Pergamon Press, 1993;161–167.
368. Storey KB. Life in a frozen state: adaptive strategies for natu-

ral freeze tolerance in amphibians and reptiles. *Am J Physiol* 1990;258:R559–R568.
369. Brannian RE, Kirk E, Williams D. Anesthetic induction of kinosternid turtles with halothane. *J Zoo Anim Med* 1987;18:115–117.
370. Jackson OF, Cooper JE. Anesthesia and surgery. In: Cooper JE, Jackson OF, eds. *Diseases of the reptilia*. Vol 2. New York: Academic Press Inc, 1981;535–549.
371. Calderwood HW. Anesthesia for reptiles. *J Am Vet Med Assoc* 1971;159:1618–1625.
372. Moberly WR. The metabolic responses of the common iguana, *Iguana iguana*, to walking and diving. *Comp Biochem Physiol* 1968;27:21–32.
373. Johlin JM, Moreland FB. Studies of the blood picture of the turtle after complete anoxia. *J Biol Chem* 1933;103:107–114.
374. Nevarez JG, Strain GM, da Cunha AF, et al. Evaluation of four methods for inducing death during slaughter of American alligators (*Alligator mississippiensis*). *Am J Vet Res* 2014;75:536–543.
375. Storey KB, Storey JM. Natural freezing survival in animals. *Annu Rev Ecol Syst* 1996;27:365–386.
376. Machin KL. Amphibian pain and analgesia. *J Zoo Wildl Med* 1999;30:2–10.
377. Stevens CW, Pezalla PD. Endogenous opioid system downregulation during hibernation in amphibians. *Brain Res* 1989;494:227–231.
378. Martin BJ. Evaluation of hypothermia for anesthesia in reptiles and amphibians. *ILAR J* 1995;37:186–190.
379. Suckow MA, Terril LA, Grigdesby CF, et al. Evaluation of hypothermia-induced analgesia and influence of opioid antagonists in Leopard frogs (*Rana pipiens*). *Pharmacol Biochem Behav* 1999;63:39–43.
380. Schaffer DO. Anesthesia and analgesia in nontraditional laboratory animal species. In: Kohn DF, Wixson SK, White WJ, et al. eds. *Anesthesia and analgesia in laboratory animals*. San Diego: Academic Press, 1997;337–378.
381. Harms CA, Greer LL, Whaley J, et al. Euthanasia. In: Gulland FMD, Dierauf LA, eds. *Marine mammal medicine*. 3rd ed. Boca Raton, Fla: CRC Press Inc, 2018;675–691.
382. Harms CA, McLellan WA, Moore MJ, et al. Low-residue euthanasia of stranded mysticetes. *J Wildl Dis* 2014;50:63–73.
383. Lockwood G. Theoretical context-sensitive elimination times for inhalational anaesthetics. *Br J Anaesth* 2010;104:648–655.
384. Drew ML. Wildlife issues. In: *Guidelines for the euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;19–22.
385. Schwartz JA, Warren RJ, Henderson DW, et al. Captive and field tests of a method for immobilization and euthanasia of urban deer. *Wildl Soc Bull* 1997;25:532–541.
386. Hyman J. Euthanasia in marine mammals. In: Dierauf LA, ed. *Handbook of marine mammal medicine: health, disease and rehabilitation*. Boca Raton, Fla: CRC Press Inc, 1990;265–266.
387. Needham DJ. Cetacean strandings. In: Fowler ME, ed. *Zoo and wild animal medicine: current therapy 3*. 3rd ed. Philadelphia: WB Saunders Co, 1993;415–425.
388. Gullett PA. Euthanasia. In: Friend M, ed. *Field guide to wildlife diseases. Volume 1: general field procedures and diseases of migratory birds*. Resource publication #167. Washington, DC: US Department of the Interior, Fish and Wildlife Service, 1987;59–63.
389. Fair JM. *Guidelines for the use of wild birds in research*. 3rd ed. Washington, DC: The Ornithological Council, 2010.
390. Sikes RS, Gannon WL, Animal Care and Use Committee of the American Society of Mammalogists. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J Mammal* 2011;92:235–253.
391. Herpetological Animal Care and Use Committee. *Guidelines for use of live amphibians and reptiles in field and laboratory research*. Miami: American Society of Ichthyologists and Herpetologists, 2004.
392. Orlans FB. *Field research guidelines: impact on animal care and use committees*. Bethesda, Md: Scientists Center for Animal Welfare, 1988.
393. McClure DN, Anderson N. Rodents and small mammals. In: *Guidelines for euthanasia of nondomestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006; 61–65.
394. Brakes P, Butterworth A, Donoghue M. *Investigating criteria for insensibility and death in firearms for euthanising stranded cetaceans in New Zealand*. IWC/58/WKM&AWI 9. Agenda item 5.2.4. Impington, England: International Whaling Commission, 2006.
395. Moore M, Walsh M, Bailey J, et al. Sedation at sea of entangled North Atlantic right whales (*Eubalaena glacialis*) to enhance disentanglement. *PLoS One* 2010;5:e9597.
396. Dunn JL. Multiple-agent euthanasia of a juvenile fin whale, *Balanoptera physalus*. *Mar Mamm Sci* 2006;22:1004–1007.
397. Hampton JO, Mawson PR, Coughran DK, et al. Validation of the use of firearms for euthanising stranded cetaceans. *J Cetacean Res Manag* 2014;14:117–123.
398. Øen EO, Knudsen SK. Euthanasia of whales: the effect of. 375 and. 458 calibre round-nosed, full metal-jacketed rifle bullets on the central nervous system of common minke whales. *J Cetacean Res Manag* 2007;9:81–88.
399. Donoghue M. *IWC 58: workshop on whale killing methods and associated welfare issues cetaceans in New Zealand*. IWC/58/WKM&AWI 10. Agenda item 4.4. Impington, England: International Whaling Commission, 2006.
400. Lawrence K. Euthanasia of stranded whales. *Vet Rec* 2003;153:540.
401. Bonner WN. Killing methods. In: Laws RM, ed. *Antarctic seals: research methods and techniques*. Cambridge, England: Cambridge University Press, 1993;150–160.
402. Sweeney JC. What practitioners should know about whale strandings. In: Kirk RW, ed. *Kirk's current veterinary therapy 10*. Philadelphia: WB Saunders Co, 1989;721–727.
403. Blackmore DK, Madie P, Bowling MC, et al. The use of a shotgun for emergency slaughter of stranded cetaceans. *N Z Vet J* 1995;43:158–159.
404. Daoust PY, Crook A, Bollinger TK, et al. Animal welfare and the harp seal hunt in Atlantic Canada. *Can Vet J* 2002;43:687–694.
405. Coughran D, Stiles I, Fuller PJ. Euthanasia of beached humpback whales using explosives. *J Cetacean Res Manag* 2012;12:137–144.
406. International Whaling Commission. *Report of the workshop on welfare issues associated with the entanglement of large whales*. IWC/62/15. Agenda item 5.2.1. Impington, England: International Whaling Commission, 2010.
407. Daoust PY, Ortenburger AI. Successful euthanasia of a juvenile fin whale. *Can Vet J* 2001;42:127–129.

# Glossary

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

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

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

**Affect**: The external expression of emotion.

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

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

**Animal**: Any nonhuman animal (Kingdom: Animalia).

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

**Avian**: Relating to birds.

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

**Chick:** A young bird.

**Cremation**: To incinerate a dead body. See INCINERATION.

**Depopulation**: The rapid destruction of a population of animals in response to urgent circumstances with as much consideration given to the welfare of the animals as practicable.

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

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

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

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

**Exsanguination**: The action of draining an animal of blood.

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

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

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

**Finfish**: a term used to describe true fish as opposed to other non-fish aquatic animals such as the invertebrates "starfish" and "cuttlefish"

**Good Death**: see EUTHANASIA.

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

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

**Incineration**: To burn completely, to ashes.

**Insensible**: See UNCONSCIOUS.

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

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

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

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

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

**Poult**: A young fowl.

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

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

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

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

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

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

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

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

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

**Unconsciousness**: Unconsciousness, defined as loss of individual awareness. This occurs when the brain's ability to integrate information is blocked or disrupted. Onset of unconsciousness is associated with loss of the righting reflex. An unconscious animal is therefore recumbent and, by definition, unable to perceive pain; however, unconscious animals may respond to noxious stimulation with spinally mediated involuntary movements depending on the degree of CNS depression present.

**Wild**: A free-roaming animal of a nondomestic species.

# Appendix 1

Agents and methods of euthanasia by species.

| Species | Acceptable | Acceptable with conditions (for adjunctive methods, see text) |
|---|---|---|
| Aquatic invertebrates | S6.3: Immersion in anesthetic solution (magnesium salts, clove oil, eugenol, ethanol) | S6.3: Adjunctive methods (second step) include 70% alcohol and neutral-buffered 10% formalin, pithing, freezing, boiling |
| Amphibians | S7.3: As appropriate by species—Injected barbiturates, dissociative agents and anesthetics as specified, topical or injected buffered MS 222 or topical benzocaine hydrochloride | S7.3: As appropriate by species—Inhaled anesthetics as specified, $CO_2$, PCB or firearm, manually applied blunt force trauma to the head, rapid freezing of small (< 4 g [0.1 oz]) individuals where immediate death occurs |
| Avians (See also Poultry) | S5: IV barbiturates | S5: Inhaled anesthetics, $CO_2$, CO, $N_2$, Ar, cervical dislocation (small birds and poultry), decapitation (small birds)<br>S7.6: Gunshot (free-ranging birds) |
| Cats | S1: IV barbiturates, injected anesthetic overdose Tributame, T-61 | S1: Barbiturates (alternate routes of administration), inhaled anesthetic overdose, CO,* $CO_2$,* gunshot* |
| Cattle | S3.2: IV barbiturates | S3.2: Gunshot, PCB |
| Dogs | S1: IV barbiturates, injected anesthetic overdose Tributame, T-61 | S1: Barbiturates (alternate routes of administration), inhaled anesthetic overdose, CO,* $CO_2$,* gunshot,* PCB* |
| Fish | S6.2: Immersion in buffered benzocaine or benzocaine hydrochloride, isoflurane, sevoflurane, quinaldine sulfate, buffered MS 222, 2-phenoxyethanol, injected pentobarbital, rapid chilling (appropriate species), ethanol | S6.2: Eugenol, isoeugenol, clove oil, $CO_2$-saturated water, decapitation/cervical transection/manually applied blunt force trauma followed by pithing or exsanguination, maceration (research setting), captive bolt (large fish) |
| Equids | S4: IV barbiturates | S4: PCB, gunshot |
| Marine mammals | S7.5 (captive): Injected barbiturates<br>S7.7 (free ranging): Injected barbiturates or anesthetic overdose | S7.5 (captive): Inhaled anesthetics<br>S7.7 (free ranging): Gunshot, manually applied blunt force trauma, implosive decerebration |
| Nonhuman primates | S2.3, S7.4: Injected barbiturates or anesthetic overdose | S2.3, S7.4 (as appropriate by species): Inhaled anesthetic, CO, $CO_2$ |
| Poultry | S3.4: Injected barbiturates and anesthetic overdose | S3.4: $CO_2$, CO, $N_2$, Ar, low-atmospheric-pressure stunning, cervical dislocation (as anatomically appropriate), decapitation, manual blunt force trauma, electrocution, gunshot, captive bolt |
| Rabbits | S2.4: IV barbiturates | S2.4: Inhaled anesthetic overdose, $CO_2$, cervical dislocation (as anatomically appropriate), PCB, NPCB |
| Reptiles | S7.3: As appropriate by species—Injected barbiturates/MS 222, dissociative agents with adjunctive method and anesthetics as specified | S7.3: As appropriate by species–Inhaled anesthetics as specified, $CO_2$, PCB or firearm, manually applied blunt force trauma, rapid freezing for animals < 4 g where immediate death occurs, spinal cord severance/destruction of brain (crocodilians) |
| Rodents | S2.2: Injected barbiturates and barbiturate combinations, dissociative agent combinations | S2.2: Inhaled anesthetics, $CO_2$, CO, tribromoethanol, ethanol, cervical dislocation, decapitation, focused beam microwave irradiation |
| Small ruminants | S3.2: Injected barbiturates | S3.2: $CO_2$ (goat kids), Gunshot, PCB, NPCB (goat kids) |
| Swine | S3.3: Injected barbiturates | S3.3: $CO_2$, CO, NO, $N_2$, Ar, gunshot, electrocution, PCB, NPCB (piglets), manually applied blunt force trauma |

*Not recommended for routine use.

## Appendix 2

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

| Agent or method | Comments |
|---|---|
| Air embolism | Air embolism may be accompanied by convulsions, opisthotonos, and vocalization. If used, it should be done only in anesthetized animals. |
| Asphyxiation | Physically preventing respiration (smothering, strangulation, dewatering) is unacceptable. |
| Burning | Chemical or thermal burning of an animal is not an acceptable method of euthanasia. |
| Chloral hydrate | Unacceptable in dogs, cats, and small mammals. |
| Chloroform | Chloroform is a known hepatotoxin and suspected carcinogen and, therefore, is extremely hazardous to personnel. |
| Cyanide | Cyanide poses an extreme danger to personnel and the manner of death is aesthetically objectionable. |
| Decompression (excluding low atmospheric pressure stunning when it can be demonstrated that it achieves euthanasia) | Decompression is unacceptable for euthanasia because of numerous disadvantages. (1) Many chambers are designed to produce decompression at a rate 15–60 times as fast as the recommended optimum for animals, resulting in pain and distress attributable to expanding gases trapped in body cavities. (2) Immature animals are tolerant of hypoxia, and longer periods of decompression are required before respiration ceases. (3) Accidental recompression, with recovery of injured animals, can occur. (4) Bleeding, vomiting, convulsions, urination, and defecation, which are aesthetically unpleasant, may develop in unconscious animals. |
| Drowning | Drowning is not a means of euthanasia and is inhumane. |
| Exsanguination | Because of the anxiety associated with extreme hypovolemia, exsanguination as a sole method of killing should be used only on unconscious animals. |
| Formaldehyde | Direct immersion of an animal into formalin, as a means of euthanasia, is inhumane with the exception of Porifera. |
| Household products and solvents | Acetone, cleaning agents, quaternary compounds (including $CCl_4$), laxatives, pesticides, dimethylketone, quaternary ammonium products, antacids, and other toxicants not specifically designed for therapeutic or euthanasia use are not acceptable. |
| Hypothermia | Hypothermia is not an appropriate method of euthanasia. |
| Insulin | Insulin causes hypoglycemia, which can lead to considerable distress (behavior changes, irritability, disorientation) before onset of hypoglycemic seizures, which may or may not result in death. |
| Magnesium sulfate and potassium chloride | Unacceptable for use as euthanasia agents in conscious vertebrate animals. |
| Manually applied blunt force trauma to the head | Generally unacceptable for most species excluding piglets and small laboratory animals. Replace, as much as possible, manually applied blunt force trauma to the head with alternate methods. |
| Neuromuscular blocking agents (nicotine, magnesium sulfate, potassium chloride, and all curariform agents) | When used alone, these drugs all cause respiratory arrest before loss of consciousness, so the animal may perceive pain and distress after it is immobilized. |
| Rapid freezing | Rapid freezing as a sole means of euthanasia is not considered to be humane with the exception of small (< 4 g) reptiles, amphibians, and < 5-day-old rodent neonates where immediate death occurs. In all other cases animals should be rendered dead or unconscious prior to freezing. (Rapid chilling of fish is not considered to be rapid freezing). |
| Strychnine | Strychnine causes violent convulsions and painful muscle contractions. |
| Thoracic compression | Not acceptable for use on a conscious animal. |

## Appendix 3

Images for the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition (illustrations by Louis Clark, bio-graphix.com).

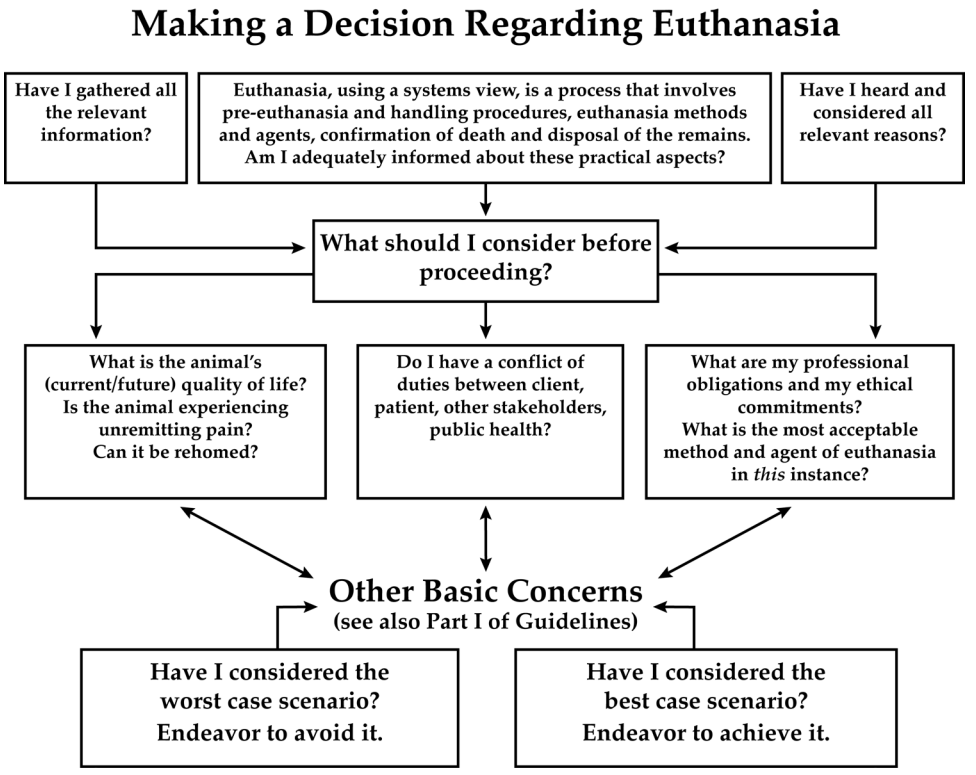


**Figure 1**—Veterinarians may appeal to this decision tree as a way to decide whether euthanasia is warranted when the proper course of action is not clear.

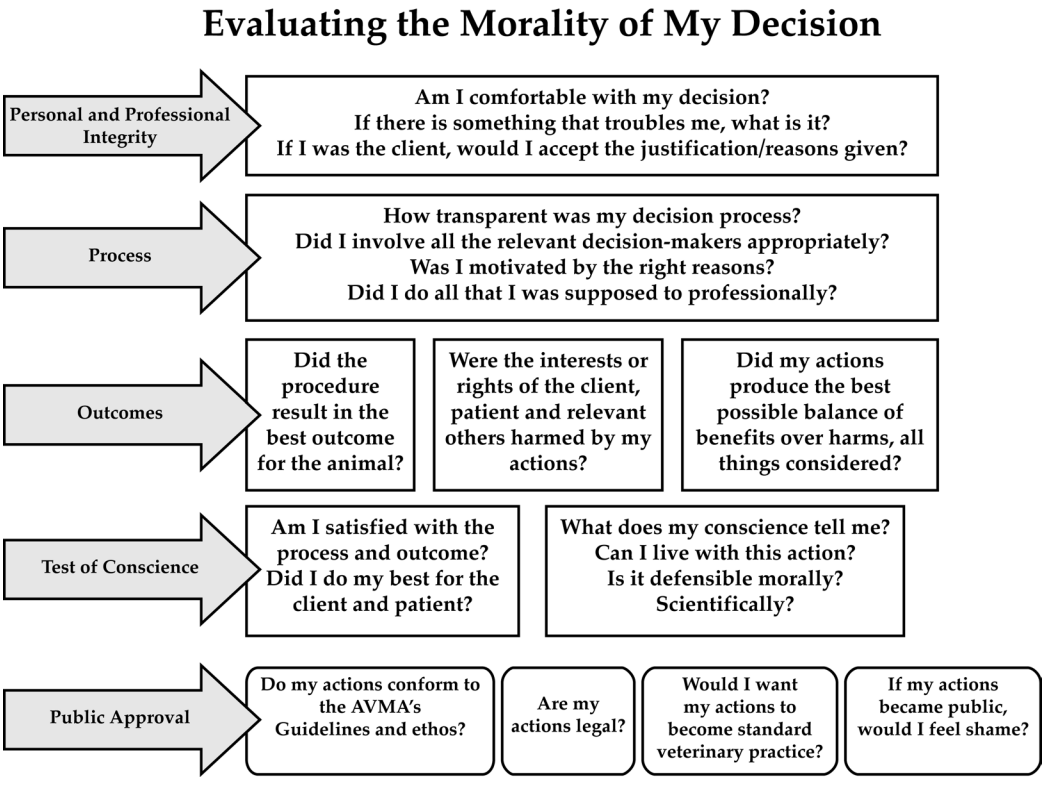


**Figure 2**—When attempting to make the best decision possible in a thorough and balanced way, veterinarians may find this decision matrix helpful. It can assist in assessing the morality of euthanasia in particular cases, especially if they are less straightforward.

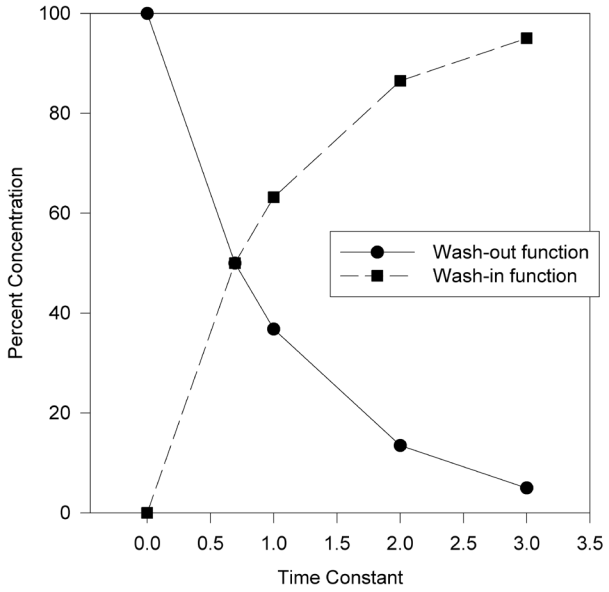


**Figure 3**—Graphic representation of the wash-in and wash-out exponential functions, using a hypothetical example of a closed container, originally filled with gas A into which gas B is introduced. The wash-in and wash-out functions are used to determine the time constant for the enclosed volume or space. The gas concentration within the container can be readily determined from the time constant, which is calculated by dividing the container volume by the gas displacement rate. Figure taken from Meyer RE, Morrow WEM. Carbon dioxide for emergency on-farm euthanasia of swine. *Journal of Swine Health and Production* 2005;13(4):210–217, 2005. Reprinted with permission.

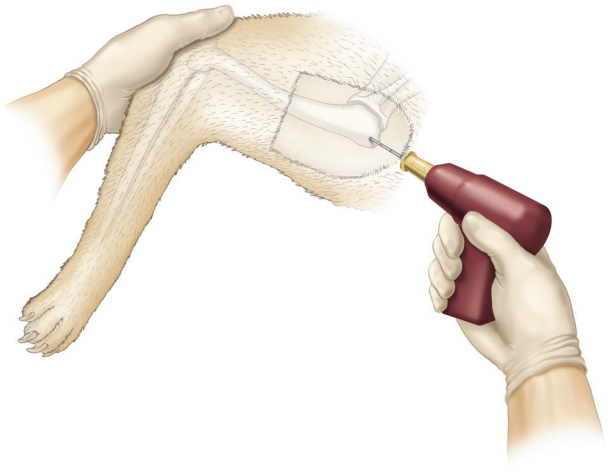

**Figure 4**—One recommended site (greater tubercle of the humerus) for administration of an intraosseous injection in adult dogs, using a bone injection gun. An alternative is to use a Jamshidi bone marrow needle or, in very young dogs, a hypodermic needle.

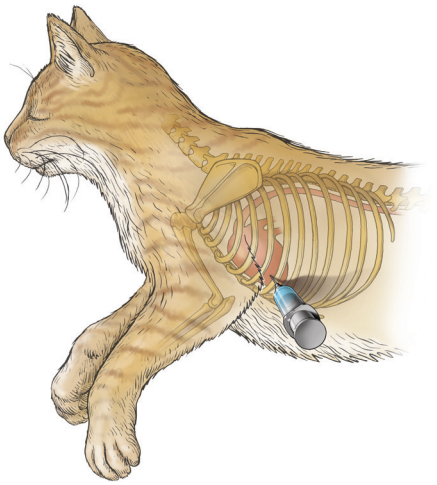

**Figure 5**—Site for administration of intracardiac injections in the cat. Intracardiac injection is appropriate only in unconscious or anesthetized animals.

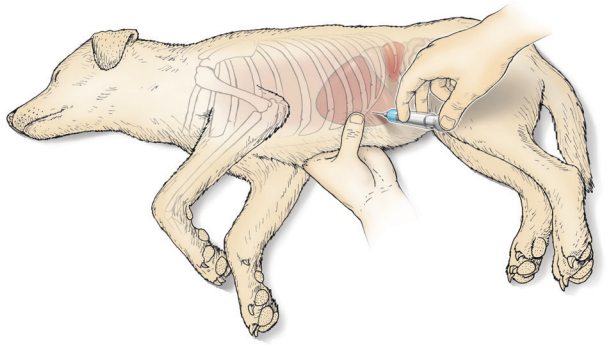

**Figure 6**—Site for administration of intrahepatic and intrasplenic injections in the dog. In this figure, the liver is being injected; the spleen is depicted in red caudal to the liver and stomach. Intrahepatic and intrasplenic injections are appropriate only in unconscious or anesthetized animals with the exception of intrahepatic injections in cats as discussed in the text.

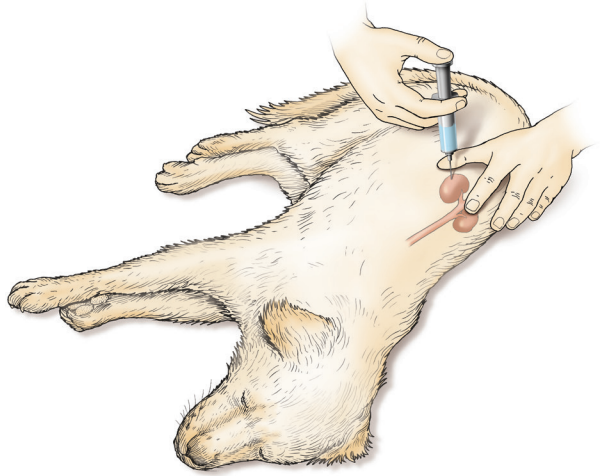

**Figure 7**—Site for administration of an intrarenal injection in the dog. Intrarenal injection is appropriate only in anesthetized or unconscious animals.

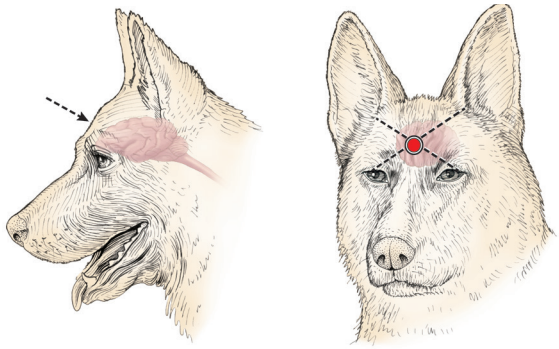

**Figure 8**—Anatomic site for gunshot in dogs is located midway between the level of the eyes and base of the ears, slightly off midline with aim directed across the dog toward the spine. (Based on Longair JA, Finley GG, Laniel MA, et al. Guidelines for the euthanasia of domestic animals by firearms. *Can Vet J* 1991;32:724–726.)

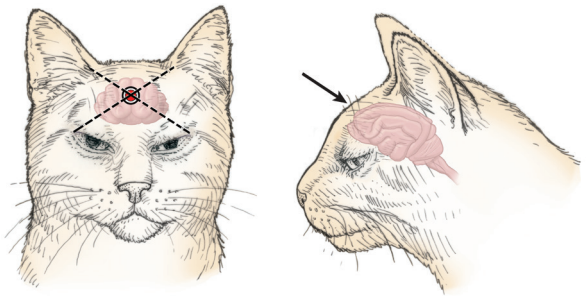

**Figure 9**—Anatomic site for gunshot in cats is a point slightly ventral to a line drawn between the medial bases of the ears (based on Longair JA, Finley GG, Laniel MA, et al. Guidelines for the euthanasia of domestic animals by firearms. *Can Vet J* 1991;32:724–726) or the intersection of lines drawn between lateral canthi of the eyes and medial bases of ears as shown.

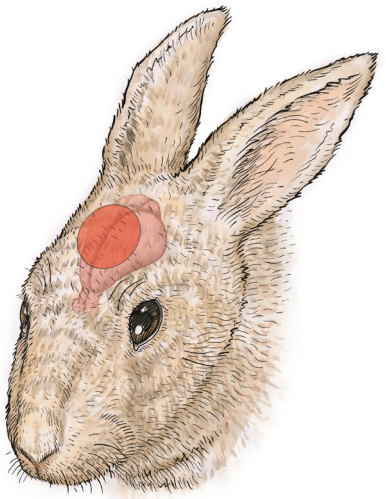

**Figure 10**—Anatomic site for placement of a captive bolt in rabbits. The device should be placed in the center of the forehead, with the barrel in front of the ears and behind the eyes. The device should be discharged twice in rapid succession at the pressure recommended for the age and size of the rabbit. (Walsh JL, Percival A, Turner PV. Efficacy of blunt force trauma, a novel mechanical cervical dislocation device, and a non-penetrating captive bolt device for on-farm euthanasia of pre-weaned kits, growers, and adult commercial meat rabbits. *Animals (Basel)* 2017;7:100.)

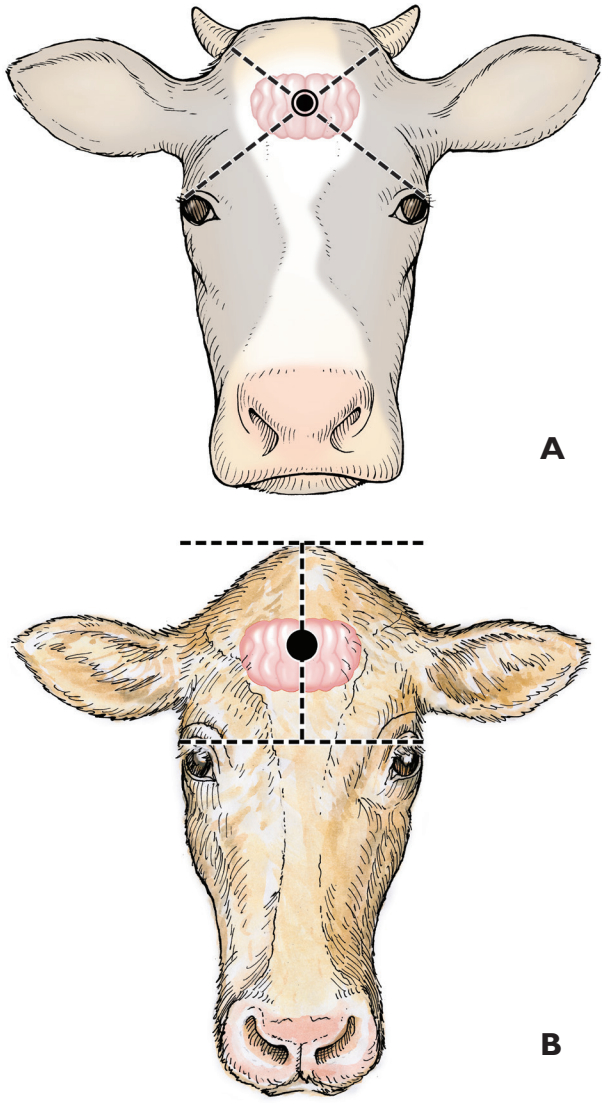


**Figure 11**—Anatomic site for gunshot or placement of a captive bolt and desired path of the projectile in cattle. The point of entry of the projectile should be at the intersection of 2 imaginary lines, each drawn from the outside corner of the eye to the center of the base of the opposite horn (A). Alternatively in long-faced cattle or young stock (B), a point on the midline of the face that is halfway between the top of the poll and an imaginary line connecting the outside corners of the eyes can be used (AVMA Guidelines for the Humane Slaughter of Animals: 2016 Edition).

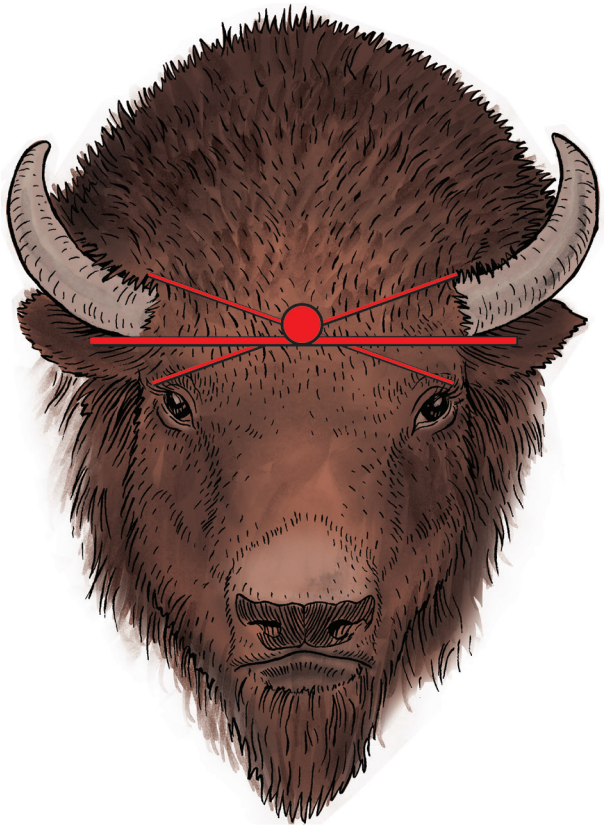

**Figure 12**—Similar to cattle, for American bison, the preferred site for gunshot euthanasia is on the forehead approximately 2.5 cm (1 inch) above an imaginary line connecting the bottom of the horns. The angle of entry should be perpendicular to the skull.

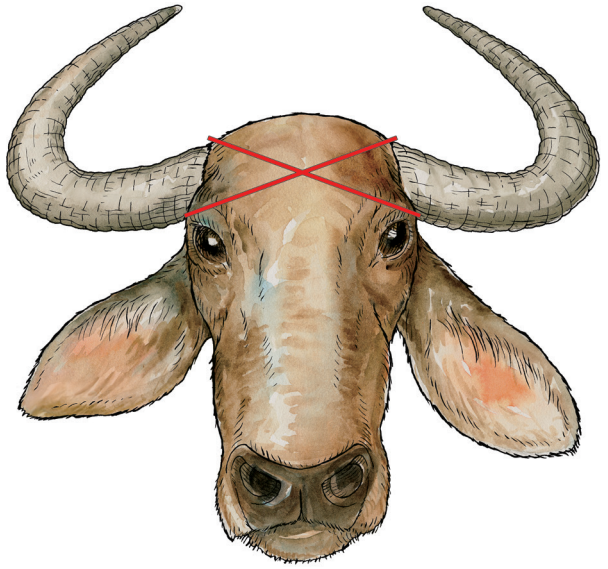

**Figure 13**—For water buffalo, the preferred anatomic site for gunshot or placement of a captive bolt is the intersection of 2 imaginary lines, each drawn from the lower edge of the horn to the upper edge of the opposite horn.

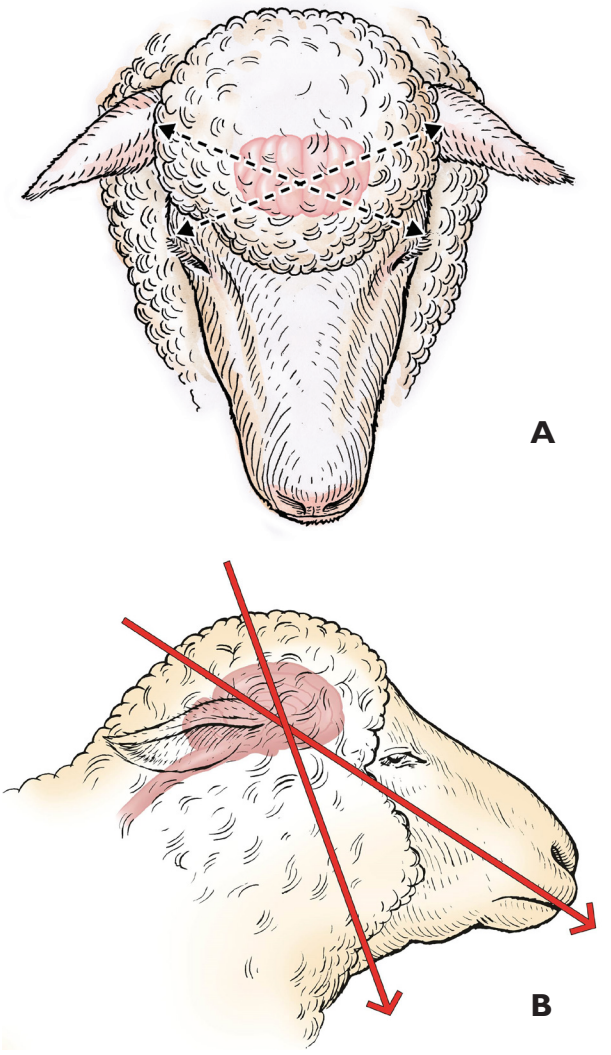


**Figure 14**—For polled sheep or goats (A), place the PCB perpendicular to the skull over the anatomic site identified as slightly caudal to the poll (the crown or the highest point on the head) at the intersection of 2 lines drawn from the outside corner of each eye to the middle of the base of the opposite ear. Alternatively, a site located on the dorsal midline of the head, which corresponds with the external occipital protuberance of the skull, may be used. When using the site associated with the external occipital protuberance, place the PCB flush with the skull at the external occipital protuberance while angling or aiming the muzzle of the PCB toward the mouth. Panel B indicates direction. (Based on observations in goats by Collins SL, Caldwell M, Hecht S, et al. Comparison of penetrating and nonpenetrating captive bolt methods in horned goats. *Am J Vet Res* 2017;78:151–157; and by Plummer PJ, Shearer JK, Kleinhenz KE, et al. Determination of anatomic landmarks for optimal placement in captive-bolt euthanasia of goats. *Am J Vet Res* 2018;79:276–281.)

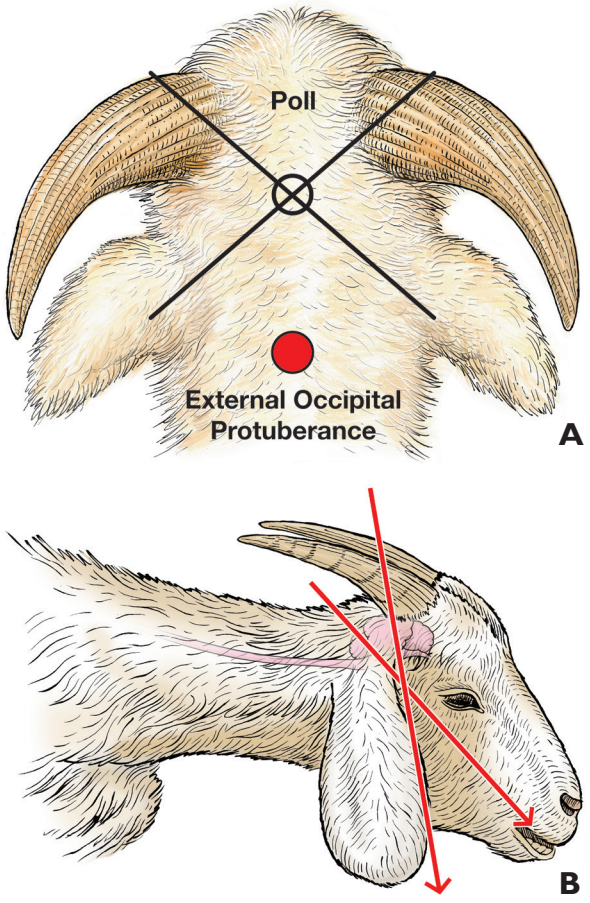


**Figure 15**—For horned sheep or goats (A), place the PCB perpendicular to the skull over the anatomic site identified as slightly caudal to the poll (also known as the crown or the highest point on the head) at the intersection of 2 lines drawn from the outside corner of each eye to the middle of the base of the opposite ear. (Plummer PJ, Shearer JK, Kleinhenz KE, et al. Determination of anatomic landmarks for optimal placement in captive-bolt euthanasia of goats. *Am J Vet Res* 2018;79:276–281.) Alternatively, a site located on the dorsal midline of the head, which corresponds with the external occipital protuberance of the skull, may be used. When using the site associated with the external occipital protuberance, place the PCB flush with the skull at the external occipital protuberance while angling or aiming the muzzle of the PCB toward the mouth. (Collins SL, Caldwell M, Hecht S, et al. Comparison of penetrating and nonpenetrating captive bolt methods in horned goats. *Am J Vet Res* 2017;78:151–157.) Panel B indicates direction.

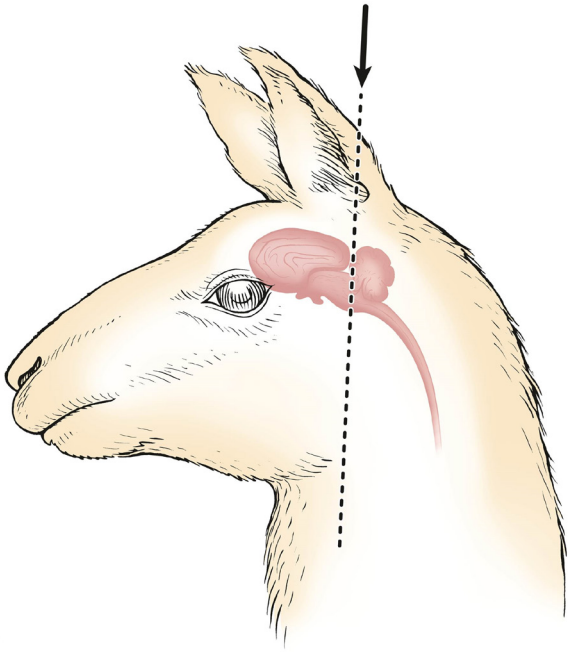

**Figure 16**—Anatomic site for placement of a captive bolt and desired path of the projectile in camelids. The device should be placed at the crown position (highest point on the head) aiming downward to the base of the jaw.

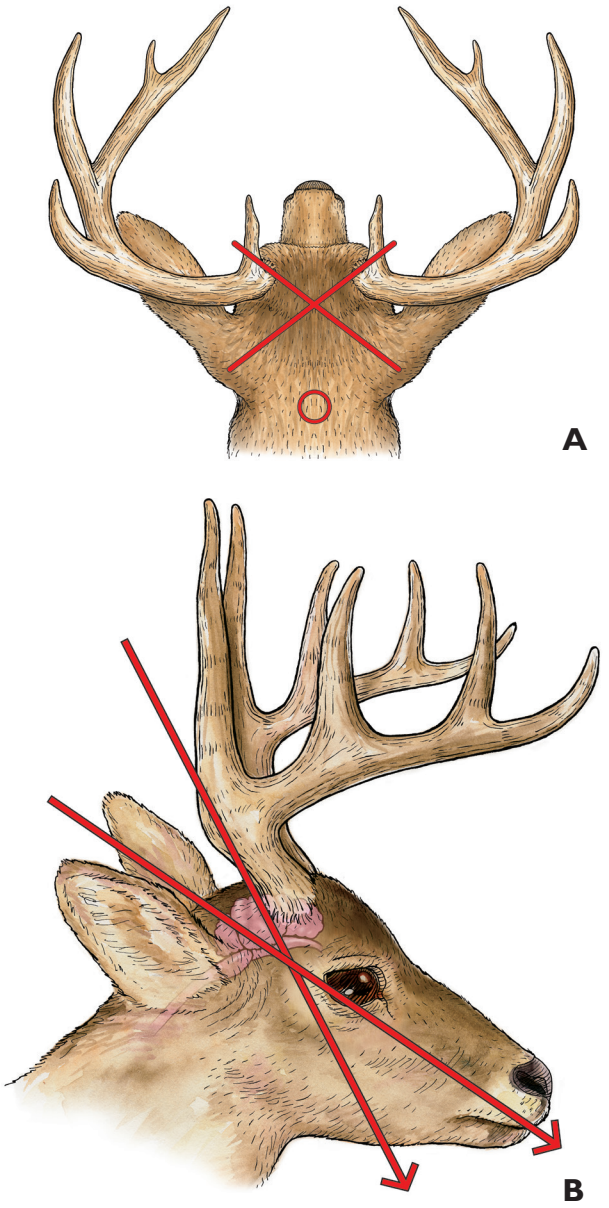


**Figure 17**—Anatomic site for placement of a captive bolt and desired path of the projectile in antlered deer. A captive bolt device with a longer bolt may be needed. The point of entry of the projectile should be at the intersection of 2 imaginary lines drawn from the outside corner of each eye to the top of the base of the ear or base of the antler (A). Alternatively, a site located on the dorsal midline of the head, which corresponds with the external occipital protuberance of the skull, may be used. When using the site associated with the external occipital protuberance, place the PCB flush with the skull at the external occipital protuberance while angling or aiming the muzzle of the PCB toward the mouth. Panel B indicates direction.

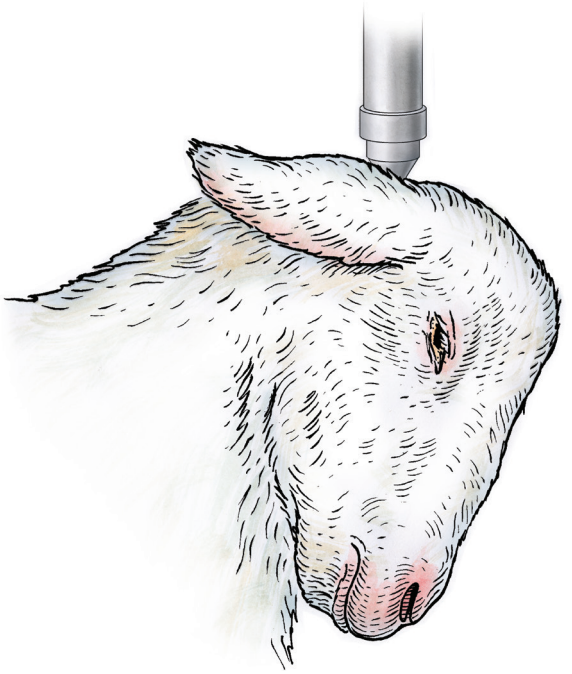

**Figure 18**—Anatomic site for placement of a captive bolt in neonatal lambs and kids. The preferred shooting position is with the muzzle of the NPCB on the midline behind the poll (ie, between the ears) with the chin tucked into the neck. (Grist A, Lines JA, Knowles TG, et al. The use of a mechanical non-penetrating captive bolt device for the euthanasia of neonate lambs. *Animals (Basel)* 2018;8:49. Sutherland MA, Watson TJ, Johnson CB, et al. Evaluation of the efficacy of a non-penetrating captive bolt to euthanase neonatal goats up to 48 hours of age. *Anim Welf* 2016;25:471–479. Grist A, Lines JA, Knowles TG, et al. Use of a non-penetrating captive bolt for euthanasia of neonate goats. *Animals (Basel)* 2018;8:58.)

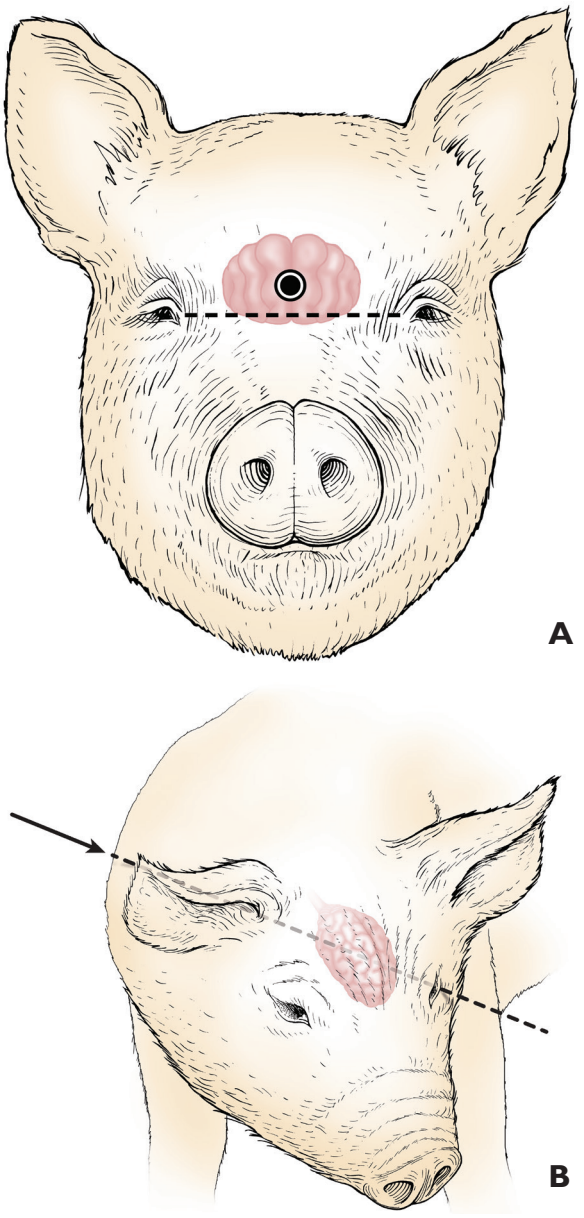


**Figure 19**—Shown is 1 possible anatomic site for PCB and 2 possible anatomic sites for gunshot application in swine. The frontal site may be used for both PCB and gunshot (A) and is located in the center of the forehead slightly above a line drawn between the eyes. The bolt or bullet should be directed toward the spinal canal. The behind-the-ear site (B) should be used only with gunshot application, and the projectile should be toward the opposite eye. The ideal target location and direction of aim may vary slightly according to the breed and age of the animal (owing to growth of the frontal sinuses).

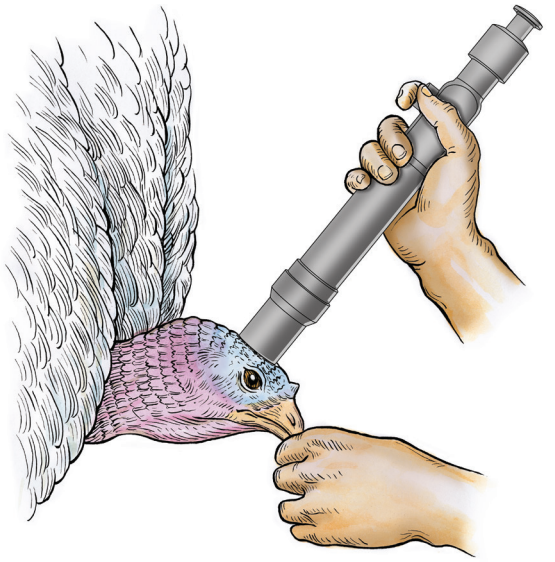

**Figure 20**—Anatomic site for placement of a captive bolt in turkeys (and poultry that lack comb development). The placement of the device should be directly on the midline of the skull and at the highest/widest point of the head with the captive bolt aimed directly down toward the brain. To ensure accurate captive bolt placement and to optimize the safety of the operator, the bird must be correctly restrained. In this illustration, the tip of the beak is held to enable the operator to safely and securely restrain the head of the bird while positioning the captive bolt with the other hand. An additional person is required to restrain the wings and/or feet of the bird (ideally the breast of the bird should rest on a solid surface to keep the bird calm) during and after the application of the captive bolt.

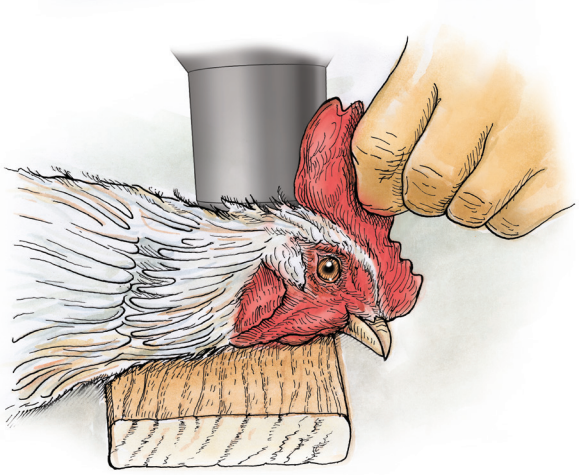

**Figure 21**—Anatomic site for placement of a captive bolt in chickens (and poultry with comb development). The placement should be directly behind the comb and on the midline of the skull with the captive bolt aimed directly down. To ensure accurate captive bolt placement and to optimize the safety of the operator, the bird must be correctly restrained. In this illustration, the comb of the bird is held to enable the operator to safely and securely restrain the head of the bird while positioning the captive bolt with the other hand. To provide additional restraint and resistance against the captive bolt strike force, the ventral portion of the bird's head should be placed against a flat surface (eg, floor or board) to ensure optimal concussion from the device.

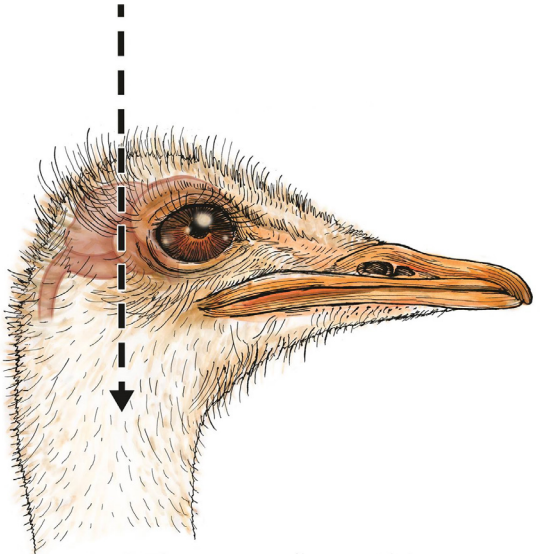

**Figure 22**—Anatomic site for placement of a captive bolt and desired path of the projectile in ratites. A captive bolt device with either an NPCB or a short penetrating bolt and the smallest charge appropriate for poultry or rabbits should be applied to the top of the head at the midpoint of an imaginary line between the outer "ear" openings.

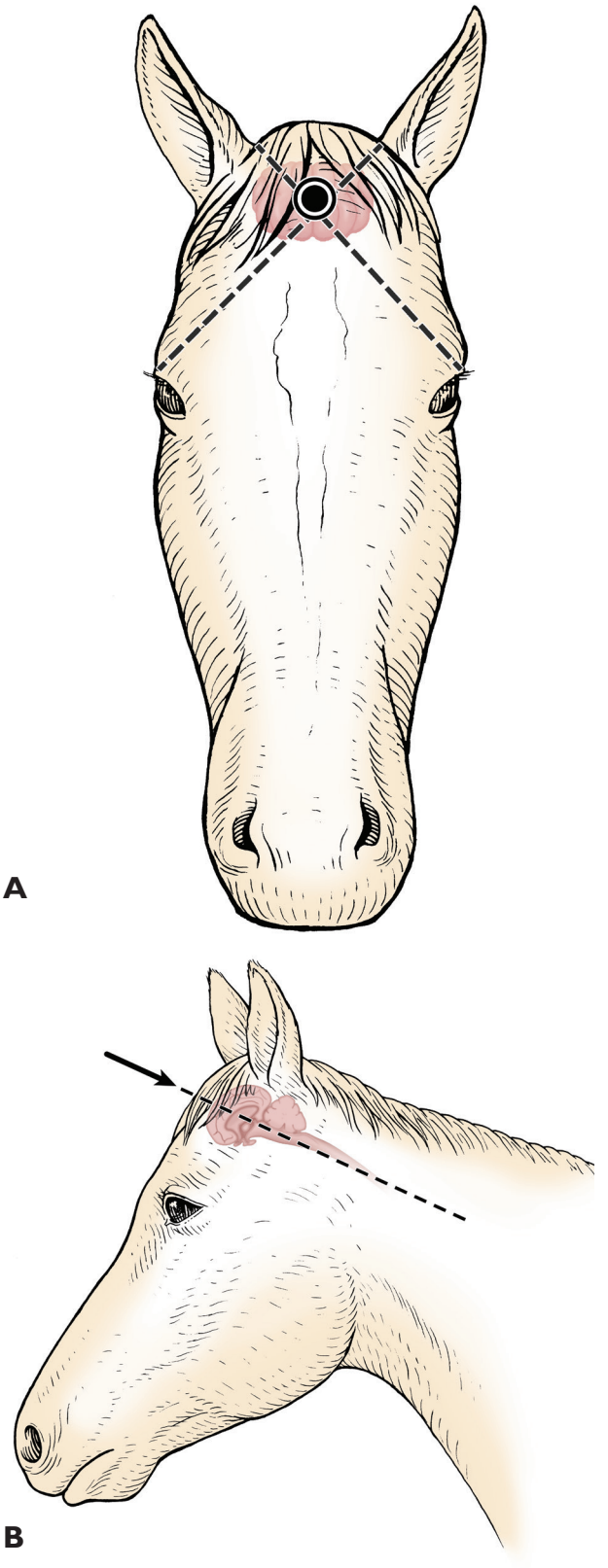


**Figure 23**—Anatomic site for the application of gunshot or PCB for euthanasia of equids (A). The point of entry of the projectile should be at the intersection of 2 imaginary lines, each drawn from the outside corner of the eye to the center of the base of the opposite ear. Panel B indicates direction.

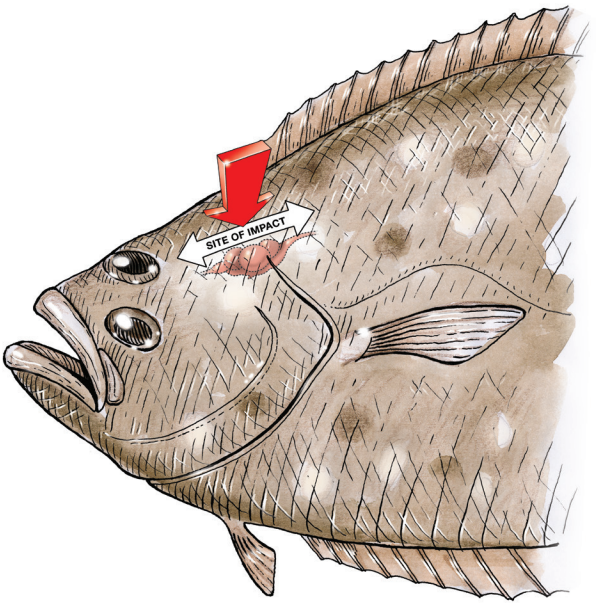


**Figure 24**—Anatomic site for the application of manually applied blunt force trauma in finfish. The finfish's size, species, and anatomy and the characteristics of the blow (including its accuracy, speed, and club mass) will determine the efficacy of manually applied blunt force trauma. The location of the blow should be targeted at the area where the brain is closest to the surface of the head and where the skull is its thinnest.

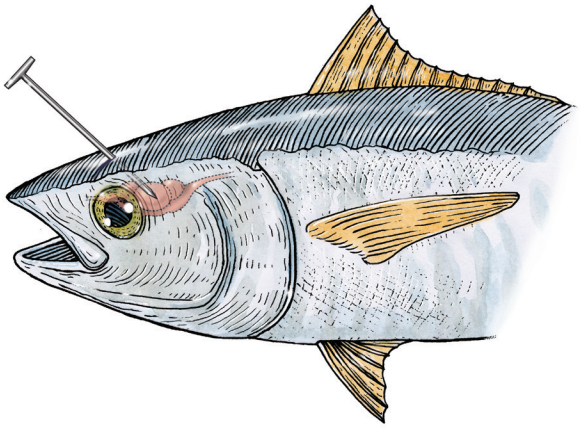

**Figure 25**—Anatomic sites for captive bolt of large fish species. The NPCB gun has either a wide mushroom-shaped head or a flat head that does not penetrate the brain. With PCB devices (including spiking), the projectile should be directed into the hindbrain of the fish; there should be a focus on maximum destruction of brain tissue.

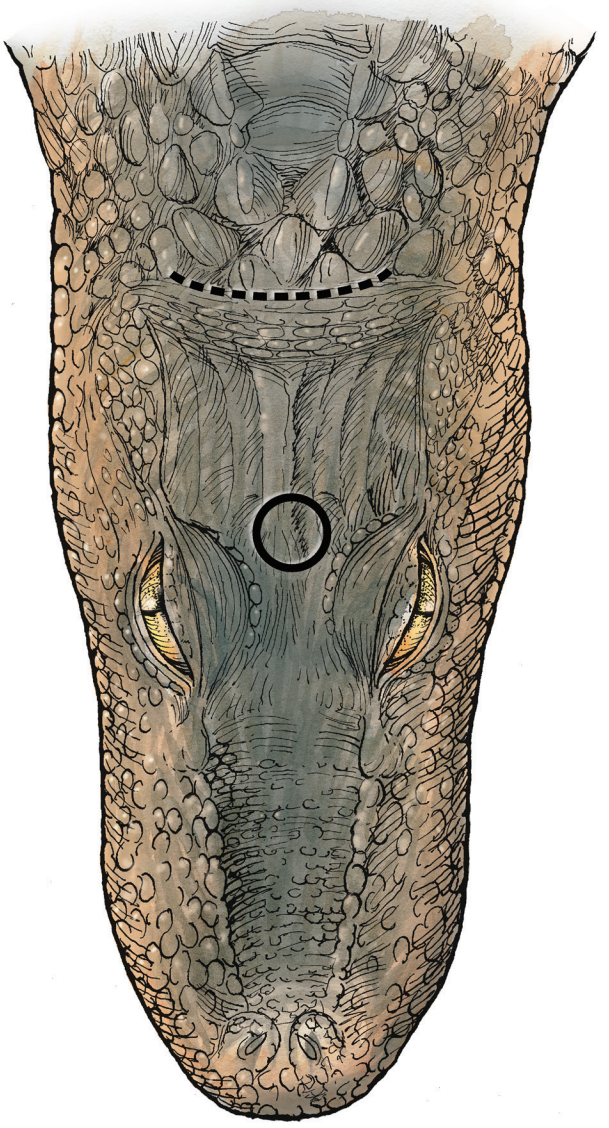

**Figure 26**—Anatomic sites for captive bolt or gunshot placement and for spinal cord severance or decapitation. The brain of the alligator is relatively small and is located immediately behind orbits and extends caudally between the supratemporal fossae. To ensure destruction of brain tissue, the captive bolt or gunshot must be placed on the midline between the orbit and the cranial aspect of the supratemporal fossae.