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American Veterinary Medical Association
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Re: AWI Comments on AVMA Draft Guidelines for the Depopulation of Animals

The Animal Welfare Institute submitted the following comments and references through the AVMA's online comment form:

Lines	Specific language to be changed	Specific language to replace current language	Rationale for suggested change	References uploaded
162-166	0.1 Depopulation as part of Unified Incident Command (Emergency Management) Operations Depopulation refers to the implementation of a unique, large-scale emergency or disaster management plan involving the rapid termination of a population(s) of animals with the least amount of negative welfare impacts to the animals.	0.1 Depopulation as part of the Disaster Management Cycle Depopulation refers to the rapid termination of a population(s) of animals with the least amount of negative welfare impacts to the animals. It typically occurs during the "response" phase of the disaster management cycle, which encompasses	A major change from the 2019 edition is introducing the topic of depopulation in terms of its role in a "large-scale emergency or disaster management plan" and introducing the concept of "unified incident command" prior to even defining depopulation. This is problematic for a number of reasons. Depopulations are often carried out without the unified incident command structure, for example, when pigs and poultry were depopulated following slaughterhouse closure due to	https://publichealth.tulane.edu/blog/disaster-management-cycle/ 2022 One Health Animal Disaster Management An Ethics of Care Approach 2021 COVID-19 effects on livestock production - a one welfare issue

		prevention, preparedness, response, and recovery phases. While this document focuses on considerations pertinent to the "response," the AVMA recognizes the importance of the other phases of the disaster management cycle, including prevention (developing measures that prevent or mitigate the risk of circumstances that result in depopulation) and preparedness (developing strategies, plans, and procedures to effectively deal with situations in which it is necessary to resort to depopulation).	COVID-19 outbreaks among workers or when depopulations are carried out due to financial considerations. Moreover, while it makes sense to invoke the disaster management cycle in the context of depopulation, much of the discussion in this section of the Guidelines assumes that considerations regarding depopulation are pertinent only in the "response" phase of the disaster management cycle. The impact of depopulation on animal welfare and human psychological well-being is often determined by what measures are taken during earlier phases of the cycle, such as prevention and preparedness.	

179-181	Thus, the destruction of animals en masse in response to an emergency should ideally be performed under the supervision of a veterinarian or appropriate veterinary service entity.	Thus, the destruction of animals en masse in response to an emergency must be performed under the supervision of a veterinarian or appropriate veterinary service entity. Operations managing large numbers of animals must proactively ensure, well before any emergency or disaster, that comprehensive prevention, planning, and preparedness measures are in place. These measures should be developed under veterinary oversight to safeguard animal welfare during emergencies, including those requiring depopulation.	Stronger language is needed to emphasize the importance of veterinary oversight. The use of "should ideally" implies that, since ideal circumstances often are not obtained, routine lack of veterinary oversight is acceptable. In addition, veterinarians will essentially "have their hands tied" if they become involved only during the "response" phase of an emergency. Veterinary involvement in earlier phases of the disaster management cycle is essential, and the importance of these phases must be made clear in the Guidelines.	2022 One Health Animal Disaster Management An Ethics of Care Approach

198-199	Ideally, the development of a depopulation plan should occur before an emergency and include the involvement of all the relevant stakeholders.	A depopulation plan must be developed well in advance of any emergency, with all necessary preparedness steps in place to ensure its successful execution. This includes securing contracts, access to equipment and supplies, training, conducting drills and exercises, and involving all relevant stakeholders.	Stronger language is needed to emphasize the importance of advanced planning. We have seen the outcome of failure to plan and prepare for depopulation— animal welfare inevitably suffers, as does human psychological well-being, because moral distress and secondary trauma are heightened when low-welfare methods of depopulation are used. For example, the attached reference on swine veterinarians' perspective on COVID-related depopulations states, "All participants talked about the lack of preparation and the need to be better equipped to manage emergency public health livestock events." In addition, as demonstrated by Figure 1 in USDA APHIS VS's "Emergency Preparedness and Response Training and Exercise Strategy and Plan," drawing up plans alone is insufficient to ensure they can be carried out if and when necessary. Rather, training, drills, and exercises must be conducted well in advance of the need to deploy depopulation methods.	2022 Mass Depopulation of Swine during COVID-19 - An Exploration of Swine Vet's Perspective 2024 USDA APHIS VS Emergency Preparedness & Response Training & Exercise Strategy & Plan

203-206	While the AVMA believes these Guidelines contain valuable information that can help safeguard animals' welfare during depopulation, it is important to understand that the primary objective of depopulation plans is to protect human well-being.	Recommend deletion of this sentence.	This runs counter to numerous statements made throughout the introduction section, which note that depopulation is often used as a strategy to end or prevent animal suffering and emphasize the importance of integrating numerous, sometimes disparate, ethical duties. For example, the statement at lines 238–241: "Emergency management operations involving depopulation includes a commitment that decisions are ethically grounded. This requires recognizing and balancing three directives: 1) protecting public health; 2) minimizing animal pain and distress and 3) safeguarding the physical and mental health of responders." Lines 732–733, in the Veterinary Ethics section, read: "The primary responsibility of the veterinary community during a public health emergency is to minimize harm to both humans and animals and to reduce their deaths." And this statement at lines 851–855: "Developing an ethical decision-making framework that accommodates animals and their welfare during an emergency broadens the scope of who counts during an	2022 One Health Animal Disaster Management An Ethics of Care Approach Morand S, Lajaunie C. Linking Biodiversity with Health and Well-being: Consequences of Scientific Pluralism for Ethics, Values and Responsibilities. Asian Bioeth Rev. 2019 Mar 12;11(2):153-168. doi: 10.1007/s41649-019-00076-4. PMID: 33717309; PMCID: PMC7747447.

			emergency, enabling alternative understandings of vulnerability and suffering. It can advance what must be done to save as many human and animal lives as possible."	
263-264	Depopulation may employ euthanasia or slaughter methods, especially when the number of animals is low or the emergency has been contained.	Depopulation must employ euthanasia or slaughter methods when the number of animals is low or the emergency has been contained.	The current wording runs counter to lines 259-260: "The AVMA Guidelines for the Euthanasia of Animals should be referred to in circumstances where the primary aim is to end the actual or anticipated suffering of individual animals." Given the moral commitments articulated in this and other AVMA guidance documents/policies, and given that depopulation methods often involve greater risks to animal welfare compared to euthanasia and slaughter methods, the Guidelines must use stronger language to ensure that euthanasia or slaughter methods are used when the	AVMA principles of veterinary medical ethics AVMA animal welfare principles AVMA statement on the roles of vets in promoting animal welfare (https://www.avma.org/resources-tools/avma-policies/joint-avma-fve-cvma-roles-veterinarians-promoting-animal-welfare)

			number of animals is small or the emergency is contained.	

269-275	While it is the responsibility of veterinarians, veterinary services and other responders to develop and deploy depopulation methods that minimize animals' pain and distress and to ultimately save as many animals' lives as possible, there may be events (e.g., an outbreak of Highly Pathogenic Avian Influenza (HPAI)) that expand faster than they can be controlled using scientifically supported depopulation methods. Using methods that currently lack broad evidentiary support, but which result in a quick death for animals and advance disease containment, may become necessary.	Once HPAI has been detected on a premises, it is the responsibility of veterinarians, APHIS Veterinary Services, and other responders to deploy depopulation methods that minimize animals' pain and distress and to ultimately save as many animals' lives as possible. This occurs during the Response phase of the disaster/emergency management cycle. However, the options available to these personnel during the Response phase will, to a large extent, be pre-determined by choices made during other phases of the cycle, particularly during Mitigation (reducing the risk that an event requiring depopulation will	It is inappropriate to point to HPAI as an example of a disease for which use of non-standard depopulation methods, which fail to minimize animals' pain and distress, is excusable. At this stage, HPAI is recognized as a constant threat in the United States, as the poultry industry has recognized that it has become endemic in wild birds in North America (see attached reference, from Jan 2024). Consistent with "responsible use" of animals, operations responsible for the health and welfare of large bird populations have an ethical obligation to ensure that, should their operation become infected with this fatal disease, they are equipped to end the lives under their care using validated methods that prioritize and maximize animal welfare. It has been almost 3 years since HPAI was confirmed on a U.S. poultry operation, so poultry operations have had ample opportunity to prepare such that the highest welfare methods of depopulation are rapidly accessible should their farm become infected. Failure to plan and prepare for known and somewhat predictable risks is	2024 Egg Industry report WATT Poultry https://training.fema.gov/emiweb/downloads/is111_unit%204.pdf

		occur) and Preparedness (ensure that sufficient planning has been undertaken, that equipment and supplies will be rapidly available if needed, and that responsible parties have been sufficiently trained to respond rapidly and competently in an emergency). For sectors in which emergency depopulations occur regularly (e.g., HPAI in the U.S. poultry industry) or are anticipated to be needed in the foreseeable future (U.S. swine industry, in anticipation of African Swine Fever), it is imperative that mitigation and preparedness are prioritized by animal owners and incentivized by appropriately	insufficient justification for using methods of depopulation that severely compromise animal welfare. This paragraph should emphasize the need for attention to other phases of the disaster management cycle, including planning, preparation and risk mitigation.	

		crafted governmental policy. Adequate mitigation and preparedness can ensure that events do not expand faster than they can be controlled using scientifically supported depopulation methods that safeguard animal welfare and the psychological well-being of responders.		

324-325	The circumstances surrounding depopulation are unusual and will involve extraordinary intervention measures.	The circumstances surrounding depopulation have historically been uncommon and, in many animal sectors, still are. However, urgent or emergency depopulation has become increasingly common in animal agriculture. Since 2020, millions or tens of millions of agricultural animals have been depopulated annually in the U.S., with most being chickens or turkeys. Animals used in food depopulation often reside in high numbers and at high stocking densities on agricultural operations, which can pose logistical challenges to depopulation. Events resulting in depopulation in recent years include animal disease	It may sometimes be the case that "the circumstances surrounding depopulation are unusual and will involve extraordinary intervention measures," but at present, emergency depopulation is a standard part of agriculture in the US. For example, it is estimated that approximately 1 million dogs are euthanized in animal shelters annually and approximately 6 million pet dogs are euthanized by veterinarians for health or welfare reasons (Pearson 2023). In contrast, well over 130 million poultry have been depopulated since Feb 2022 due to HPAI. All evidence suggests that HPAI is now endemic in wild birds in North America, meaning that depopulation of poultry will remain a regular part of poultry production for the foreseeable future. To continue to treat depopulation as an unusual occurrence and use that as justification for the use of methods that cause pain and suffering is inconsistent with the veterinary profession's commitment to advancing animal welfare and providing "a humane death."	https://www.aphis.usda.gov/sites/default/files/hpai-2022-2023-summary-depop-analysis.pdf. - https://www.aphis.usda.gov/media/document/2086/file - https://www.meatpoultry.com/articles/31000-former-pure-prairie-poultry-chickens-depopulated - https://arkansasadvocate.com/2024/09/25/arkansas-chicken-growers-sue-poultry-execs-for-damages-from-closure-depopulated-flocks/ - https://www.avma.org/resources-tools/avma-policies/avma-animal-welfare-principles - https://avmajournals.avma.org/view/journals/javma/262/2/javma.23.07.0366.xml - 2021 Marchant - Covid 19 - one welfare paper - 2020 NPPC The Tragic Impact of COVID-19 on US Hog Farmers - The Need to Euthanize

		outbreaks, delays in slaughter or movement of animals, corporate bankruptcy or other financial issues, barn fires, and feed contamination events.	Online References: - https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/wild-birds - https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.6195	

460-462	Each facility where depopulation is performed must ensure that their personnel receive appropriate training beforehand, including in the humane restraint of the species of animal.	Each facility where depopulation is performed must ensure that their personnel receive appropriate training beforehand, including in the humane restraint of the species of animal. Any facility with a large number of animals (i.e., more than could be reasonably and rapidly euthanized in the event of an emergency) must preemptively engage in planning and preparation to ensure that the highest welfare method(s) of depopulation are readily accessible within hours after a potential need for them is identified.	This paragraph opens with a discussion of factors that impact the selection of the most appropriate method of depopulation. Research strongly supports the contention that insufficient planning and preparedness, and waiting to provide personnel training until a depopulation is imminent, results in the use of lower welfare (non-preferred) depopulation methods, as well as depopulation delays. For example, Baysinger's paper on VSD+ demonstrates how VSD+TH ended up being relied upon due to the inability to access, over the course of a month, higher welfare methods of depopulation. During the on-going HPAI outbreak, state records show that, in some cases, higher welfare methods like whole house gassing were not utilized due to lack of equipment, supplies, and training. The following are quotations explaining why VSD+ needed to be used, from p. 9 of the attached Delaware public records: "We have never tried to access CO_2 trucks or manifolds until this moment," "We have not been able to get an answer on how long it will take to get CO_2	Delaware state records Baysinger - VSD+TH paper 2022 Mass Depopulation of Swine during COVID-19 - An Exploration of Swine Vet's Perspective

			trucks, or even if they are available," and "CO2 requires personnel knowledgeable about setting up a manifold system and we do not have anyone in the state with that type of training." Furthermore, research on the experience of swine veterinarians involved in depopulation supports a strong emphasis on better preparedness, e.g., the top theme that emerged from interviews with swine vets regarding depopulation during COVID-19 as "the need to be better prepared for crisis events."	
479-480	The POD gave serious consideration to the following criteria in their assessment of the appropriateness of depopulation methods: 1. Ability to induce loss of consciousness followed by death with minimum pain or distress	The POD gave serious consideration to the following criteria in their assessment of the appropriateness of depopulation methods: 1. Ability to induce loss of consciousness followed by death with minimum	The current wording fails to provide a framework for assessing animal welfare impacts of the depopulation method. Such a framework must include consideration of all potential negative affective states, their intensity, and duration, from the point at which the depopulation process is initiated until the animal loses consciousness. This consideration is well-recognized by animal welfare experts as essential in determining the	2022 Challenges of animal welfare assessment for controlled atmosphere killing methods; 2015 Advantages & limitations of the Five Domains model for assessing welfare impacts assoc w vertebrate pest control 2016 A good death? report of the second newcastle meeting on lab animal euth

		pain and distress, where this is assessed by considering the intensity and duration of negative affective states including but not limited to pain, anxiety, overheating, fear, nausea, and breathlessness (dyspnea).	relative "humaneness" or level of animal welfare associated with killing methods (see attached references; a list of negative affective states can be found in Figure 1 of Mellor 2016). In determining the appropriateness of depopulation methods, and how to categorize them, this consideration seems to be at least as important as how long the method requires to cause death. Since this framework for assessing animal welfare associated with depopulation was not provided to the POD, it's unclear what edits would be appropriate in this section. Asking the Panel to reconsider each method in light of this framework is a suggested option.	
498-499	In this edition, the POD recommends a tier system to guide veterinarians identify the most effective depopulation method.	In this edition, the POD recommends a tier system to guide veterinarians in identifying the best depopulation method for the specific circumstance.	Presumably, any method that results in most or all of an animal population being killed within a relatively short window is considered "effective." The intention of the Guidelines is (or should be) to aid veterinarians in choosing the method that is best in terms of animal welfare while also being feasible given the myriad considerations that must be considered, including available resources, personnel,	

			and the accessibility of the animals.	
506-508	The decisions about depopulation methods should be made with consideration of professional, ethical, and technical aspects as well as the availability of infrastructure, equipment, and trained personnel; human and animal welfare; and disposal and environmental outcomes.	The disaster management cycle includes several different phases, including planning, risk mitigation, and preparedness. During the response phase of an emergency, the depopulation options available will often be predetermined by the degree to which those responsible for populations of animals have attended to other phases of the disaster management cycle, especially planning and preparedness. Veterinarians must advocate for prioritization of animal welfare considerations during each of the	Research strongly supports the contention that insufficient planning and preparedness, and waiting to provide personnel training until a depopulation is imminent, results in use of lower welfare (non-preferred) depopulation methods, as well as depopulation delays. For example, Baysinger's paper (attached) demonstrates how VSD+TH ended up being relied upon due to the inability to access, over the course of a month, higher welfare methods of depopulation. During the on-going HPAI outbreak, state records show that, in some cases, higher welfare methods like whole house gassing were not utilized due to lack of equipment, supplies, and training. The following are quotations explaining why VSD+ needed to be used, from p. 9 of the attached Delaware public records: "We have never tried to access CO_2 trucks or manifolds until this moment," "We have not been able to get an answer on	2022 One Health Animal Disaster Management An Ethics of Care Approach Delaware state records Baysinger - VSD+TH paper 2022 Mass Depopulation of Swine during COVID-19 - An Exploration of Swine Vet's Perspective

		phases of the disaster management cycle. Once the decision to depopulate has been made, the choices regarding depopulation methods should be made with consideration of professional, ethical, and technical aspects as well as the availability of infrastructure, equipment, and trained personnel; human and animal welfare; and disposal and environmental outcomes.	how long it will take to get CO2 trucks, or even if they are available," and "CO2 requires personnel knowledgeable about setting up a manifold system and we do not have anyone in the state with that type of training." Furthermore, research on the experience of swine veterinarians involved in depopulation supports a strong emphasis on better preparedness, e.g., the top theme that emerged from interviews with swine vets regarding depopulation during COVID-19 as "the need to be better prepared for crisis events."	

512-519	Tier 1 Methods These methods are given highest priority and should be utilized preferentially when developing emergency response plans and when circumstances allow reasonable implementation during emergencies. These methods are supported by multiple sources of evidence suggesting that they result in rapid loss of consciousness and optimize animal welfare outcomes. They may correspond to methods within the AVMA Guidelines for the Euthanasia of Animals or the AVMA Guidelines for the Humane Slaughter of Animals, but with adjustments so that they may be applied in more challenging situations.	Tier 1 Methods These methods are given highest priority and must be utilized when developing emergency response plans and preparedness activities are undertaken. Every effort should be made to utilize Tier 1 methods when depopulation is required. These methods are supported by multiple sources of evidence suggesting that they result in rapid loss of consciousness and optimize animal welfare outcomes. They may correspond to methods within the AVMA Guidelines for the Euthanasia of Animals or the AVMA Guidelines for the Humane Slaughter of Animals, but with adjustments so that they may be applied	Changing "should" to "must" emphasizes the importance of engaging in planning and preparedness such that the most humane methods (Tier 1 methods) can be utilized during an emergency. Strengthening the wording in the description of Tier 1 ensures that the Guidelines are not misinterpreted to express approval of the routine use of lower-tier methods in the circumstances most likely to result in depopulation. Because Tier 1 methods generally require a higher level of preparedness than lower-tier methods, the description of Tier 1 should underscore the importance of planning and preparedness.	https://www.avma.org/resources-tools/avma-policies/joint-avma-fve-cvma-roles-veterinarians-promoting-animal-welfare

		to situations involving groups of animals.		

520-528	Tier 2 Methods These methods have moderate to limited evidence available to demonstrate rapid loss of consciousness or have other constraints that do not support their prioritization to tier 1. Potential constraints that might result in use of methods in this category include, but are not limited to, zoonotic disease risk, response time, human safety, depopulation efficiency, deployable resources, equipment, animal access, disruption of infrastructure, disease transmission risk, environmental concerns, and carcass disposal.	Tier 2 Methods These methods must result in rapid loss of consciousness, but may (1) have limited evidence available regarding their animal welfare impacts, (2) be associated with negative animal welfare outcomes, such as negative affective states, for a brief period of time, or (3) have a negative impact on human operators, or (4) have other constraints that do not support their prioritization to Tier 1. Potential constraints that might result in the use of methods in this category include, but are not limited to, zoonotic disease risk that cannot be adequately mitigated, response time, human safety, lack of deployable	It appears that the intention of Tier 1 is to identify methods that maximize animal welfare so that those responsible for the welfare of an animal population will work to ensure that such methods are rapidly accessible in an emergency. Tier 2 methods are available in situations where Tier 1 methods cannot be used, but should nonetheless protect animal welfare, if by no other means than to ensure suffering is brief. If a method requires animals to experience negative affective states for a prolonged period prior to loss of consciousness, such a method belongs in Tier 3. Therefore, the description of what constitutes a Tier 2 method should be clarified. In addition, the circumstances in which Tier 2 methods can be used must be clarified. As we have seen in the past few years, a method classified by the AVMA as "permitted in constrained circumstances" (VSD+Heat) rapidly became the primary method used to depopulate commercial egg farms impacted by HPAI (USDA report). This transpired despite language in the 2019 Guidelines that explicitly stated, "The use of less	- USDA report - The rise of heatstroke paper

		resources or equipment, lack of animal access, disruption of infrastructure, or disease transmission risk that cannot be mitigated.	preferred methods should not become synonymous with standard practice" (p 7). This occurred at least in part because of the overly broad list of justifications for the use of lower-tier methods. The "constraints" described in the 2019 edition, and proposed in the revised version, cover virtually all scenarios in which depopulation is likely to be required. It is crucial that the same mistake not be made in the revised edition of the Guidelines.	

529-537	Tier 3 Methods These methods have limited to no evidence to support their use or evidence may be contrary to good animal welfare. Tier 3 should be considered only when circumstances preclude the reasonable implementation of any of the Tier 1 or Tier 2 methods and when the risk of doing nothing is likely to have a reasonable chance of resulting in significantly more animal suffering than that associated with the proposed depopulation method. Examples of such situations include, but are not limited to, structural collapse or compromise of buildings housing animals, complete inability to safely access animals for a prolonged period of time or any circumstance that poses a severe threat to human life.	Tier 3 Methods These methods have limited to no evidence to support their use or are likely to cause animals to experience significant negative affective states for a prolonged period prior to loss of consciousness. Tier 3 must be considered only when all options for implementing Tier 1 or Tier 2 methods have been exhausted and when the risk of doing nothing is likely to result in significantly more animal suffering than that associated with the proposed depopulation method. Tier 3 methods are not recommended by the American Veterinary Medical Association. Examples of situations in which Tier 3 methods	It is essential that the impact of Tier 3 methods on animal welfare be clear in this description. The current wording indicates that a method will be classified in Tier 3 if there is evidence that it "may be contrary to good animal welfare." However, Tier 2 methods do not provide "good" animal welfare; rather they compromise animal welfare for a limited time (e.g., CO2-based methods) or at least risk doing so (e.g., long-range gunshot). In addition, because Tier 3 methods may cause significant animal suffering, strong language must be used to discourage their use in all but the most extreme situations. Given advancements in depopulation methods, the examples of circumstances in which Tier 3 methods might need to be considered should be revised. The example of "structural collapse or compromise of buildings housing animals" should be removed because foaming methods (including high expansion nitrogen foam) can, with the use of machinery, be utilized in at least some situations involving structural	

		might nonetheless be considered include (1) complete inability to safely access animals or their housing areas for a prolonged period of time or (2) any circumstance that would pose a severe threat to human life if Tier 1 or Tier 2 methods were to be employed, and where the threat to human life can only be mitigated by application of a Tier 3 method.	compromise of buildings. In addition, for the sake of clarity, it is important to specify that the use of Tier 3 methods is ethically justified only if such use mitigates the threat to human life, while the use of Tier 1 or 2 methods does not.	

550-551	3) direct depression of neurons necessary for life function (e.g., CO2, hyperthermia)	3) direct or indirect depression of neurons necessary for life function (e.g., CO2, hyperthermia)	In the case of hyperthermia, death is not effected by direct depression of neurons, at least not at the temperatures described for VSD+heat/humidity. Necropsies of animals who have expired from heatstroke indicate that the cause of death is not brain injury but rather hypovolemic or distributive shock, DIC, SIRS, and respiratory failure due to accumulation of frothy, hemorrhagic fluid in the airways.	The Rise of Heatstroke as a Method of Depopulating Pigs and Poultry; Romanucci, M.; Salda, L.D. Pathophysiology and pathological findings of heatstroke in dogs. Vet. Med. Auckl. NZ 2013, 4, 1–9. ; Bruchim, Y.; Horowitz, M.; Aroch, I. Pathophysiology of heatstroke in dogs—Revisited. Temperature 2017, 4, 356–370.; Bruchim, Y.; Loeb, E.; Saragusty, J.; Aroch, I. Pathological findings in dogs with fatal heatstroke. J. Comp. Pathol. 2009, 140, 97–104.; Epstein, Y.; Yanovich, R. Heatstroke. N. Engl. J. Med. 2019, 380, 2449–2459.; Gonzalez-Rivas, P.A.; Chauhan, S.S.; Ha, M.; Fegan, N.; Dunshea, F.R.; Warner, R.D. Effects of heat stress on animal physiology, metabolism, and meat quality: A review. Meat Sci. 2020, 162, 108025. ; Xie, S.; Nicholson, A.; Woolford, L.; McWhorter, T.J. Physiological, biochemical and

				histopathological changes associated with heatstroke in the galah (Eolophus roseicapilla) and rock dove (Columba livia). Avian Pathol. 2019, 48, 57–72.

641-643	Veterinarians and front-line personnel involved in killing animals due to a large-scale incident can experience a heavy moral burden and be substantially impacted psychologically.	Veterinarians and front-line personnel involved in killing animals due to a large-scale incident can experience a heavy moral burden and be substantially impacted psychologically. Moral distress is defined as arising "when individuals are aware of the right action but are hindered by institutional constraints" (Baysal et al., 2024). There is some evidence that depopulation workers experience a higher level of moral distress, mental distress, and burnout when lower-welfare depopulation methods are used, particularly when they must be resorted to due to lack of adequate preparation.	Baysinger et al. found that veterinarians who were involved in depopulating pigs with VSD+ (a low-welfare depopulation method) experienced greater psychological distress (as measured by the validated distress scales) and burnout. Bussolari et al. found in a study of veterinarians involved in COVID-related depopulation of pigs that all veterinarians "talked about the lack of preparation and the need to be better equipped." Research on moral distress has found that it is distinct from other types of negative psychological impacts. Because the options available to veterinarians and other frontline workers involved in depopulation are in large part determined by decisions made by others in the past (for example, whether they have prepared such that higher-welfare depopulation methods are rapidly accessible when needed), they would appear to be at high risk for moral distress, in addition to other psychological impacts such as secondary/vicarious trauma, perpetration-induced traumatic stress, post-traumatic stress	2011 Perpetration-induced traumatic stress—a risk for veterinarians involved in destruction of healthy animals; 2022 Mental Health Impact of Mass Depopulation of Swine on Veterinarians During COVID-19 Infrastructure Breakdown; 2022 Mass depopulation of swine during COVID-19: An exploration of swine veterinarians' perspectives; 2024 Moral distress measurement in animal care workers: a systematic review 2020 Compassion Fatigue in Animal Care Workers

			disorder, compassion fatigue, etc.	
747-749	Veterinarians who serve as members on an emergency team should have operational experience in animal welfare, ideally at the group level, and familiarity with incident command principles.	Veterinarians who serve as members on an emergency team must have operational experience in animal welfare, ideally at the group level, and familiarity with incident command principles.	This change emphasizes that these qualifications are non-negotiable and critical for effective emergency response. Requiring operational experience in animal welfare at the group level ensures that veterinarians are prepared to address the complex and often time-sensitive challenges encountered during emergencies, minimizing the risk of errors that could lead to compromised animal welfare.	
802-803	An emergency that involves depopulation is thus not an ordinary situation.	While many emergencies that involve depopulation are not ordinary situations, depopulation is a regular occurrence	At this stage, depopulation due to HPAI is common and occurs frequently; with HPAI likely endemic in wild birds in North America (O'Keefe 2024), it is likely that depopulation will continue to be a routine component of poultry-based	2024 Egg Industry report WATT Poultry

		in some sectors of animal agriculture, particularly as high pathogenicity avian influenza has become endemic in wild birds (Watt Poultry).	agriculture. Because the predictability of situations involving depopulation is an important ethical consideration, this should be made clear.	
2305-2306	Development and exercise of detailed response plans before their use is extremely beneficial to a depopulation response.	Development and exercise of detailed response plans well in advance of an actual emergency is crucial.	Mandating such plans ensures that teams are well-trained, coordinated, and capable of executing humane, safe, and efficient actions in emergency situations. By making it a requirement rather than an option, it establishes a consistent standard of care and preparedness, enhancing the effectiveness and ethical integrity of response efforts.	
2313	A needs assessment for personnel and resources should be included in the planning phase.	A needs assessment for personnel and resources must be included in the planning phase.	Requiring a needs assessment for personnel and resources helps ensure operational readiness, effective coordination, and humane outcomes by guaranteeing the necessary tools and staff are in place before an emergency response.	
2378-2379	Availability and procurement of needed equipment is an important component of planning and execution.	Because availability and procurement of needed equipment is an important component of planning and execution, it is	Both COVID-19- and HPAI-related depopulations have demonstrated that "failure to plan" equates to "planning to fail," at least when it comes to protecting animal welfare. All operations responsible for	

		essential that well before there is any identified need for depopulation, operations caring for large numbers of bovids secure access to all needed equipment and ensure they have a means of rapidly securing needed supplies.	caring for significant numbers of animals must ensure they have the means of implementing higher-welfare depopulation methods, including access to personnel, equipment, and supplies. This will help ensure that methods that compromise animal welfare are not resorted to.	
2406-2407	A psychological first-aid field guide training module for first responders is available.27	A psychological first-aid field guide training module for first responders is available. Planning and preparation far in advance of any need for depopulation can help ensure that higher welfare depopulation methods are easily accessible and this, in turn, can reduce moral distress and secondary trauma for personnel involved in implementing depopulation.	Evidence suggests that depopulation workers experience a higher level of moral distress, mental distress, and burnout when lower-welfare depopulation methods are used, particularly when such methods are required due to inadequate preparation. For example, Baysinger et al. found that veterinarians who were involved in depopulating pigs with a low-welfare method (VSD+) experienced greater psychological distress (as measured by the validated distress scales) and burnout. Bussolari et al. found in a study of veterinarians involved in COVID-related depopulation of pigs that all veterinarians "talked about the lack of preparation and the need to be better	2011 Perpetration-induced traumatic stress—a risk for veterinarians involved in destruction of healthy animals; 2022 Mental Health Impact of Mass Depopulation of Swine on Veterinarians During COVID-19 Infrastructure Breakdown; 2022 Mass depopulation of swine during COVID-19: An exploration of swine veterinarians' perspectives; 2024 Moral distress measurement in animal care workers: a systematic review

			equipped." Moral distress is defined as arising "when individuals are aware of the right action but are hindered by institutional constraints" (Baysal et al., 2024). Research on moral distress has found that it is distinct from other types of negative psychological impacts. Because the options available to veterinarians and other frontline workers involved in depopulation are in large part determined by decisions made by others in the past (for example, whether they have prepared such that higher-welfare depopulation methods are rapidly accessible when needed), they would appear to be at high risk for moral distress, in addition to other psychological impacts such as secondary/vicarious trauma, perpetration-induced traumatic stress, post-traumatic stress disorder, compassion fatigue, etc.	

2629-2632	In cases where known exposure to adulterants or intoxicants has occurred but the animals do not demonstrate clinical signs of illness or suffering, are not a threat to human health, and are not likely to negatively affect the environment, then depopulation may not need to occur immediately.	In cases where known exposure to adulterants or intoxicants has occurred but the animals do not demonstrate clinical signs of illness or suffering, are not a threat to human health, and are not likely to negatively affect the environment, then depopulation may not need to occur immediately; in such instances, euthanasia or slaughter methods, rather than depopulation methods, must be used.	It is essential to include language emphasizing that depopulation methods should not be used in non-urgent circumstances. Doing so upholds the veterinary profession's responsibility to protect animal welfare and helps ensure the coherence of the Guidelines. At lines 263–264, the draft Guidelines currently say, "Depopulation may employ euthanasia or slaughter methods, especially when the number of animals is low or the emergency has been contained." At 164–166, the definition of depopulation specifies, "Depopulation refers to the implementation of a unique, large-scale emergency or disaster management plan involving the rapid termination of a population(s) of animals." The example cited here specifically indicates that there is not a need for depopulation to occur "rapidly"; therefore, use of depopulation methods would be inappropriate and, given that "not all depopulation methods meet the AVMA criteria for euthanasia" (lines 263–265) or slaughter, and are thus more likely to compromise animal welfare, this would be unethical.	

			These changes would be consistent with lines 5552–5555 in the poultry section, which indicate that euthanasia methods, not depopulation methods, should be used for culling of end-of-lay hens on commercial egg operations because this situation is not an emergency.	
2663-2665	Decisions to depopulate potentially contaminated animals may occur even if no clinical signs of illness are demonstrated, owing to public perception that the animal protein may not be wholesome or because of risk aversion	Decisions to depopulate potentially contaminated animals may occur even if no clinical signs of illness are demonstrated, owing to public perception that the animal protein may not be wholesome or because of risk aversion. In such a circumstance, methods of euthanasia and slaughter, rather than depopulation	It is essential to include language emphasizing that depopulation methods should not be used in non-urgent circumstances. Doing so upholds the veterinary profession's responsibility to protect animal welfare and is necessary for the Guidelines to be logically coherent. At lines 263–264, the draft Guidelines currently say, "Depopulation may employ euthanasia or slaughter methods, especially when the number of animals is low or the emergency has been contained." At 164–166, the definition of depopulation specifies, "Depopulation refers to the implementation of a	

		methods, must be used.	unique, large-scale emergency or disaster management plan involving the rapid termination of a population(s) of animals." The example cited here specifically indicates that there is not a need for depopulation to occur "rapidly"; therefore, use of depopulation methods would be inappropriate and, given that "not all depopulation methods meet the AVMA criteria for euthanasia" (lines 263–265) or slaughter, and are thus more likely to compromise animal welfare, this would be unethical. These changes would be consistent with lines 5552–5555 in the poultry section, which indicate that euthanasia methods, not depopulation methods, should be used for culling of end-of-lay hens on commercial egg operations because this situation is not an emergency.	
2761-2763	Anatomic landmarks for the proper placement of the muzzle of captive bolt devices can be referenced from the 2020 AVMA Euthanasia Guidelines.44	Anatomic landmarks for the proper placement of the muzzle of captive bolt devices can be referenced from the 2020 AVMA Euthanasia Guidelines. Bison have a very thick	Because this section refers to bovids rather than only cattle, it is important to note species differences that may significantly affect welfare.	https://www.hsa.org.uk/downloads/technical-notes/tn25-slaughter-and-killing-of-minority-farmed-species.pdf

		frontal bone (up to six times that of a domestic bovine at any given age), therefore effective, captive-bolt stunning might be difficult to achieve and must be avoided.		

2867	Water-based foam	Move to tier 3	Water-based foam as a depopulation method should be downgraded to Tier 3. It is currently identified as Tier 2 for cattle on the basis of one sole study in this species. Available evidence indicates that welfare is severely compromised when death occurs via obstruction of the airway (Beausoleil 2015; Ludders 1999). The expert Panel on Animal Health and Welfare of the European Food Safety Authority (EFSA) has found that water-based foam should not be used because it is "highly painful" and, as a "method designed to cause occlusion of the trachea," is "equivalent to death by drowning or suffocation." The AVMA's 2020 Guidelines for the Euthanasia of Animals list both asphyxiation and drowning as methods that are "unacceptable as primary methods of euthanasia," noting specifically that drowning is "inhumane." In addition, the United Kingdom's governmental Animal Welfare Committee states that water-based foam should not be used for killing animals, noting that "[w]elfare concerns arise from this mode of action which	16 Beausoleil, N. J., & Mellor, D. J. (2015). Introducing breathlessness as a significant animal welfare issue. New Zealand Veterinary Journal, 63(1), 44–51. https://doi.org/10.1080/00480169.2014.940410 17 Ludders, J. W., Schmidt, R. H., Dein, F. J., & Klein, P. N. (1999). Drowning Is not euthanasia. Wildlife Society Bulletin, 27(3), 666–670. 18 EFSA Panel on Animal Health and Welfare (2020). Welfare of pigs during killing for purposes other than slaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195. https://doi.org/10.2903/j.efsa.2020.6195 19 EFSA Panel on Animal Health and Welfare. (2019). Killing for purposes other than slaughter: poultry. EFSA Journal. European Food Safety Authority, 17(11), e05850. https://doi.org/10.2903/j.efsa.2019.5850 20 EFSA Panel on Animal Health and Welfare. (2024). The use of

			is equivalent to drowning or suffocation . . . neither of which are recognised as humane under European legislation nor the 2018 World Organisation for Animal Health guidelines on the killing of animals for disease control purposes." Further, even precautions such as ensuring the foam level rapidly rises to two times the animal's head height do not decrease the average time to unconsciousness much below three minutes from the start of foaming (Campler 2023, Korenyi-Both 2022)—a relatively long period for animals to suffer pain, respiratory distress, fear, anxiety, and helplessness. In fact, the sole study performed on the use of water-based foam in cattle suggests it takes even longer for cattle to lose consciousness: the container containing the cattle required an average of 89 seconds to fill, and cattle did not cease moving for an average of 2.5 to 3 minutes after the container was full (Capria 2023). There is thus significant evidence that using water-based foam to depopulate cattle (or other livestock) is "contrary to	high expansion foam for stunning and killing pigs and poultry. EFSA Journal. European Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855. 21 AVMA. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Page 112. 22 United Kingdom Animal Welfare Committee (2024). Opinion on the use of high expansion nitrogen foam delivery systems for depopulation of poultry flocks affected by notifiable disease in the UK. Available at: https://www.gov.uk/government/publications/awc-opinion-on-high-expansion-nitrogen-foam-for-cullingpoultry/awc-opinion-on-the-use-of-high-expansion-nitrogen-foam-for-culling-poultry 23 Campler, M. R., Cheng, T.-Y., Arruda, A. G., Flint, M., Kieffer, J. D., Youngblood, B., & Bowman, A. S. (2023). Refinement of water-based foam depopulation procedures for finisher pigs during field conditions: Welfare implications and logistical

			good animal welfare." For this reason, and because of the availability of other practical, scalable, higher-welfare methods, the use of water-based foam should be designated a Tier 3 method.	aspects. Preventive Veterinary Medicine, 217, 105974. https://doi.org/10.1016/j.prevetmed.2023.105974 24 Korenyi-Both, J., Vidaurre, J., Held, T., Campler, M. R., Kieffer, J., Cheng, T. Y., Moeller, S. J., Bowman, A. S., & Arruda, A. G. (2022). Description of electroencephalographic data gathered using water-based medium-expansion foam as a depopulation method for nursery pigs. Scientific Reports, 12(1), 16798. https://doi.org/10.1038/s41598-022-21353-7 Capria VM, Arruda AG, Cheng TY, et al. Water-based medium-expansion foam depopulation of adult cattle. Trans An Sci. 2023:7(1):txad065

2888-2892	Complications with the use of water-based foam in cattle include the time to unconsciousness of the animals undergoing depopulation. While cessation of movement (COM) is a current proxy for time to unconsciousness in cattle, further research is needed to determine the timepoint at which cattle become unconscious as agonal movement can continue for several minutes after loss of postural tone.	A sole paper investigating the use of water-based foam in cattle did not investigate animal welfare outcomes, but did note that "Necropsies of a subset of cattle revealed foam extending to at least the tracheal bifurcation in all cattle and distal to this level in 67% (8/12) animals" (Capria 2023). As with other causes of tracheal obstruction, obstructive asphyxiation from water-based foam results in severe noncardiogenic pulmonary edema. Disadvantages of water-based foam in cattle include the time to unconsciousness of the animals undergoing depopulation and the severe negative	Water-based foam as a depopulation method should also be downgraded to Tier 3. It is currently identified as Tier 2 for cattle on the basis of one study in this species. According to the Guidelines, Tier 1 methods "are supported by multiple sources of evidence suggesting that they result in rapid loss of consciousness and optimize animal welfare outcomes." However, available evidence indicates that welfare is severely compromised when death occurs via obstruction of the airway (Beausoleil 2015; Ludders 1999). The expert Panel on Animal Health and Welfare of the European Food Safety Authority (EFSA) has found that water-based foam should not be used because it is "highly painful" and, as a "method designed to cause occlusion of the trachea," is "equivalent to death by drowning or suffocation." The AVMA's 2020 Guidelines for the Euthanasia of Animals list both asphyxiation and drowning as methods that are "unacceptable as primary methods of euthanasia," noting specifically that drowning is "inhumane." In addition, the United Kingdom's governmental	-2023 Water-based medium-expansion foam depopulation of adult cattle -Beausoleil, N. J., & Mellor, D. J. (2015). Introducing breathlessness as a significant animal welfare issue. NewZealand Veterinary Journal, 63(1), 44–51. https://doi.org/10.1080/00480169.2014.940410 -Ludders, J. W., Schmidt, R. H., Dein, F. J., & Klein, P. N. (1999). Drowning Is not euthanasia. Wildlife SocietyBulletin, 27(3), 666–670. - United Kingdom Animal Welfare Committee (2024). Opinion on the use of high expansion nitrogen foam deliverysystems for depopulation of poultry flocks affected by notifiable disease in the UK. Available at:https://www.gov.uk/government/publications/awc-opinion-on-high-expansion-nitrogen-foam-for-cullingpoultry/awc-opinion-on-the-use-of-high-expansion-nitrogen-foam-for-culling-poultry - 2016 Nondrowning asphyxia in veterinary forensic pathology_ suffocation, strangulation & mech asphyxia

		affective states experienced by the animals prior to loss of consciousness. The type of dyspnea caused by airway obstruction—characterized by an increased drive to breathe due to hypercarbia, along with an inability to expand the lungs—is known to result in "air hunger" in mammals. Air hunger is described by humans as the most unpleasant form of respiratory distress (Beausoleil 2015); killing via such a method is considered highly likely to be painful by UK and EU governmental animal welfare expert panels, and its use in these countries is not permitted. While cessation of movement (COM) is a current proxy for time to	Animal Welfare Committee states that water-based foam should not be used for killing animals, noting that "[w]elfare concerns arise from this mode of action which is equivalent to drowning or suffocation . . . neither of which are recognised as humane under European legislation nor the 2018 World Organisation for Animal Health guidelines on the killing of animals for disease control purposes." Further, even precautions such as ensuring the foam level rapidly rises to two times the animal's head height do not decrease the average time to unconsciousness much below three minutes from the start of foaming (Campler 2023, Korenyi-Both 2022)—a relatively long period for animals to suffer pain, respiratory distress, fear, anxiety, and helplessness. In fact, the sole study performed on use of water-based foam in cattle suggests it takes even longer for cattle to lose consciousness: the container containing the cattle required an average of 89 seconds to fill, and cattle did not cease moving for an average of 2.5 to 3 minutes ***after*** the container was full	

		unconsciousness in cattle, further research is needed to determine the time point at which cattle become unconscious, as agonal movement can continue for several minutes after loss of postural tone.	(Capria 2023). Thus, there is significant evidence that using water-based foam to depopulate cattle (or other livestock) is "contrary to good animal welfare." For this reason, and because of the availability of other practical, scalable, higher-welfare methods, the use of water-based foam should be designated a Tier 3 method. Online references (too large to attach): -EFSA Panel on Animal Health and Welfare (2020). Welfare of pigs during killing for purposes other than slaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195. https://doi.org/10.2903/j.efsa.2020.6195 - EFSA Panel on Animal Health and Welfare. (2019). Killing for purposes other than slaughter: poultry. EFSA Journal. European Food Safety Authority, 17(11), e05850. https://doi.org/10.2903/j.efsa.2019.5850 - EFSA Panel on Animal Health and Welfare. (2024). The use of high expansion foam for stunning and killing pigs and	

			poultry. EFSA Journal. European Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855. - AVMA. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Page 112. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf	

2922-2924	While no research studies are available to examine the use of nitrates or nitrites in cattle as depopulation agents, one such study in swine indicates the variability in performance of sodium nitrite as a depopulation agent.	No research studies are available to examine the use of nitrates or nitrites in cattle as depopulation agents. Several sodium nitrite studies have been performed on pigs, but both the unreliability of this killing method and its potential to negatively impact animal welfare by causing respiratory distress and vomiting have resulted in sodium nitrite being categorized as Tier 3 and "not recommended" for pigs in this updated version of the AVMA Depopulation Guidelines. Porcine studies should not be extrapolated to cattle because of key physiologic species differences that impact the metabolism of sodium nitrite and	Pigs are very susceptible to sodium nitrite because they have uniquely low levels of methemoglobin reductase (Cowled 2008). Pigs have significantly lower natural levels of methemoglobin reductase than cattle, and cattle have been found to be less sensitive than pigs to nitrite poisoning (Cowled 2008). Given its negative animal welfare impacts (vomiting and prolonged respiratory distress) and unreliability as a killing method in pigs, sodium nitrite is categorized as Tier 3 for pigs. Since it is known to be even less effective for killing in cattle, it must not be listed or suggested for use in bovids.	2008 Additional toxins for feral pig

		make it even less effective as a killing agent for cattle than it is for pigs.		
2924-2926	Pepin et al98 reported a 181.4 mg/lb oral dose (delivered by gavage at 1x, 2x, 2.5x and 3x this rate) with success (death) rates ranging from 60 to 90% and time to death ranging from 34 to 83 minutes.	Delete this sentence.	This sentence is irrelevant given the physiologic and metabolic differences between pigs and cattle. Pigs are very susceptible to sodium nitrite because they have uniquely low levels of methemoglobin reductase (Cowled 2008). Pigs have significantly lower natural levels of methemoglobin reductase than cattle, and cattle have been found to be less sensitive than pigs to nitrite poisoning (Cowled 2008). Given its negative animal welfare impacts (vomiting and prolonged respiratory distress) and unreliability as a killing method in pigs, sodium nitrite is categorized as Tier 3 for pigs. Since it is known to be even less effective for killing in cattle, it	2008 Additional toxins for feral pig

			must not be listed or suggested for use in bovids.	
2942-2943	Water-based foam, while efficacious for adult cattle, has yet to be validated at this stage of life for bovines.	Water-based foam, while efficacious for adult cattle, negatively impacts animal welfare prior to loss of consciousness. In addition, it has not been validated at this stage of life for bovines. Therefore water-based foam is not recommended.	Water-based foam as a depopulation method should be downgraded to Tier 3. It is currently identified as Tier 2 for cattle on the basis of one study in this species. According to the Guidelines, Tier 1 methods "are supported by multiple sources of evidence suggesting that they result in rapid loss of consciousness and optimize animal welfare outcomes." However, available evidence indicates that welfare is severely compromised when death occurs via obstruction of the airway (Beausoleil 2015; Ludders 1999). The expert Panel on Animal Health and Welfare of the European Food Safety Authority (EFSA) has found that water-based foam should not be used because it is "highly painful" and, as a "method designed to cause occlusion of the trachea," is "equivalent to death by drowning or suffocation." The AVMA's 2020	16 Beausoleil, N. J., & Mellor, D. J. (2015). Introducing breathlessness as a significant animal welfare issue. New Zealand Veterinary Journal, 63(1), 44–51. https://doi.org/10.1080/00480169.2014.940410 17 Ludders, J. W., Schmidt, R. H., Dein, F. J., & Klein, P. N. (1999). Drowning Is not euthanasia. Wildlife Society Bulletin, 27(3), 666–670.. United Kingdom Animal Welfare Committee (2024). Opinion on the use of high expansion nitrogen foam delivery systems for depopulation of poultry flocks affected by notifiable disease in the UK. Available at: https://www.gov.uk/government/publications/awc-opinion-on-high-expansion-nitrogen-foam-for-cullingpoultry/awc-opinion-on-the-use-of-high-expansion-

			Guidelines for the Euthanasia of Animals list both asphyxiation and drowning as methods that are “unacceptable as primary methods of euthanasia,” noting specifically that drowning is “inhumane.” In addition, the United Kingdom’s governmental Animal Welfare Committee states that water-based foam should not be used for killing animals, noting that “[w]elfare concerns arise from this mode of action which is equivalent to drowning or suffocation . . . neither of which are recognised as humane under European legislation nor the 2018 World Organisation for Animal Health guidelines on the killing of animals for disease control purposes.” Further, even precautions such as ensuring the foam level rapidly rises to two times the animal’s head height do not decrease the average time to unconsciousness much below three minutes from the start of foaming (Campler 2023, Korenyi-Both 2022) —a relatively long period for animals to suffer pain, respiratory distress, fear, anxiety, and helplessness. In fact, the sole study performed	nitrogen-foam-for-culling-poultry Campler, M. R., Cheng, T.-Y., Arruda, A. G., Flint, M., Kieffer, J. D., Youngblood, B., & Bowman, A. S. (2023). Refinement of water-based foam depopulation procedures for finisher pigs during field conditions: Welfare implications and logistical aspects. Preventive Veterinary Medicine, 217, 105974. https://doi.org/10.1016/j.prevetmed.2023.105974 Korenyi-Both, J., Vidaurre, J., Held, T., Campler, M. R., Kieffer, J., Cheng, T. Y., Moeller, S. J., Bowman, A. S., & Arruda, A. G. (2022). Description of electroencephalographic data gathered using water-based medium-expansion foam as a depopulation method for nursery pigs. Scientific Reports, 12(1), 16798. https://doi.org/10.1038/s41598-022-21353-7 Capria VM, Arruda AG, Cheng TY, et al. Water-based medium-expansion foam depopulation of adult cattle. Trans An Sci. 2023:7(1):txad065

			on use of water-based foam in cattle suggests it takes even longer for cattle to lose consciousness: the cattle containing the cattle required an average of 89 seconds to fill, and cattle did not cease moving for an average of 2.5 to 3 minutes after the container was full (Capria 2023). There is thus significant evidence that using water-based foam to depopulate cattle (or other livestock) is "contrary to good animal welfare." For this reason, and because of the availability of other practical, scalable, higher-welfare methods, the use of water-based foam should be designated a Tier 3 method. Online references (too large to attach): -EFSA Panel on Animal Health and Welfare (2020). Welfare of pigs during killing for purposes other than slaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195. https://doi.org/10.2903/j.efsa.2020.6195 - EFSA Panel on Animal Health and Welfare. (2019). Killing for	

			purposes other than slaughter: poultry. EFSA Journal. European Food Safety Authority, 17(11), e05850. https://doi.org/10.2903/j.efsa.2019.5850 - EFSA Panel on Animal Health and Welfare. (2024). The use of high expansion foam for stunning and killing pigs and poultry. EFSA Journal. European Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855. - AVMA. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Page 112. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf	
3277	...number of US commercial swine operations in 2022 totaled 60,809,000.1 As of March 1, 2024, the...	...number of US commercial swine operations in 2022 totaled 60,809. As of March 1, 2024, the...	This is a typo. According to the NASS 2022 Census on Agriculture (p 20), there were 52,984 independent hog growers, 652 contractor or integrator hog farms, and 7,273 contract growers.	

			Online references – too big to upload: 2022 NASS Census on Agriculture - https://www.nass.usda.gov/Publications/AgCensus/2022/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf	
3571	Carbon dioxide	Carbon dioxide should be moved to Tier 2.	This section fails to discuss the welfare concerns associated with use of CO2, including pain, respiratory distress, anxiety and other negative affective states. Inclusion of such a discussion would indicate why gassing with CO2 should be moved into Tier 2, rather than Tier 1. For example, "Based on the time to loss of SEPs [somatosensory evoked potentials], it is concluded that during killing with a high concentration of carbon dioxide, pigs would have to endure a moderate to severe respiratory distress induced with this gas for a considerable period of time prior to the loss of brain responsiveness" (Raj 1999). "The results indicated that exposure to 2 per cent oxygen in argon (anoxia) induced minimal respiratory distress, 30 per cent carbon dioxide in argon with 2 per cent residual oxygen induced a moderate distress and exposure to all the	- 1997 Raj Welfare Implications of Gas Stunning Pigs: 3. the Time to Loss of Somatosensory Evoked Potentials & Spontaneous Electrocorticogram of Pigs During Expsoure to Gases - 1996 Raj Welfare Implications of the gas stunning of pigs: 2 stress of induction of anesthesia." - 2018 Strangulation, Suffocation, and Asphyxia book chapter:

			concentrations of carbon dioxide in air induced severe respiratory distress in the pigs" (Raj 1996). "Inhaled CO2 causes respiratory acidosis and is painful due to the formation of carbonic acid on mucous membranes of the respiratory tract and conjunctiva. It also causes breathlessness (air hunger) and induces a fear response due to its effect on the amygdala" (McEwen 2018). And "[i]t has been demonstrated that pigs find CO2 in high concentrations aversive and, given a free choice, they avoid such atmospheres (Raj and Gregory, 1995; EFSA, 2004). CO2 itself causes irritation of the nasal mucosa, and exposure induces a painful sensation (Steiner et al., 2019). CO2 has the potential to cause welfare consequences via three 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 (Raj, 2006; Beausoleil and Mellor, 2015; AVMA, 2020)"	

			Online reference - too big to upload: EFSA Panel on Animal Health and Welfare (AHAW), Nielsen SS, Alvarez J, et al. Welfare of pigs at slaughter. EFSA J. 2020;18(6):e06148. Published 2020 Jun 17. doi:10.2903/j.efsa.2020.6148	

3572-3573	Carbon dioxide is a practical means for depopulation provided certain criteria are met to address the numbers and size of swine and overall throughput.	Carbon dioxide has been considered a practical means for depopulation provided certain criteria are met to address the numbers and size of swine and overall throughput. However, carbon dioxide is controversial due to its reported impacts on animal welfare, specifically its aversive nature and the fear, pain, and air hunger it causes in animals such as pigs (Steiner 2019). Research has found that, relative to other gaseous methods of inducing unconsciousness, CO2 causes pigs "moderate to severe respiratory distress induced…for a considerable period of time prior to the loss of brain responsiveness" (Raj 1999). Another study characterized	This section omits discussion of the affective states, such as pain, respiratory distress, and fear, experienced prior to loss of consciousness in pigs killed with CO2. Given that one role of the Guidelines is to guide veterinarians and others in comparing depopulation methods on the basis of animal welfare, it is essential that this issue be clearly and comprehensively presented. Online reference - too big to upload: EFSA Panel on Animal Health and Welfare (AHAW), Nielsen SS, Alvarez J, et al. Welfare of pigs at slaughter. EFSA J. 2020;18(6):e06148. Published 2020 Jun 17. doi:10.2903/j.efsa.2020.6148	2019 Humanely Ending the Life of Animals - Research Priorities to Identify Alternatives to C02 - 1997 Raj Welfare Implications of Gas Stunning Pigs: 3. the Time to Loss of Somatosensory Evoked Potentials & Spontaneous Electrocorticogram of Pigs During Expsoure to Gases - 1996 Raj Welfare Implcations of the gas stunning of pigs: 2 stress of induction of anesthesia." - 2018 Strangulation, Suffocation, and Asphyxia book chapter:

		the respiratory distress caused by CO_2 in pigs to be "severe " (Raj 1996). Another study reports: "Inhaled CO_2 causes respiratory acidosis and is painful due to the formation of carbonic acid on mucous membranes of the respiratory tract and conjunctiva. It also causes breathlessness (air hunger) and induces a fear response due to its effect on the amygdala" (McEwen 2018). In its report on pig slaughter, the EFSA evaluates CO_2 and reports: "It has been demonstrated that pigs find CO_2 in high concentrations aversive and, given a free choice, they avoid such atmospheres" and they identify three different mechanisms by which it negatively		

		impacts animal welfare: "(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 (Raj, 2006; Beausoleil and Mellor, 2015; AVMA, 2020)" (EFSA 2020 Welfare of pigs at slaughter - doi: 10.2903/j.efsa.2020 .6148).		

3729	Manual blunt force trauma	This method should be moved to Tier 3 and the Guidelines should specify that it should only be used on small numbers of animals.	Manual blunt force trauma for pigs should be reassigned from Tier 1 to Tier 3 and described as “not recommended” for killing large numbers of animals. This method should be used only as a last resort for depopulation of piglets. It must be described accurately in the Guidelines. Manual blunt force trauma is typically performed “by striking the animal’s head with a hammer” or “swinging the young animal against the floor or a wall" (Dalla Costa 2020; Grist 2018). While this method of killing may result in instantaneous loss of consciousness when performed perfectly, it carries a high risk of negative animal welfare outcomes because: (1) a high level of skill is required to perform it properly; (2) it can lead to prolonged and significant pain and distress when performed imperfectly; and (3) operators are highly prone to fatigue (Velarde 2018, Dalla Costa 2020, Anderson 2022). The AVMA’s Euthanasia Guidelines explain that “[f]atigue can lead to inconsistency in application, creating humane concerns about its efficacious application to large numbers of	- Dalla Costa, F. A., Gibson, T. J., Oliveira, S. E. O., Gregory, N. G., Coldebella, A., Faucitano, L., Ludtke, C. B., Buss, L. P., & Dalla Costa, O. A. (2020). Evaluation of physical euthanasia for neonatal piglets on-farm. Journal of Animal Science, 98(7), skaa204. https://doi.org/10.1093/jas/skaa204 - Grist, A., Lines, J. A., Knowles, T. G., Mason, C. W., & Wotton, S. B. (2018). The Use of a Non-Penetrating Captive Bolt for the Euthanasia of Neonate Piglets. Animals: an open access journal from MDPI, 8(4), 48. https://doi.org/10.3390/ani8040048 - Velarde, A. & Dalmau, A. (2018). Chapter 10 - Slaughter of pigs. In M. Špinka (Ed.) Advances in Pig Welfare. Woodhead Publishing, pp. 295-322. https://doi.org/10.1016/B978-0-08-101012-9.00010-1 34 AVMA. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf. Page

			animals." As a result, "the AVMA encourages those using manually applied blunt force trauma to the head as a euthanasia method to actively search for alternate approaches." Research has found that determining consciousness can be difficult when manual blunt force trauma is used as a killing method. Thus, piglets killed by this method often receive repeated blows—even under controlled research conditions. The EFSA notes that, because this method of killing is "prone to error . . . the probability of achieving an immediate and humane killing in all cases is low." In recognition that incomplete concussion leads to "pain and fear," the EFSA's expert animal welfare panel does not recommend manual blunt force trauma as an on-farm killing method. In the European Union, this method is not permitted to be used routinely, but only "where there are no other methods available" (Council Directive 1099/2009. 2009. Council Regulation No. 1099/2009 on the protection of animals at the time of killing. Off. J. Eur. Union L303:1–30).	42. 35 AVMA. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf. Page 42. - Whiting, T. L., Steele, G. G., Wamnes, S., & Green, C. (2011). Evaluation of methods of rapid mass killing of segregated early weaned piglets. The Canadian Veterinary Journal = La Revue Veterinaire Canadienne, 52(7), 753–758. - EFSA Panel on Animal Health and Welfare. (2020). Welfare of pigs during killing for purposes other than slaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195. https://doi.org/10.2903/j.efsa.2020.6195 - Dalla Costa, F. A., Gibson, T. J., Oliveira, S. E. O., Gregory, N. G., Coldebella, A., Faucitano, L., & Dalla Costa, O. A. (2019). On-farm pig dispatch methods and stockpeople attitudes on their use. Livestock Science, 221, 1–5. https://doi.org/10.1016/j.livsci.2019.01.007

			In addition to its impact on animals, performing manual blunt force trauma on a large number of animals carries unacceptable risks to the psychological well-being of operators. For this reason, under E.U. regulations, no one is permitted to kill more than 70 animals per day by this method. Accordingly, to protect both animal and human welfare, manual blunt force trauma should be designated a Tier 3 method.	
3734	This approach can have significant impact on mental health of employees.	This approach can have a significant impact on the mental health of employees. Because of this, under European Union regulations,	The discussion around psychological impacts on workers should be expanded. See page 23 of attached reference for requirements when “percussive blow to the head” is utilized in the EU.	COUNCIL REGULATION (EC) No 1099_2009

		workers are not permitted to kill more than 70 animals per day with manual blunt force trauma (Council Directive 1099/2009. 2009. Council Regulation No. 1099/2009 on the protection of animals at the time of killing. Off. J. Eur. Union L303:1–30.)		

3736	Water based foams	Water-based foam should be moved to Tier 3.	Water-based foam kills by obstructive asphyxia and/or drowning and should be downgraded to Tier 3. It is currently identified as Tier 1 for pigs. According to the draft Guidelines, Tier 1 methods “are supported by multiple sources of evidence suggesting that they result in rapid loss of consciousness and optimize animal welfare outcomes." However, available evidence indicates that welfare is severely compromised when death occurs via obstruction of the airway. The expert Panel on Animal Health and Welfare of the European Food Safety Authority (EFSA) has found that water-based foam should not be used because it is “highly painful” and, as a “method designed to cause occlusion of the trachea,” is “equivalent to death by drowning or suffocation.” The AVMA’s 2020 Guidelines for the Euthanasia of Animals list both asphyxiation and drowning as methods that are “unacceptable as primary methods of euthanasia,” noting specifically that drowning is “inhumane.” In addition, the United Kingdom’s	Sign on letter -Beausoleil, N. J., & Mellor, D. J. (2015). Introducing breathlessness as a significant animal welfare issue. NewZealand Veterinary Journal, 63(1), 44–51. https://doi.org/10.1080/00480169.2014.940410 -Ludders, J. W., Schmidt, R. H., Dein, F. J., & Klein, P. N. (1999). Drowning Is not euthanasia. Wildlife SocietyBulletin, 27(3), 666–670. -EFSA Panel on Animal Health and Welfare (2020). Welfare of pigs during killing for purposes other thanslaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195. https://doi.org/10.2903/j.efsa.2020.6195 -EFSA Panel on Animal Health and Welfare. (2019). Killing for purposes other than slaughter: poultry. EFSAJournal. European Food Safety Authority, 17(11), e05850. https://doi.org/10.2903/j.efsa.2019.5850-EFSA Panel on Animal Health and Welfare. (2024). The use of high expansion foam for stunning and killing pigsand poultry. EFSA Journal. European

			governmental Animal Welfare Committee states that water-based foam should not be used for killing animals, noting that "[w]elfare concerns arise from this mode of action which is equivalent to drowning or suffocation . . . neither of which are recognised as humane under European legislation nor the 2018 World Organisation for Animal Health guidelines on the killing of animals for disease control purposes." Further, even precautions such as ensuring the foam level rapidly rises to two times the animal's head height do not decrease the average time to unconsciousness much below three minutes from the start of foaming—a relatively long period for animals to suffer pain, respiratory distress, fear, anxiety, and helplessness. There is thus significant evidence that using water-based foam to depopulate pigs (or other livestock) is "contrary to good animal welfare." For this reason, and because of the availability of other practical, scalable, higher-welfare methods, the use of water-based foam should be designated a Tier 3 method.	Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855. -AVMA. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Page 112. -United Kingdom Animal Welfare Committee (2024). Opinion on the use of high expansion nitrogen foam deliverysystems for depopulation of poultry flocks affected by notifiable disease in the UK. Available at:https://www.gov.uk/government/publications/awc-opinion-on-high-expansion-nitrogen-foam-for-cullingpoultry/awc-opinion-on-the-use-of-high-expansion-nitrogen-foam-for-culling-poultry - Campler, M. R., Cheng, T.Y., Arruda, A. G., Flint, M., Kieffer, J. D., Youngblood, B., & Bowman, A. S. (2023).Refinement of water-based foam depopulation procedures for finisher pigs during field conditions: Welfareimplications and logistical aspects. Preventive Veterinary Medicine, 217, 105974.https://doi.org/10.1016/j.prevetmed.2023.10597424 Korenyi-Both, J., Vidaurre, J., Held, T., Campler, M. R., Kieffer,

				J., Cheng, T. Y., Moeller, S. J., Bowman, A. S., &Arruda, A. G. (2022). Description of electroencephalographic data gathered using water-based medium-expansionfoam as a depopulation method for nursery pigs. Scientific Reports, 12(1), 16798. https://doi.org/10.1038/s41598-022-21353-7

3740-3741	Death associated with water-based foam is due to occlusion of the airway.	Death associated with water-based foam is believed to be due to occlusion of the airway, however, this has not been definitively determined to be the case for all animals; drowning and laryngospasm (dry drowning) have not been ruled out as additional mechanisms. While post-mortem lesions include the presence of a large amount of pulmonary edema fluid (Campler 2025 preprint), this finding is compatible with both obstructive asphyxia and drowning as a cause of death (McEwen 2018, McEwen 2016, Pellegrino 2023).	Airway obstruction has not been confirmed to be the sole mechanism of deathin pigs. Drowning or laryngospasm (dry drowning) may be a cause of death in some pigs. Necropsies found "thick pink liquid containing bubbles derived from pulmonary edema" and alveolar hemorrhage in pigs killed with water-based foam (Campler 2025, a preprint which has been submitted ot the panel), findings that are consistent with death via obstructive asphyxia (McEwen 2016, McEwen 2018). However, these findings can also be seen with drowning (Lunetta 2005). For example, Pellegrino (2023) reports: "During drowning, the individual holds their breath consciously until the internal boost to inspire becomes irrepressible and they inhale water. The fluid reaches the airways, stimulating the cough reflex and laryngospasm. At this point, water causes surfactant loss, leading to consumption of the alveolar-capillary membrane, with an increase in its permeability and subsequent generalized pulmonary edema." Lunetta (2005) describes “interstitial and intraalveolar edema and	- 2023 Epidemiology, clinical aspects, and management of pediatric drowning: - 2018 Strangulation, suffocation, and asphyxia - Lunetta P, Modell JH. Macroscopical, microscopical, and laboratory findings in drowning victims a comprehensive review. In: Tsokos M, editor. Forensic pathology reviews. Totowa, NJ: Humana Press; 2005. p. 3–77. - 2018 Drowning and Bodies Recovered from Water book chapter - 2016 McEwen BJ. Nondrowning asphyxia in veterinary forensic pathology: suffocation, strangulation, and mechanical asphyxia

			hemorrhages" as among the "main light microscopic (LM) signs of drowning" and also describes the pathophysiology of pulmonary edema in drowning ("The penetration of drowning media into the respiratory system increases airway pressures and causes a reactive pulmonary edema.") McEwen et al. (2018) notes that pulmonary edema is often found during necropsy and is considered part of the "drowning process." McEwen (2016) discusses how the pulmonary lesions resulting from strangulation, suffocation, and drowning can appear similar. Whether death occurs due to airway obstruction with foam or airway obstruction with water, the presence of a large amount of pulmonary edema fluid deep in the airways of animals killed with water-based foam supports the assertion by the expert Panel on Animal Health and Welfare of the European Food Safety Authority (EFSA) that water-based foam should not be used because it is "highly painful" and, as a "method designed to cause occlusion of the trachea," is "equivalent to death by drowning or suffocation."	

			Online references (too large to attach): -EFSA Panel on Animal Health and Welfare (2020). Welfare of pigs during killing for purposes other than slaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195. https://doi.org/10.2903/j.efsa.2020.6195 - EFSA Panel on Animal Health and Welfare. (2019). Killing for purposes other than slaughter: poultry. EFSA Journal. European Food Safety Authority, 17(11), e05850. https://doi.org/10.2903/j.efsa.2019.5850 - EFSA Panel on Animal Health and Welfare. (2024). The use of high expansion foam for stunning and killing pigs and poultry. EFSA Journal. European Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855.	

3770-3771	Time to unconsciousness in sows is 2 min with brain death and 3 min and 10 seconds.30	Time to unconsciousness has been estimated in various studies by time until cessation of movement and, in one case, by EEG readings. Interpretation of research findings is complicated by the decision of researchers to begin counting after the container holding the animals has been completely filled, to a height twice the animals' heads, which may take up to 4 minutes depending on the size of the container and the number of foam generators. Because negative animal welfare impacts likely begin when the foam reaches snout height, or perhaps earlier, time to loss of consciousness would better be measured from the initiation of foam	The cited reference (Arruda 2022) states, "The average time (SD) to cessation of movement and fatal arrhythmia post foam-filling completion was 2.2 min (34.8 s) and 8.7 min (138.0 s), respectively." Since "[f]oam was delivered until a visible overflow of either the bulk container or trailer occurred" and "[t]he average time (mean ± SD) to fill the enclosed trailer with foam was 83.0 ± 12.0 s (minimum = 67.0, maximum = 96.0," animals would have experienced the negative affective states (fear, anxiety, respiratory distress, and possibly pain) for an average of 3.7 minutes. Many of the other studies on water-based foam have adopted the same convention, "starting the clock" after the container is filled and the animals have been submerged for some time (Lorbach 2021, Capria 2023, Korenyi-Both 2022). One study that started measuring at the start of fill reported a range of 178-204 seconds until cessation of motion, when the most rapid speed of container filling was used (Campler 2023).	- 2022 Reliability of water-based medium-expansion foam as a depopulation method for nursery pigs and cull sows - Lorbach J N, Campler MR, Youngblood B, et al. Comparison of gaseous and water-based medium-expansion foam depopulation methods in cull sows. Animals (Basel). 2021;11(11):3179. doi:10.3390/ani11113179 - Capria VM, Arruda AG, Cheng TY, et al. Water-based medium-expansion foam depopulation of adult cattle. Trans An Sci. 2023:7(1):txad065 - Campler MR, Cheng TY, Arruda AG, et al. Refinement of water-based foam depopulation procedures for finisher pigs during field conditions: Welfare implications and logistical aspects. Prev Vet Med. 2023:217:105974. - Korenyi-Both J, Vidaurre J, Held T, et al. Description of electroencephalographic data gathered using water-based medium-expansion foam as a depopulation method for nursery pigs. Scientific Reports. 2022;12(1):16798 - 2023 Water-based foam – cattle – Capria

		generation. The studies on water-based foam indicate that, even with rapid fill, time to loss of consciousness cannot be decreased much below 3 minutes.		

3776-3777	Further work is needed on the meaning of the presence of foam in the trachea to understand the implications and the mechanism by which water-based foam causes death.	Necropsies of animals depopulated with water-based foam demonstrate foam in the airway, and post-mortem lesions include a large amount of hemorrhagic froth throughout the bronchial tree; such lesions can be found in animals who die from airway obstruction or drowning. Based on what is known about the physiologic and clinical response to airway occlusion (see attached references), it is likely that animals experience fear, anxiety, and pain prior to losing consciousness. These negative animal welfare impacts require that higher welfare methods be used in preference to water-based foam, and that water-based	Water-based, medium expansion foam causes death in poultry by occluding the airway, resulting in obstructive asphyxiation. The porcine research on this method has not definitively determined whether airway occlusion with foam (i.e., obstructive asphyxiation), drowning, or laryngospasm (i.e., “dry drowning”)—or all three—are its mechanism(s) of killing pigs. As clinicians, many of us are familiar with patients experiencing acute partial airway obstruction and/or pulmonary edema; in either instance, such animals are typically in significant distress, showing signs of fear, anxiety, and even panic. In emergency veterinary medicine, sedation is a standard part of treatment to alleviate "fear and anxiety" in patients with upper airway obstruction (Rozanski 2018). Research sheds light on the physiological mechanisms underlying these affective states. For example, a 2015 paper (Beausoleil) reviewing the animal welfare impacts of “breathlessness” (often called “dyspnea” in American publications) explains: “During both drowning and asphyxia,	- Rozanski, E., Drobatz, K. J., Hopper, K., & Silverstein, D. C. (2018). Respiratory Distress. In Textbook of Small Animal Emergency Medicine (pp. 18–21). John Wiley & Sons, Inc. https://doi.org/10.1002/9781119028994.ch4 - Beausoleil, N. J., & Mellor, D. J. (2015). Introducing breathlessness as a significant animal welfare issue. New Zealand Veterinary Journal, 63(1), 44–51. https://doi.org/10.1080/00480169.2014.940410- 17 Ludders, J. W., Schmidt, R. H., Dein, F. J., & Klein, P. N. (1999). Drowning Is not euthanasia. Wildlife Society Bulletin, 27(3), 666–670. - 2022 Complete tracheal obstruction during anaesthesia for ventral slot decompression- - United Kingdom Animal Welfare Committee (2024). Opinion on the use of high expansion nitrogen foam deliverysystems for depopulation of poultry flocks affected by notifiable disease in the UK. Available at:https://www.gov.uk/government/publications/awc-opinion-on-

		foam be used only when the suffering associated with the method is likely to be significantly less than the suffering resulting from doing nothing.	airflow is occluded and thoracic volume expansion is impossible. Pulmonary gas exchange stops and carbon dioxide and oxygen tensions equilibrate between blood and static air (or water) in the lungs (Ludders et al. 1999). Cellular metabolism continues, first using up the available oxygen and producing an accumulation of carbon dioxide; progressive hypoxaemia and hypercapnia will stimulate automatic drive to breathe. As oxygen is depleted, cells switch to glycolysis which generates a lactacidosis, further enhancing drive to breathe. The complete lack of afferent feedback from pulmonary stretch receptors, superimposed on rapidly rising automatic drive to breathe will produce severe air hunger before loss of consciousness (Ludders et al. 1999).” Rapid onset of hypercarbia has been demonstrated with airway occlusion (Murray 2022). The negative welfare impacts of airway occlusion and drowning are the reason water-based foam is considered “highly painful” and “equivalent to death by drowning or suffocation" by European animal welfare experts. This method is	high-expansion-nitrogen-foam-for-cullingpoultry/awc-opinion-on-the-use-of-high-expansion-nitrogen-foam-for-culling-poultry

			not permitted by European legislation nor the 2018 World Organisation for Animal Health guidelines on the killing of animals for disease control purposes. Online references (too large to upload): - EFSA Panel on Animal Health and Welfare (2020). Welfare of pigs during killing for purposes other than slaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195. https://doi.org/10.2903/j.efsa.2020.6195 - EFSA Panel on Animal Health and Welfare. (2019). Killing for purposes other than slaughter: poultry. EFSAJournal. European Food Safety Authority, 17(11), e05850. https://doi.org/10.2903/j.efsa.2019.5850 - EFSA Panel on Animal Health and Welfare. (2024). The use of high expansion foam for stunning and killing pigsand poultry. EFSA Journal. European Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855.	

3778	Nitrogen filled foam	High expansion nitrogen gas-filled foam [Note: This is a nitrogen/anoxia based method and, while it should be included under Tier 1, it should **not** be categorized as a water-based foam. It should be moved to Inhaled Methods.]	Extensive research has documented that, properly deployed such that bubble size and expansion ratio are correct, high expansion foam filled with nitrogen gas causes death via creating an anoxic environment; the bubbles are a carrier for the gas. Its mechanism of killing is very different from water-based foam, which relies on airway occlusion (obstructive asphyxia). Physiologic changes, necropsy findings, and affective states differ considerably. To avoid confusion, it is essential that this method be appropriately categorized. When high expansion nitrogen foam is used to create an anoxic or near-anoxic environment, the animals continue to be able to inhale and exhale, and they continue to be able to offload carbon dioxide. Interestingly, creating an anoxic environment via the introduction of nitrogen gas to displace oxygen has been advocated by groups that advance medically assisted dying. In Switzerland, where this practice is legal, a nitrogen capsule is now available to terminally ill human patients seeking this option	2023 Culhane presentation 2022 complete tracheal obstruction… 2015 introducing breathlessness 2016 nondrowning asphyxia in veterinary forensic 2018 strangulation, suffocation, and asphyxia

			(https://www.exitinternational.net/sarco/concept/). In contrast, when the airway is occluded, both oxygen exchange and carbon dioxide removal are prevented. It is well understood that, when the airway occluded, blood levels of carbon dioxide rise rapidly (see Murray 2022, attached, which states, "[d]uring airway obstruction, failure to eliminate CO2 will cause the arterial partial pressure of CO2 (PaCO2) to increase, resulting in hypercarbia and respiratory acidosis."). We know that air hunger, a particular type of respiratory distress, results from the combination of rising CO2 levels and the inability to expand the lungs. As described by Beausoleil (2015 - also attached): "During both drowning and asphyxia, airflow is occluded and thoracic volume expansion is impossible. Pulmonary gas exchange stops and carbon dioxide and oxygen tensions equilibrate between blood and static air (or water) in the lungs (Ludders et al. 1999). Cellular metabolism continues, first using up the available oxygen and producing an accumulation	

			of carbon dioxide; progressive hypoxaemia and hypercapnia will stimulate automatic drive to breathe. As oxygen is depleted, cells switch to glycolysis which generates a lactacidosis, further enhancing drive to breathe. The complete lack of afferent feedback from pulmonary stretch receptors, superimposed on rapidly rising automatic drive to breathe will produce severe air hunger before loss of consciousness (Ludders et al. 1999).” This paper also reports that, in humans, air hunger is "reported to be the most unpleasant sensation and probably has the greatest potential to compromise animal welfare.” The attached presentation by Marie Culhane (2023) has an informative slide comparing the different types of foam and their mechanisms (slide 13/38). Until recently, we didn't have good necropsy comparisons for high expansion N2 foam [HENF] and water-based foam [WBF]. A soon-to-be-released paper (Campler 2025 – preprint we believe has been submitted to the Depopulation Panel) that	

			compared limited necropsy findings from pigs killed by these methods. Unlike pigs killed with HENF and CO2, pigs killed with WBF were most likely to have pulmonary edema and alveolar hemorrhage. This makes sense, given that previous veterinary forensics research indicates that the lesions associated with obstructive asphyxiation differ from those seen with anoxia (sometimes called nonobstructive suffocation). For example, McEwen (2018, attached) states, "Non-cardiogenic pulmonary edema occurs in dogs with partial, total, or intermittent airway obstruction," while "nonobstructive suffocation" (due to either replacement of O2 with other gases or with depletion of O2 in the environment) are nonspecific. McEwen (2016, attached) notes "there are often no macroscopic lesions in nonobstructive suffocation," whereas "partial, total or intermittent airway obstruction causes noncardiogenic pulmonary edema."	

3809	At the publication of this document, there are no Tier 2 methods to describe.	Add carbon dioxide gassing and carbon monoxide to this Tier	Carbon dioxide- and carbon monoxide-based methods should be moved to Tier 2 for animal welfare reasons. Please see comments related to the text of these sections.	- 2019 Humanely Ending the Life of Animals - Research Priorities to Identify Alternatives to C02 - 1997 Raj Welfare Implications of Gas Stunning Pigs: 3. the Time to Loss of Somatosensory Evoked Potentials & Spontaneous Electrocorticogram of Pigs During Expsoure to Gases - 1996 Raj Welfare Implications of the gas stunning of pigs: 2 stress of induction of anesthesia." - 2018 Strangulation, Suffocation, and Asphyxia book chapter:

3812-3814	Sodium nitrite High doses of sodium nitrite have been used in various bait forms for the control of feral swine through the induction of methemoglobinemia when an adequate amount of bait is ingested.	Sodium nitrite Sodium nitrite converts hemoglobin in red blood cells to methemoglobin and thus prevents oxygen transport (Lower 2020). At high doses, it can lead to death by brain and tissue hypoxia. High doses of sodium nitrite have been used in various bait forms for the control of feral swine through the induction of methemoglobinemia when an adequate amount of bait is ingested. However, use of sodium nitrite to kill pigs results in severe welfare compromise. Because of its mechanism of action, pigs who ingest sodium nitrite experience dyspnea (labored breathing or breathlessness) that begins about 30 to 60 minutes after	This section lacks a discussion of the negative affective states associated with methemoglobinemia and vomiting. Discussion of the animal welfare impacts of different methods is essential if the Guidelines are to serve their stated purpose.	2015 Introducing breathlessness as a significant animal welfare issue 5. Lower A. Evaluation of Sodium Nitrite for Mass Euthanasia of Commercial Pigs – NPB #20-118. Carthage Veterinary Services, LTD; 2020. https://porkcheckoff.org/wp-content/uploads/2021/02/20-118-LOWER-final-rpt.pdf 6. Institute of Medical and Veterinary Science. Assessing the Humaneness and Efficacy of a New Feral Pig Bait in Domestic Pigs, Report for the Australian Government Department of the Environment, Water, Heritage and the Arts.; 2010. 7. Pepin B. Determine Effective Oral Dosing of Sodium Nitrite for Efficient Euthanasia of Adult Swine Using Oral Drench Technique – NPB #20-122. Pipestone Veterinary Services; 2020. https://porkcheckoff.org/wp-content/uploads/2021/02/20-122-PEPIN-final-rpt.pdf 8. Cowled BD, Elsworth P, Lapidge SJ. Additional toxins for feral pig (Sus scrofa) control: identifying and testing Achilles' heels. Wildl Res. 2008;35(7):651. doi:10.1071/WR07072

		ingestion and progressively worsens until they die (Pepin 2020, Cowled 2008). As the dyspnea is the result of prolonged hypoxemia (low oxygen levels in the blood), it is experienced as “air hunger” (Beausoleil 2015). Described as “extremely unpleasant and distressing” by humans, air hunger is regarded as the type of breathlessness with the greatest potential to compromise animal welfare. In addition, sodium nitrite poisoning causes multiple episodes of vomiting in roughly half to two-thirds of pigs who ingest it [Lower 2020, Institute of Medical and Veterinary Science 2010, Pepin 2020, Cowled 2008), and pigs killed with		

		sodium nitrite exhibit a large increase in the stress hormone cortisol prior to death (Institute of Medical and Veterinary Science 2010).		

3839-3843	Ventilation shut down plus heat and humidity (VSD+) Recognizing the limitations of VSD and the desire to achieve a quicker time to death, the use of additional techniques/tools have been added (such as heat and humidity) to VSD resulting in what is often referred to as VSD plus. It is recommended that VSD plus approaches be prioritized as much as possible over VSD.	Ventilation shut down plus heat and humidity (VSD+) Ventilation shutdown plus heat and humidity involves confining pigs to a barn previously retrofitted for VSD+ and adding heat and steam to induce heatstroke in the animals (Baysinger 2021, Reyes-Illg 2023). Killing via heatstroke severely compromises animal welfare for a prolonged period (Reyes-Illg 2023). For example, clinical sequelae of heatstroke are described as including "distributive shock, gastrointestinal bleeding and sloughing with attendant vomiting and hemorrhagic diarrhea, abdominal organomegaly, rhabdomyolysis, acute respiratory	This section fails to include a discussion of the welfare impacts of VSD+ and the potential for pigs to sustain burns of the skin and airway at the temperature and humidity conditions created by VSD+TH. It is essential that veterinarians and others considering implementing this method be aware of this information, as it is essential for determining, for all Tier 3 methods, whether "the risk of doing nothing is likely to have a reasonable chance of resulting in significantly more animal suffering than that associated with the proposed depopulation method" (lines 532-533).	- the rise of heatstroke- -Baysinger 2021 VSD+TH - Bruchim, Y.; Horowitz, M.; Aroch, I. Pathophysiology of heatstroke in dogs—Revisited. Temperature 2017, 4, 356–370. [Google Scholar] [CrossRef] [PubMed] [Green Version] -Bruchim, Y.; Loeb, E.; Saragusty, J.; Aroch, I. Pathological findings in dogs with fatal heatstroke. J. Comp. Pathol. 2009, 140, 97–104. [Google Scholar] [CrossRef] [PubMed] -Koljonen, V. Hot air sauna burns—Review of their etiology and treatment. J. Burn Care Res. 2009, 30, 705–710. [Google Scholar] [CrossRef] -Kluger, N.; Laipio, J.; Virolainen, S.; Ranki, A.; Koljonen, V. A Fatal Case of Hot Air Sauna Burn in an Elderly Patient Initially Misdiagnosed as Bullous Pemphigoid. Acta Derm. Venerol. 2011, 91, 732–733. [Google Scholar] [CrossRef] [Green Version] -Ghods, M.; Corterier, C.; Zindel, K.; Kiene, M.; Rudolf, K.; Steen, M. Hot air sauna burns. Burns 2008, 34, 122–124. [Google Scholar] [CrossRef] [PubMed]

		distress syndrome, brain injury and neurological abnormalities, multiorgan dysfunction, and coagulopathies, including disseminated intravascular coagulation (DIC), frequently ending in hemorrhagic diathesis. In dogs, the cause of death in fatal heat stroke is typically shock and respiratory failure due to accumulation of frothy, hemorrhagic fluid in the airways" (Reyes-Illg 2023). In addition, the high levels of temperature and humidity that have been used in an attempt to reduce the time to death in pigs subjected to VSD+ raise concerns about the potential for burns. Reyes-Illg (2023) notes: "It can be		- Koski, A.; Koljonen, V.; Vuola, J. Rhabdomyolysis caused by hot air sauna burn. Burns 2005, 31, 776–779. [Google Scholar] [CrossRef] [PubMed] - Papp, A. Sauna-related burns: A review of 154 cases treated in Kuopio University Hospital Burn Center 1994-2000. Burn. J. Int. Soc. Burn Inj. 2002, 28, 57–59. [Google Scholar] [CrossRef] [PubMed] - Kudchadkar, S.R.; Hamrick, J.T.; Mai, C.L.; Berkowitz, I.; Tunkel, D. The heat is on… thermal epiglottitis as a late presentation of airway steam injury. J. Emerg. Med. 2014, 46, e43–e46. [Google Scholar] [CrossRef] [PubMed] - Hathaway, P.B.; Stern, E.J.; Harruff, R.C.; Heimbach, D.M. Steam inhalation causing delayed airway occlusion. Ajr Am. J. Roentgenol. 1976, 166, 322. [Google Scholar] [CrossRef] [Green Version] - Zhai, L.; Adlhart, C.; Spano, F.; Innocenti Malini, R.; Piątek, A.K.; Li, J.; Rossi, R.M. Prediction of Steam Burns Severity using Raman Spectroscopy on ex vivo Porcine Skin. Sci. Rep. 2018, 8, 6946. [Google Scholar] [CrossRef] [Green Version]

		speculated that, in pigs, VSD+TH [VSD+high temperature and humidity] may cause severe burns prior to LOC [loss of consciousness], especially at the higher reported temperature ranges. Pigs are frequently used in burn research because of the extensive anatomical and physiological similarities between porcine and human skin. Temperature conditions at the high end of the range reported for VSD+TH are similar to those known to cause second- and third-degree hot air sauna burns (HASB) and rhabdomyolysis in humans who lose consciousness or become immobile in a sauna for as little as 30 min. As discussed above, when VSD+TH is		- Holm, S.; Engström, O.; Melander, M.; Horvath, M.C.S.; Fredén, F.; Lipcsey, M.; Huss, F. Cutaneous steam burns and steam inhalation injuries: A literature review and a case presentation. Eur. J. Plast. Surg. 2022, 45, 881–896. [Google Scholar] [CrossRef] - Shamohammadi, H.; Mehrabi, S.; Sadrizadeh, S.; Yaghoubi, M.; Abouali, O. 3D numerical simulation of hot airflow in the human nasal cavity and trachea. Comput. Biol. Med. 2022, 147, 105702. [Google Scholar] [CrossRef] - Wan, J.; Zhang, G.; Qiu, Y.; Wen, C.; Fu, T. Heat dissipation by blood circulation and airway tissue heat absorption in a canine model of inhalational thermal injury. Burns 2016, 42, 548–555. [Google Scholar] [CrossRef] - Moritz, A.R.; Henriques, F.C.; McLean, R. The Effects of Inhaled Heat on the Air Passages and Lungs: An Experimental Investigation. Am. J. Pathol. 1945, 21, 311–331. [Google Scholar]

		performed, the temperature within the barn may be raised as high as 76.7 °C (170.1 °F), well within the range of temperatures found in saunas (70–100 °C, or 158–212 °F). The introduction of steam with VSD+TH to raise the humidity to a minimum of 90% creates humidity levels similar to those of a steam room. Because of the heat-carrying capacity of steam, steam rooms are typically kept at cooler temperatures than saunas, around 43.3–48.9 °C (110–120 °F), to prevent thermal discomfort (https://www.saunasociety.org/sauna-types). Research using ex vivo porcine skin has shown that temperature and humidity conditions similar to those created during		

		VSD+TH (70 °C or 158 °F, relative humidity of 75%) increase the permeability of the stratum corneum as much as 50 times compared to room temperature and lead to burns of the underlying dermis before damage to the epidermis is apparent. Such steam burns are generally considered more severe than burns from hot dry air. Both HASB and steam burns may initially present with limited visually apparent skin changes, which would make it difficult for veterinarians overseeing VSD+TH to recognize such injuries post-mortem. While researchers in the VSD+TH report indicate that they took care to avoid		

		burning pigs with steam as it was discharged from the steam generators, these measures may not protect against burns from hot, highly humidified air. Research on humans and dogs suggests that, at the high end of the temperature range of VSD+TH, inhalation burns may also be possible. This risk may be heightened by the high level of humidity, which carries heat deeper into the respiratory tract." Necropsies have not been performed in pigs killed with VSD+. Because of its prolonged and severely negative impact on animal welfare, VSD+ is not recommended as a depopulation method.		

3869-3870	When ventilation systems fail, pigs may suffer distress or death due to lack of oxygen or excessive CO2.40	In addition to severely compromising animal welfare for a prolonged period (Reyes-Illg 2023), use of VSD alone was found ineffective as a depopulation method (Baysinger 2021).	The cited reference provides no support for this sentence. While the author of the cited reference conjectures that "If ventilation fails, pigs may suffer distress or death by what is commonly called 'suffocation'" - implying lack of oxygen or excessive CO2 - the reference states that "neither O2 nor CO2 levels" were monitored during the accidental failure of the ventilation systems in the two cases. In one of the case studies, none of the animals died. In the other study, "around 30 sows died" after 16 hours, during which barn temperature was documented to rise significantly, to over 95 °F (35 °C). All available research suggests that heatstroke is the cause of death with VSD, and the "clinical sequelae of heatstroke are concerning. Across species, they include distributive shock, gastrointestinal bleeding and sloughing with attendant vomiting and hemorrhagic diarrhea, abdominal organomegaly, rhabdomyolysis, acute respiratory distress syndrome, brain injury and neurological abnormalities, multiorgan dysfunction, and	- The Rise of Heatstroke as a Method of Depopulating Pigs and Poultry: Implications for the US Veterinary Profession - Baysinger - VSD+TH - Bruchim, Y.; Horowitz, M.; Aroch, I. Pathophysiology of heatstroke in dogs—Revisited. Temperature 2017, 4, 356–370. [Google Scholar] [CrossRef] [PubMed] [Green Version] - Bruchim, Y.; Loeb, E.; Saragusty, J.; Aroch, I. Pathological findings in dogs with fatal heatstroke. J. Comp. Pathol. 2009, 140, 97–104. [Google Scholar] [CrossRef] [PubMed] - Pathophysiology and pathological findings of heatstroke in dogs

			coagulopathies, including disseminated intravascular coagulation (DIC), frequently ending in hemorrhagic diathesis. In dogs, the cause of death in fatal heat stroke is typically shock and respiratory failure due to accumulation of frothy, hemorrhagic fluid in the airways [68]" (Reyes-Illg 2023).	
4117-4119	In rare cases, the use of sedatives or anesthesia delivered via a dart gun might be considered before the depopulation method is applied. The perceived benefit should be weighed against the risk of incomplete sedation and increased stress induced by this method.	In rare cases, the use of sedatives or anesthesia delivered via a dart gun might be considered before the depopulation method is applied. The perceived benefit should be weighed against the risk of incomplete sedation and increased stress induced by this method. Completion of regular training	With proper training, administration of sedatives/anxiolytics/anesthetic via dart gun should reduce stress significantly in non-tame animals. Proper training, such as through a Safe-Capture course, is required.	

		programs in safe administration of chemical immobilization and anesthesia via darting is recommended in scenarios in which this might be needed (e.g., Safe-Capture (R) via San Diego Zoo Wildlife Alliance Academy - https://sdzwaacademy.org/safecapture/index.php).		
4314-4316	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.	While both landmarks are considered acceptable for captive bolt placement, the crown site is strongly recommended due to the higher risk of incomplete concussion when the frontal site is used, particularly if an increased restraint (which may negatively impact welfare) is not employed (Ginson 2015).	Given that the research paper cited concluded: "The behavioural/cranial/spinal responses and the gross pathology results of this study suggest that the preferred shooting position for alpacas is on midline on top of the head (crown position). Shooting in this position maximises the probability of the bolt penetrating into or damaging the parietal/occipital lobes, thalamus, midbrain, pons or medulla. Damage to these structures was found to be associated with decreased odds of incomplete concussion," this position should be strongly recommended in the	Gibson TJ, Whitehead C, Taylor R, Sykes O, Chancellor NM, Limon G. Pathophysiology 4482 of penetrating captive bolt stunning in Alpacas (Vicugna pacos). Meat Science. 2015;100:227-231. 4483 doi:10.1016/j.meatsci.2014.10.022

			Depopulation Guidelines, rather than concluding that either site is acceptable.	
4337-4341	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% CO2 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.13 All kids entering the chamber of 70% CO2 lost consciousness while consuming the meal.	Carbon dioxide inhalation as a form of euthanasia has been evaluated in young goat kids (< 2 weeks of age). Aversion testing suggests that concentrations below 30% CO2 lead to a level of aversion insufficient to prevent feeding in these neonates. However, the authors concluded, "It is likely that pain was experienced by all kids during exposure to all CO2 concentrations" (Withrock 2015). The authors speculate that pain perception in this study may have been impacted by concurrent consumption of	The single study cited is a master's thesis that only tested CO2 concentrations up to 30%, not 70%. It concluded that "It is likely that pain was experienced by all kids during exposure to all CO2 concentrations. This is supported by the increase in treatment vocalizations during gas treatment days compared to both baseline and washout days, which agrees with previous literature reporting vocalizations as an indicator of stress in kids (Price and Thos, 1980, Lyons et al., 1993, Siebert et al., 2011)...Although 30% CO2 had fewer vocalizations than 20% CO2, the difference in vocalization frequency can likely be attributed to the shortened latency to ataxia and unconsciousness during 30%." It also noted that consuming food while being exposed to the elevated CO2 concentrations may have impacted pain perception, "a circumstance	- 2015 The use of carbon dioxide (CO2) as an alternative euthanasia method for goat kids- - 2019 Humanely Ending the Life of Animals - Research Priorities to Identify Alternatives to C02 -1997 Raj Welfare Implications of Gas Stunning Pigs: 3. the Time to Loss of Somatosensory Evoked Potentials & Spontaneous Electrocorticogram of Pigs During Expsoure to Gases - 1996 Raj Welfare Implications of the gas stunning of pigs: 2 stress of induction of anesthesia." - 2018 Strangulation, Suffocation, and Asphyxia book chapter:

		food, something unlikely to occur during depopulation. Exposure to moderate to high levels of CO2 has been confirmed to be painful and causes affective states such as respiratory distress and anxiety in other species (see attached references).	that would likely not occur in practice." The kids in the study were "acquired between 1-7 days of age" and "received at least 3 days of acclimation," that is, they were extremely young animals who may not have been developed sufficiently to demonstrate other signs of aversion or may be more tolerant of hypercarbia/hypercapnia than even slightly older animals. This section fails to discuss the welfare concerns associated with the use of CO2, including pain, respiratory distress, anxiety, and other negative affective states in other species. For example: -"Based on the time to loss of SEPs [somatosensory evoked potentials], it is concluded that during killing with a high concentration of carbon dioxide, pigs would have to endure a moderate to severe respiratory distress induced with this gas for a considerable period of time prior to the loss of brain responsiveness" (Raj 1999). - "The results indicated that exposure to 2 per cent oxygen in argon (anoxia) induced minimal	

			respiratory distress, 30 per cent carbon dioxide in argon with 2 per cent residual oxygen induced a moderate distress and exposure to all the concentrations of carbon dioxide in air induced severe respiratory distress in the pigs" (Raj 1996). - "Inhaled CO_2 causes respiratory acidosis and is painful due to the formation of carbonic acid on mucous membranes of the respiratory tract and conjunctiva. It also causes breathlessness (air hunger) and induces a fear response due to its effect on the amygdala" (McEwen 2018). And - "It has been demonstrated that pigs find CO_2 in high concentrations aversive and, given a free choice, they avoid such atmospheres (Raj and Gregory, 1995; EFSA, 2004). CO_2 itself causes irritation of the nasal mucosa and exposure is therefore inducing a painful sensation (Steiner et al., 2019). CO_2 has the potential to cause welfare consequences via three 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 (Raj, 2006; Beausoleil and Mellor, 2015; AVMA, 2020)" (EFSA 2020 Welfare of pigs at slaughter - doi: 10.2903/j.efsa.2020.6148). Online references (too large to attach): - EFSA Panel on Animal Health and Welfare (AHAW), Nielsen SS, Alvarez J, et al. Welfare of pigs at slaughter. *EFSA J*. 2020;18(6):e06148. Published 2020 Jun 17. doi:10.2903/j.efsa.2020.6148 - EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen SS, Alvarez J, et al. Welfare of sheep and goats during killing for purposes other than slaughter. *EFSA J*. 2024;22(6):e8835. Published 2024 Jun 26. doi:10.2903/j.efsa.2024.8835	

4343-4344	Death by CO_2 inhalation has not been evaluated in older goats or sheep.	Death via inhalation of carbon dioxide has been evaluated in juvenile and adult sheep and has been determined to cause respiratory distress, fear, pain, and stress, similar to other mammalian species (Rodriguez 2019; EFSA Welfare of Sheep and Goats During Killing for Purposes Other than Slaughter 2024)	The Guidelines suggest it is not clear whether CO_2 is aversive to small ruminants of different age groups. However, it fails to incorporate some current research. According to Rodriguez (2016), "Exposure to CO_2 at high concentration induces effective stunning in sheep for a period of 124 s. However, during exposure, the animals exhibited signs of aversion and breathlessness." Specifically, it found, "During the CO_2 exposure, 93% of the lambs exhibited head-shake and sneezing, when brain activity is not depressed and lambs are still conscious, indicating aversion to the carbon dioxide concentration. These results are consistent across a variety of species, such as chickens (*Gallus gallus domesticus*), turkeys (*Meleagris gallopavo*), pigs and mink (*Neovison vison*) (Raj & Gregory 1995, 1996; Raj 1996; Cooper et al 1998), which have shown that they perceive carbon dioxide as being aversive. This aversion to CO_2 has been reported to be greater than motivation to feed (in a CO_2 atmosphere) after overnight	- 2016 Assessment of aversion and unconsciousness during exposure to carbon dioxide at high concentration in lambs - EFSA Welfare of Sheep and Goats During Killing for Purposes Other than Slaughter.

			fasting in pigs, poultry and rats (Raj & Gregory 1995; Raj 1996; Kirkden et al. 2005). It also agrees with Cantieni (1976), who found that the majority of the pigs tested preferred to go without water for 72 h rather than endure exposure to carbon dioxide again. The aversive effect of 90% CO_2 is due to stimulation of highly sensitised CO_2 nociceptors in the nasal mucosae and lungs (Peppel & Anton 1993), where the presence of CO_2-sensitive chemoreceptors has been described (Manning & Schwartzstein 1995). Additionally, carbon dioxide induces severe respiratory distress causing hyperventilation and a sense of breathlessness during the induction phase prior to loss of consciousness (Gregory et al. 1990; Danneman et al .1997). Gasping was also exhibited by 42% of the lambs before loss of consciousness (at 21 s), and occurred at the same time that pCO_2 increased and pO_2 and blood pH decreased compared to basal levels. Gasping is a rudimentary respiratory activity occurring through the mouth, and is associated with	

			breathlessness during the inhalation of CO2 (Raj & Gregory 1996; Llonch et al. 2012). Afterwards, hypercapnia increased respiration rate (from 22.8 [± 0.93] to 37.3 [± 0.90] respiratory movements per min) at 23 s of CO2 exposure, provoking respiratory distress (Raj & Gregory 1996)." Rodriguez (2016) goes on to note that lambs don't vocalize in painful or fearful situations, but they react to aversion and breathlessness via headshaking with sneezing, gasping, and increased RR. It concludes: "The fact that these behaviours occur when the animal is conscious is evidence that induction of CO2 anaesthesia is not immediate and lambs may suffer from fear, pain and/or stress during immersion into gas. The presence of these behaviours clearly indicates aversion to exposure to an atmosphere with a high concentration of CO2." The recently released EFSA Welfare of Sheep and Goats during killing for purposes other than slaughter concurs: "exposure to CO2 at high concentrations does not cause immediate loss of	

			consciousness and lambs may experience discomfort, pain, fear and/or distress." It should be noted that the EFSA's 2024 scientific opinion, Welfare of Sheep and Goats During Killing for Purposes Other than Slaughter, cites numerous studies on the subject of CO_2 gassing in small ruminants that have not been included in the draft Depopulation Guidelines, but should be. The EFSA expert panel concluded with 90–100% certainty that the following statement would apply to at least 50% of sheep and goats: "Exposure to CO_2 at high concentrations (higher than 90% by volume) causes pain and fear in sheep and goats such in other species like pigs."	

4363	Water-based foams	Water-based foam should be moved to Tier 3	Water-based foam as a depopulation method should be downgraded to Tier 3. Available evidence indicates that welfare is severely compromised when death occurs via obstruction of the airway (Beausoleil 2015; Ludders 1999). The expert Panel on Animal Health and Welfare of the European Food Safety Authority (EFSA) has found that water-based foam should not be used because it is "highly painful" and, as a "method designed to cause occlusion of the trachea," is "equivalent to death by drowning or suffocation." The AVMA's 2020 Guidelines for the Euthanasia of Animals list both asphyxiation and drowning as methods that are "unacceptable as primary methods of euthanasia," noting specifically that drowning is "inhumane." In addition, the United Kingdom's governmental Animal Welfare Committee states that water-based foam should not be used for killing animals, noting that "[w]elfare concerns arise from this mode of action which is equivalent to drowning or suffocation . . . neither of which are recognised as humane under European legislation nor the	Sign on letter 16 Beausoleil, N. J., & Mellor, D. J. (2015). Introducing breathlessness as a significant animal welfare issue. New Zealand Veterinary Journal, 63(1), 44–51. https://doi.org/10.1080/00480169.2014.940410 17 Ludders, J. W., Schmidt, R. H., Dein, F. J., & Klein, P. N. (1999). Drowning Is not euthanasia. Wildlife Society Bulletin, 27(3), 666–670. 18 EFSA Panel on Animal Health and Welfare (2020). Welfare of pigs during killing for purposes other than slaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195. https://doi.org/10.2903/j.efsa.2020.6195 19 EFSA Panel on Animal Health and Welfare. (2019). Killing for purposes other than slaughter: poultry. EFSA Journal. European Food Safety Authority, 17(11), e05850. https://doi.org/10.2903/j.efsa.2019.5850 20 EFSA Panel on Animal Health and Welfare. (2024). The use of high expansion foam for stunning and killing pigs and poultry. EFSA Journal.

			2018 World Organisation for Animal Health guidelines on the killing of animals for disease control purposes." Further, even precautions such as ensuring the foam level rapidly rises to two times the animal's head height do not decrease the average time to unconsciousness much below three minutes from the start of foaming—a relatively long period for animals to suffer pain, respiratory distress, fear, anxiety, and helplessness—and there have been no studies carried out on use of water-based foam in sheep or goats. There is significant evidence that using water-based foam to depopulate mammals is "contrary to good animal welfare." For this reason, and because of the availability of other practical, scalable, higher-welfare methods, the use of water-based foam should be designated a Tier 3 method.	European Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855. 21 AVMA. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Page 112. 22 United Kingdom Animal Welfare Committee (2024). Opinion on the use of high expansion nitrogen foam delivery systems for depopulation of poultry flocks affected by notifiable disease in the UK. Available at: https://www.gov.uk/government/publications/awc-opinion-on-high-expansion-nitrogen-foam-for-cullingpoultry/awc-opinion-on-the-use-of-high-expansion-nitrogen-foam-for-culling-poultry - Campler, M. R., Cheng, T.-Y., Arruda, A. G., Flint, M., Kieffer, J. D., Youngblood, B., & Bowman, A. S. (2023). Refinement of water-based foam depopulation procedures for finisher pigs during field conditions: Welfare implications and logistical aspects. Preventive Veterinary Medicine, 217, 105974. https://doi.org/10.1016/j.prevetmed.2023.105974 - Korenyi-Both, J., Vidaurre, J., Held, T., Campler, M. R., Kieffer,

				J., Cheng, T. Y., Moeller, S. J., Bowman, A. S., & Arruda, A. G. (2022). Description of electroencephalographic data gathered using water-based medium-expansion foam as a depopulation method for nursery pigs. Scientific Reports, 12(1), 16798. https://doi.org/10.1038/s41598-022-21353-7 - Capria VM, Arruda AG, Cheng TY, et al. Water-based medium-expansion foam depopulation of adult cattle. Trans An Sci. 2023:7(1):txad065 - EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen SS, Alvarez J, et al. Welfare of sheep and goats during killing for purposes other than slaughter. EFSA J. 2024;22(6):e8835. Published 2024 Jun 26. doi:10.2903/j.efsa.2024.8835

4363-4369	Water based foams Studies have evaluated the use of water-based foams and provided sufficient evidence of their efficacy to include them as a viable tier 2 option for sheep and goats. Phos-check Class A AFFF concentrate mixed according to label directions can be used in an any solid side vessel or pen. Dwell times of 15 minutes should be used to assure efficacy. These techniques have not been validated in captive cervids but deserve additional study. It is important to utilize foam that is free of PFAFs as these pose significant environmental and public health risks.15	Water based foams Studies have evaluated the use of water-based foams in poultry and pigs, although no studies have focused primarily on the welfare impacts of this method. This technique has not been validated in captive cervids or small ruminants. While submerging animals in water-based foam is effective at killing pigs after several minutes, this mechanism of killing (obstructive asphyxia) results in an extremely increased drive to breathe (due to rapidly worsening hypercarbia (Murray 2022)) combined with an inability to expand the lungs. Such physiologic changes in a conscious animal result in severe air hunger, a type of	There has been no research on use of water-based foam for sheep and goats. While it has shown to kill most pigs after a sufficient dwell time, animal welfare is severely compromised prior to losing consciousness. Accurate information about its mechanism of killing and the likely accompanying affective states must be provided for an accurate assessment of this method.	EFSA Welfare of Sheep and Goats During Killing for Purposes Other than Slaughter. - 2022 Complete tracheal obstruction during anaesthesia for ventral slot decompression - Beausoleil, N. J., & Mellor, D. J. (2015). Introducing breathlessness as a significant animal welfare issue. New Zealand Veterinary Journal, 63(1), 44–51. https://doi.org/10.1080/00480169.2014.940410 - Ludders, J. W., Schmidt, R. H., Dein, F. J., & Klein, P. N. (1999). Drowning Is not euthanasia. Wildlife Society Bulletin, 27(3), 666–670. - EFSA Panel on Animal Health and Welfare (2020). Welfare of pigs during killing for purposes other thanslaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195. https://doi.org/10.2903/j.efsa.2020.6195 - EFSA Panel on Animal Health and Welfare. (2019). Killing for purposes other than slaughter: poultry. EFSAJournal. European Food Safety Authority, 17(11), e05850. https://doi.org/10.2903/j.efsa.2019.5850

		respiratory distress that negatively and severely impacts animal welfare (Beausoleil 2015). Airway occlusion or drowning (a potential concern if the foam has a very high water content/low expansion ratio) results in pulmonary edema (McEwen 2018, Lunetta 2005, McEwen 2016, McEwen 2018). Both airway obstruction and pulmonary edema result in anxiety and fear. Because of the negative affective states caused by obstructive asphyxia, methods relying on this mechanism of killing are not legally permitted in the EU or UK, and are not recognized as humane under the 2018 World Organisation for Animal Health		- EFSA Panel on Animal Health and Welfare. (2024). The use of high expansion foam for stunning and killing pigs and poultry. EFSA Journal. European Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855. - United Kingdom Animal Welfare Committee (2024). Opinion on the use of high expansion nitrogen foam deliverysystems for depopulation of poultry flocks affected by notifiable disease in the UK. Available at:https://www.gov.uk/government/publications/awc-opinion-on-high-expansion-nitrogen-foam-for-cullingpoultry/awc-opinion-on-the-use-of-high-expansion-nitrogen-foam-for-culling-poultry - 2018 Drowning and Bodies Recovered from Water book chapter - 2005 Macroscopical, microscopical, and laboratory findings in drowning victims a comprehensive review-Macroscopical, microscopical, and laboratory findings in drowning victims a comprehensive review - 2016 Nondrowning asphyxia in veterinary forensic pathology_ suffocation, strangulation & mech asphyxia

		guidelines on the killing of animals for disease control purposes. Water-based foam that relies on obstructive asphyxia and/or drowning is therefore categorized as a Tier 3 method whose use should be avoided unless it is the only possible option and will relieve more suffering than it causes. Phos-check Class A AFFF concentrate mixed according to label directions can be used in any solid side vessel or pen. Dwell times of 15 minutes should be used to assure efficacy. It is important to utilize foam that is free of PFAFs as these pose significant environmental and public health risks.		- 2018 strangulation, suffocation & asphyxia

4374-4375	Due to low numbers of animals, these settings can generally be handled with Tier 1 methods.	Due to low numbers of animals, these settings can generally be handled with euthanasia methods.	If numbers are sufficiently low, use of euthanasia methods, rather than depopulation methods, must be recommended. This sentence must be changed to be coherent with other parts of the Guidelines. For example, at lines 263–264, the draft Guidelines currently say, "Depopulation may employ euthanasia or slaughter methods, especially when the number of animals is low or the emergency has been contained." At 164–166, the definition of depopulation specifies, "Depopulation refers to the implementation of a unique, large-scale emergency or disaster management plan involving the rapid termination of a population(s) of animals." Low number of animals or ending animals' lives when there is no large-scale emergency requires use of euthanasia, not depopulation, methods.	

4387-4389	… in some cases, it may be appropriate to require depopulation of the livestock guard animal. In such cases, the guardian should be the first animal depopulated.	cases, it may be appropriate to require euthanasia of the livestock guard animal. In such cases, the guardian should be the first animal euthanized.	If numbers are sufficiently low, use of euthanasia methods, rather than depopulation methods, must be recommended. This sentence must be changed to be coherent with other parts of the Guidelines. For example, at lines 263–264, the draft Guidelines currently say, "Depopulation may employ euthanasia or slaughter methods, especially when the number of animals is low or the emergency has been contained." At 164–166, the definition of depopulation specifies, "Depopulation refers to the implementation of a unique, large-scale emergency or disaster management plan involving the rapid termination of a population(s) of animals." Low number of animals or ending animals' lives when there is no large-scale emergency requires use of euthanasia, not depopulation, methods.	
4460-4461	4. AVMA. AVMA guidelines for the euthanasia of animals: 2013 edition. Accessed Feb 7, 2019. www.avma.org/KB/Policies/Documents/ euthanasia.pdf	4. AVMA. AVMA Guidelines for the Euthanasia of Animals: 2020 edition.	Outdated reference cited. Please confirm that the updated reference supports all claims it is being used to substantiate.	

4462-4463	5. AVMA. AVMA guidelines for the humane slaughter of animals: 2016 edition. Accessed Feb 7, 2019. www.avma.org/KB/Resources/Reference/AnimalWelfare/Documents/Humane-4463 Slaughter- Guidelines.pdf.	5. AVMA. AVMA Guidelines for the Humane Slaughter of Animals: 2024 edition.	Outdated reference cited. Please confirm that the updated reference supports all claims it is being used to substantiate.	
4568	Cage/aviary poultry housing systems	Divide this discussion into 2 sections, one on conventional cage housing and one on aviary housing.	There are different considerations for cage v. aviary housing in terms of depopulation. Catching birds and the risk of workers falling from a height is a challenge of aviary housing. High expansion foam hasn't been tested in cage housing yet.	
4647-4649	As recent poultry disease outbreaks in the United States (i.e., HPAI in 2014-2015 and 2022-2024) have demonstrated, the necessity for meticulous advanced planning and preparation is of paramount importance.	As recent poultry disease outbreaks in the United States (i.e., HPAI in 2014–2015 and 2022–2025 and ongoing) have demonstrated, the necessity for meticulous advanced planning and preparation is of paramount importance. Given that HPAI now appears to be endemic in wild birds in North America (O'Keefe, Watt Poultry, 2023),	The HPAI outbreak that began in 2022 is ongoing. This section could be strengthened by noting that HPAI appears to now be endemic in wild birds in North America, depopulations of poultry are now a regular part of agriculture, and are likely to continue to be for the foreseeable future. At a minimum "2024" should be changed to "2025." Online reference: https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-	https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/commercial-backyard-flocks - 2024 Watt Poultry

		depopulation of millions of poultry annually is likely to continue for the foreseeable future, unless measures such as HPAI vaccination of poultry are adopted.	detections/commercial-backyard-flocks	
4654-4657	Additionally, during the preparedness phase, investments in equipment, supplies and contracts should be incentivized to ensure that Tier 1 methods can be readily deployed. Inadequate planning that results in unnecessary animal suffering is unacceptable.	Additionally, during the preparedness phase, investments in equipment, supplies and contracts must be prioritized by producers and animal owners, and incentivized by governmental programs, to ensure that Tier 1 methods can be readily deployed. Inadequate planning that results in unnecessary animal suffering is unacceptable.	This is a great start. However, given that HPAI is an ever-present risk and HPAI-related depopulation is a foreseeable concern, stronger language (e.g., "must" rather than "should") is needed.	

4698-4701	6.3 Events That May Require Depopulation Fortunately, animal health or safety incidents that require depopulation methods to eradicate or prevent disease, protect public health, or maintain a secure food supply are relatively uncommon.	6.3 Events That May Require Depopulation Unfortunately, animal health or safety incidents that require depopulation methods to eradicate or prevent disease, protect public health, or maintain a secure food supply have become common in the poultry sector, largely because HPAI has become endemic in wild bird populations. For example, in 2022, approximately 11% of layer hens were depopulated due to HPAI (O'Keefe, Watt Poultry, 2024). In addition, there has also been a recent increase in economic issues (e.g., bankruptcy) resulting in urgent depopulation. While unplanned depopulations were previously rare	Particularly in the poultry industry, it is not the case that animal health incidents that require depopulation are relatively uncommon. Since the start of the current HPAI outbreak in the US, over 145,000,000 birds have died or been depopulated. For any given hen raised for egg production, the odds that she will be depopulated due to HPAI in any given year is approximately 10%. This is a crucial ethical consideration because those responsible for animal care have a stronger obligation to prevent end-of-life suffering when the circumstances surrounding it are likely and foreseeable compared to when they are rare and unpredictable. Online reference: https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/commercial-backyard-flocks	Graber, R. (2024). Iowa Pure Prairie Poultry chickens depopulated. WATTPoultry. Available at: https://www.wattagnet.com/broilers-turkeys/broilers/article/15706894/iowa-pure-prairie-poultry-chickens-depopulated - 2024 Former Pure Prairie chickens depopulated - Brosch, C.; Cartanza, G. COVID-19 Acutely Impacted the Delmarva Poultry Industry in Early 2020. Del. J. Public Health 2021, 7, 38–39. [Google Scholar] [CrossRef] - Hauser, C. Nearly 2 Million Chickens Killed as Poultry Workers Are Sidelined. The New York Times. 28 April 2020. Available online: https://www.nytimes.com/2020/04/28/us/coronavirus-chicken-poultry-farm-workers.html (accessed on 20 September 2022). - Arkansas chicken growers sue poultry execs for damages from closure, depopulated flocks By: Antoinette Grajeda: https://arkansasadvocate.com/2024/09/25/arkansas-chicken-growers-sue-poultry-execs-for-damages-from-closure-depopulated-flocks/

		occurrences in animal agriculture, this is no longer the case. As these practices have become a regular part of commercial production, the need for appropriate planning, preparation, and preparedness has increased, and increased attention to risk mitigation is also required.		
4999-5000	Utilization of higher expansion ratio foam (>200 and beyond) improves the effectiveness, efficiency, and humaneness of the depopulation process.	Utilization of higher expansion ratio foam (>300 and beyond) improves the effectiveness, efficiency, and humaneness of the depopulation process.	Too small an expansion ratio increases the risk of bubbles entering and occluding the airway.	EFSA HEFT opinion AWC high exp N2 foam opinion Culhane presentation
5334	Ventilation Methods	Heatstroke-based methods or Hyperthermia-based methods	This would be a more accurate descriptor, as the ventilation system must also be shut down for different types of whole-house gassing. The category of	

			method should reflect its mechanism of killing, rather than one step in its deployment.	
5335	Ventilation Shutdown Plus Heat and Humidity (VSD+)	[This method should be moved to Tier 3 and described as "not recommended."]	In the poultry section, Tier 1 methods are those that, properly deployed, result in immediate loss of consciousness or rapid loss of consciousness with no pain and very little, if any, fear, distress, and other negative affective states. With the exception of VSD+, other Tier 2 methods generally cause pain and/or distress for a short period. However, VSD+ involves conscious animals experiencing a wide range of negative affective states, including overheating, pain, frustration, anxiety, fear, fatigue, exhaustion, and respiratory distress for a prolonged period. For this reason, it should only be used when the risk of doing nothing is likely to result in more suffering than utilizing the method—which is the criteria described for Tier 3. For both pigs and poultry, if VSD+ continues to be listed in the Guidelines, it must be described as "not recommended." According to the Guidelines, the temperatures required for VSD+ are "painful for the birds," and	- Reyes-Illg, G., Martin, J. E., Mani, I., Reynolds, J., & Kipperman, B. (2023). The rise of heatstroke as a method of depopulating pigs and poultry: Implications for the US veterinary profession. Animals, 13(1), 140. https://doi.org/10.3390/ani13010140 - United Kingdom Department for Environment, Food and Rural Affairs - Animal Welfare Committee. (2023). Advice on emergency culling for the depopulation of poultry affected by high pathogenic avian influenza (HPAI) – consideration of ventilation shutdown (VSD). Available at: https://www.gov.uk/government/publications/advice-onemergency-culling-for-the-depopulation-of-poultry-affected-by-high-pathogenic-avian-influenza-hpai - VIN survey - EFSA Panel on Animal Health and Welfare (2020). Welfare of pigs during killing for purposes other than slaughter. EFSA Journal. European Food Safety Authority, 18(7), e06195.

			time to death is prolonged, ranging from 53 minutes in controlled laboratory settings to over 300 minutes under simulated field conditions. Further, even after several hours, VSD+ frequently fails to kill every bird. According to the U.S. Department of Agriculture, 74% of layer hen houses employing VSD+ report survivors, whose subsequent killing via a secondary depopulation may take up to five days. Thus, there is substantial evidence that using VSD+ to depopulate poultry is "contrary to good animal welfare," and it must, therefore, be designated a Tier 3 method.	https://doi.org/10.2903/j.efsa.2020.6195 - EFSA Panel on Animal Health and Welfare. (2019). Killing for purposes other than slaughter: poultry. EFSA Journal. European Food Safety Authority, 17(11), e05850. https://doi.org/10.2903/j.efsa.2019.5850

5369-5372	Hyperthermia is an elevation in core body temperature above the accepted normal range, which in poultry is 105-107 °F (40.6-41.7 °C)112,113 to the lethal core body temperatures. The effect of elevated heat and/or heat and humidity on the bird depends on age, body weight, environment, and species as well as past management experiences.114	Hyperthermia is an elevation in core body temperature above the accepted normal range, which in poultry is 105–107 °F (40.6–41.7 °C), to the lethal core body temperatures. In mammals, death due to hyperthermia is typically due to distributive shock and respiratory failure due to accumulation of frothy, hemorrhagic fluid in the airways (Reyes-Illg 2023; Brunchim 2009). Other lesions noted with lethal hyperthermia include "gastrointestinal bleeding and sloughing with attendant vomiting and hemorrhagic diarrhea, abdominal organomegaly, rhabdomyolysis, acute respiratory distress syndrome, brain injury and neurological	This paragraph ostensibly describes the mechanism of death with hyperthermia, however it neglects a large amount of information necessary for understanding the mechanism of death and likely accompanying affective states. For example, conditions like rhabdomyolysis, which have been observed in birds depopulated with VSD+ (Zhao 2019), are known to be painful (Stanley 2025), and videos of birds destroyed with VSD+ under experimental conditions demonstrate that they experience respiratory distress prior to loss of consciousness. See: North Carolina State University VSD Videos 5 (5 videos), VSD Videos 6 (7 videos), VSD Videos 8 (the following videos: VSD- Camera 2 VH 1-21-16 (1), VSD- Camera 2 VH 1-21-16 (2), VSD- Camera 2b VH 2-3-16 (1), VSD- Camera 2b VH 2-3-16 (2), VSD- Camera 2b VH 2-3-16 (3), VSD- Camera 2b VH 2-3-16 (4), VSD- Camera 2b VH 2-3-16 (5)) obtained via request by Animal Outlook under North Carolina Public Records Law, G.S. §132-1. Available online: https://drive.google.com/drive/f	The rise of heatstroke, other papers on the pathophysiology of heatstroke. 2019 Modelling and validating the indoor environment and supplemental heat requirement during ventilation shutdown for hens and turkeys re rhabdomyolysis. - Stanley M, Chippa V, Aeddula NR, et al. Rhabdomyolysis. [Updated 2024 Dec 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK448168/

		abnormalities, multiorgan dysfunction, and coagulopathies, including disseminated intravascular coagulation (DIC), frequently ending in hemorrhagic diathesis" (Reyes-Illg 2023). The effect of elevated heat and/or heat and humidity on the bird depends on age, body weight, environment, and species, as well as past management experiences.	olders/1Ocvpj6kcc1w-oHEw6yQUHs2DkK-WbzT7	

5372-5381	When ambient temperatures increase, the bird attempts to regulate its core body temperature through sensible and insensible heat loss. Sensible heat loss is the dissipation of body heat to the surrounding environment through radiation, conduction, and convection.115 The proportion of sensible heat loss is dependent on the temperature difference between the core body temperature of the bird and the temperature of its environment. With sensible heat loss, birds do not need to drastically alter their normal behavior patterns or metabolism.116 Insensible heat loss (Latent heat loss) is the transfer of heat when water within the bird's respiratory tract is converted to water vapor.115 Dissipation of body heat into water vapor requires the use of energy creating metabolic heat through hyperventilation, which causes respiratory alkalosis, or a shift in bodily fluids towards an alkaline pH.116	When ambient temperatures increase, the bird attempts to regulate its core body temperature through sensible and insensible heat loss. Sensible heat loss is the dissipation of body heat to the surrounding environment through radiation, conduction, and convection. The proportion of sensible heat loss is dependent on the temperature difference between the core body temperature of the bird and the temperature of its environment. With sensible heat loss, birds do not need to drastically alter their normal behavior patterns or metabolism. Insensible heat loss (Latent heat loss) is the transfer of heat	Hodgson 2022, on page 94 of 99 in Figure 3.5 (C), notes that birds killed with VSDH had a blood pH of about 7.54 at the final time point. Montesinos notes that this is within the normal range for most birds.	- 2013 Acid-base status in the Avian Patient - 2022 Physiology of Poultry during Ventilation Shut Down (+) in Response to a Foreign Animal Disease Outbreak

		when water within the bird's respiratory tract is converted to water vapor. Dissipation of body heat into water vapor requires the use of energy creating metabolic heat through hyperventilation, which causes respiratory alkalosis, or a shift in bodily fluids towards an alkaline pH. However, the resulting acid-base imbalance does not appear severe enough to result in death (Hodgson 2022, Montesinos 2013).		

5381-5388	In severe hyperthermia, death is caused by circulatory and/or respiratory collapse and/or metabolic imbalance.114 With an increase in heat and humidity the insensible heat loss methods become ineffective and accelerate the increase in core body temperature. By utilizing supplemental heat and potentially humidity, hyper-thermic depopulation exploits the limits of sensible and insensible heat loss leading to the loss of consciousness and death. The sensory perception of the brain begins decreasing due to denaturation of lipid and protein that begins at approximately 45 C (113 F) with cell damage increasing in a linear rate as temperature increases.119	In severe hyperthermia, death is caused by circulatory and/or respiratory collapse. Prior to loss of consciousness, negative affective states may include: anxiety, debility, dyspnea, disorientation, exhaustion, fear, frustration, helplessness, nausea, malaise, overheating, pain, panic, and thirst (Reyes-Illg 2023). With an increase in heat and humidity the insensible heat loss methods become ineffective and accelerate the increase in core body temperature. By utilizing supplemental heat and potentially humidity, hyper-thermic depopulation exploits the limits of sensible and insensible heat loss	It is essential that the pathophysiology of environmental hyperthermia (heatstroke or VSD+) be clearly described, as this helps ensure that the affective states that animals experience prior to loss of consciousness can be accurately assessed. A depopulation method's impact on animal welfare depends in large part on the affective states experienced by the animal prior to loss of consciousness. (See discussion re potential for delayed loss of consciousness in porcine and gallinaceous species at 2.1 in Reyes-Illg 2023; cited references are attached.)	- Reyes-Illg et al Rise of Heat Stroke - Romanucci, M.; Salda, L.D. Pathophysiology and pathological findings of heatstroke in dogs. *Vet. Med. Auckl. NZ* **2013**, *4*, 1–9. - Bruchim, Y.; Horowitz, M.; Aroch, I. Pathophysiology of heatstroke in dogs—Revisited. *Temperature* **2017**, *4*, 356–370. - Yarmolenko, P.S.; Moon, E.J.; Landon, C.; Manzoor, A.; Hochman, D.W.; Viglianti, B.L.; Dewhirst, M.W. Thresholds for thermal damage to normal tissues: An update. *Int. J. Hyperth.* **2011**, *27*, 320–343. - Lassche, G.; Frenzel, T.; Mignot, M.H.; Jonker, M.A.; van der Hoeven, J.G.; van Herpen, C.M.L.; Scheffer, G.J. Thermal distribution, physiological effects and toxicities of extracorporeally induced whole-body hyperthermia in a pig model. *Physiol. Rep.* **2020**, *8*, e14366. 1. - McKechnie, A.E. Regulation of body temperature. In *Sturkie's Avian Physiology*; Elsevier: London, UK, 2022; pp. 1231–1264. ISBN 978-0-12-819770-7. [Google Scholar] [CrossRef] 2. Porter, W.R.; Witmer, L.M. Avian Cephalic Vascular

		leading to the loss of consciousness and death. The sensory perception of the brain begins decreasing due to denaturation of lipid and protein that begins at approximately 45 °C (113 °F) with cell damage increasing in a linear rate as temperature increases. Some species, such as chickens, turkeys, and pigs, have anatomic cooling systems that protect the brain from extreme heat, likely delaying the onset of unconsciousness compared to species without this adaptation. (Reyes-Illg 2023); this potentially worsens the animal welfare impacts of VSD+. For this reason, VSD+ is considered "not recommended" as a depopulation method.		Anatomy, Sites of Thermal Exchange, and the Rete Ophthalmicum. *Anat. Rec.* **2016**, *299*, 1461–1486. [Google Scholar] [CrossRef] [Green Version] 3. Richards, S.A. Brain temperature and the cerebral circulation in the chicken. *Brain Res.* **1970**, *23*, 265–268. [Google Scholar] [CrossRef] 4. Kilgore, D.L.; Birchard, G.F.; Boggs, D.F. Brain temperatures in running quail. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **1981**, *50*, 1277–1281. [Google Scholar] [CrossRef] 5. Bech, C.; Midtgård, U. Brain temperature and the rete mirabile ophthalmicum in the Zebra finch (Poephila guttata). *J. Comp. Physiol. B* **1981**, *145*, 89–93.

5427	Oral anesthetics	Oral anesthetics, anxiolytics and/or analgesics [This section should be relocated to a separate section, perhaps entitled "Other Handling Considerations" to make its application clear]	This section should be appropriately titled to encompass the various oral medications described in the text. In addition, oral anesthetics and anxiolytics are NOT being proposed as a means of depopulation, but rather as a means of minimizing/eliminating negative affective states associated with depopulation methods, for example, fear at the buildup of high expansion nitrogen foam or handling for other methods. Therefore, it is inappropriate to place this measure in Tier 3, as tiers are reserved for depopulation methods. Locating this discussion in Tier 3 means that (1) it might not be reviewed by those seeking to implement higher-tier methods, and (2) it gives the impression the Panel has a negative opinion about providing poultry with anesthetic/anxiolytic medications prior to depopulation. Including it in a separate section on animal handling ensures that it is recognized as an adjunctive means of minimizing animal welfare harms. Relocating the discussion to a different section (not under "Tiers") would also be	

			consistent with how other sections of the Guidelines handle the issue of providing animals with anesthetics or sedation prior to depopulation. For example, at lines, 2982–2983, the statement "Chemical restraint may be necessary to improve efficacy and lower the risk of human injury" is included under a subsection titled "Dangerous Animals" under the "Bovids" section. In the section on Small Ruminants, Cervids, and Camelids, at lines 4103-4106, pre-killing sedation is not put in Tier 3 due to limited research but is rather discussed in the section "Other Handling Considerations": "In cases where cervids are very fractious and restraint poses a safety risk to the animals or personnel, the use of feed or water delivered pre-sedation is sometimes could be considered. Acepromazine maleate, diazepam, and haloperidol administered orally have all been shown to reduce stress, aggression, and fear of humans in captive cervidae." Finally, relocating this section would also make the Depopulation Guidelines more consistent with the 2020 AVMA Euthanasia Guidelines, which	

			state, "Apart from delineating appropriate methods and agents, these Guidelines also recognize the importance of considering and applying appropriate pre-euthanasia (e.g., sedation) and animal handling practices."	
5428-5430	The use of anesthetic, anxiolytic, and/or analgesic agents delivered via the water system is a potential means of improving animal welfare at the end of life by mitigating negative affective states, such as pain and fear	The use of anesthetic, anxiolytic, and/or analgesic agents delivered via the water or food system is a potential means of improving	Some medications may be best provided in feed rather than water.	

	associated with depopulation methods.	animal welfare at the end of life by mitigating negative affective states, such as pain and fear associated with depopulation methods.		
5459-5460	The foam quality is poor, bubbles are too strong to burst and release their gases, and the method of death is mechanical hypoxia, rather than gas exposure.	The foam quality is poor, bubbles are too strong to burst and release their gases, and the method of death is airway occlusion (obstructive asphyxia), rather than gas exposure.	"Mechanical hypoxia" is an ambiguous and inaccurate term for describing airway occlusion. Per the attached reference (McEwen 2016), mechanical asphyxiation is defined as one of the following: (1) positional asphyxia (position of the animal compromises the ability to breathe) or (2) traumatic asphyxia (external chest or abdominal compression by a heavy object preventing respiration). Furthermore, airway occlusion results not only in hypoxia but also in hypercarbia. In a conscious animal, elevated PaCO2 increases drive to breathe and, based on our understanding of types of dyspnea in humans, increases the intensity of the unpleasant sensation of air hunger when coupled with inability to bring in more air. The terms "airway occlusion" or "obstructive asphyxia" are more accurate and precise	2016 Nondrowning asphyxia in veterinary forensic pathology_ suffocation, strangulation & mech asphyxia; 2015 Beausoleil Mellor Introducing Breathlessness

			descriptors for the mechanism of death of both compressed air form and low- or medium-expansion water-based foam.	
5464	Ventilation shutdown alone (VSD)	[This proposed depopulation method should not be listed in the Guidelines. If the Panel on Depopulation elects to continue to list the method, it should be made clear that it is "not recommended."]	Live burning, live burial and other methods that cause severe, prolonged animal suffering are not included in the Guidelines. VSD alone is on par with such methods and therefore should not be included. State public records (pages 4, 6, & 8) indicate that, at present, VSD alone is sometimes being used for HPAI depopulation, but is being described as VSD+ in order for animal owners to avail themselves of indemnity payments.	Iowa records indicating that the only heat provided was lights being left on.
5508	In summary, VSD alone does not meet the AVMA depopulation expectation of >95% death rate in < 1 hour.	In summary, VSD is ineffective as a depopulation method, as it does not reliably kill a large percentage of the birds. It also results in a	This is the only place in this document that a 95% death rate in less than one hour is discussed. If this is an expectation, then the section on VSD+ should also include this language, given that state records indicate that, even	

		prolonged period of negative animal welfare. It must not be used.	performed according to parameters described by USDA and AVMA, VSD+ as used in poultry typically does not result in 95% death rate in less than one hour.	
5533-5535	Bird embryos that have attained > 80% incubation should be euthanized by methods similar to those used in avian neonates. Eggs at < 80% incubation may be destroyed by prolonged exposure (> 20 minutes) to CO2, cooling (< 4°C for 4 hours) or freezing.130	Bird embryos that have attained > 50% incubation should be euthanized by methods similar to those used in avian neonates. Eggs at < 50% incubation may be destroyed by prolonged exposure (> 20 minutes) to CO2 or freezing (< −20 °C for 4 hours).	The reference used to support this statement (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.) is outdated and does not reflect current science that embryonic chickens are able to consciously feel pain by day 13 of incubation (13/21 = 62% of hatch), as described in the UK governmental Animal Welfare Committee's recent report, which is based on a review of all the existing literature. Therefore, referencing 80% of the hatching period is inappropriate. Furthermore, recent references note that freezing is not appropriate as a euthanasia technique for fertilized eggs beyond 50% of the incubation period. In addition, the temperature required for freezing as a method of terminating fertilized eggs is misstated. Finally, every effort	2023 awc-opinion-alternatives-culling-newly-hatched-chicks-poultry-industry 2015 Ethical Euthanasia and Short-Term Anesthesia of the Chick Embryo 2018 Evaluation of Alternative Euthanasia Methods of Neonatal Chickens

			should be made to specify a concentration of carbon dioxide that would be effective for both sentient and non-sentient chick embryos. Studies have tended to support very high concentrations of CO2 (75%–90%) for neonatal chickens and also support nitrogen anoxia as well as low atmospheric pressure stunning (LAPS). (See Gurung 2018)	

Lines	Specific language to be changed	Specific language to replace current language	Rationale for suggested change	References uploaded
318-320	The POD is committed to ensuring that no unnecessary pain or distress is inflicted on conscious animals during an emergency like depopulation. When significant effort to save animals has been exhausted, it is imperative that animals are destroyed, and their carcasses be disposed consonant with high ethical standards.	The POD is committed to ensuring that no unnecessary pain or distress is inflicted on conscious animals during an emergency like depopulation. If alternatives to depopulation are deemed impossible through a collaborative, robust ethical decision-making process and significant effort to save animals has been exhausted, animals must be destroyed in the most humane methods available, and their carcasses be disposed consonant with high ethical standards.	It would be preferable for the AVMA Depopulation Guidelines to begin with a discussion about the ethical basis for the decision to depopulate rather than pursue other alternatives. The Canadian Veterinary Medical Association has a position statement on this issue. There have also been books and academic papers written about this topic. It is essential that the veterinary profession not merely provide technocratic "solutions" when robust discussion and debate about the underlying ethical or scientific issues has not occurred.	https://www.canadianveterinarians.net/policy-and-outreach/position-statements/statements/decision-making-for-mass-depopulation-of-domesticated-animals/ 2016 Ethical and societal considerations on killing animals

682-684	Periodic professional continuing education on the latest methods, methods, and materials available for both euthanasia and depopulation is highly encouraged.	Periodic professional continuing education on the latest methods, techniques, and materials available for both euthanasia and depopulation is required.	Stronger language should be used to ensure that professionals involved in euthanasia and depopulation are held to a standard of ongoing competence, ensuring they are aware of the most recent advancements, ethical considerations, and regulatory changes.	
810-812	Ethical reasoning should not be suspended or ignored and is essential in guiding crisis management and depopulation procedures.	Ethical reasoning must not be suspended or ignored and is essential in guiding crisis management and depopulation procedures.	"Must" is used in the language of a nearly identical statement at the top of the Veterinary Ethics section in 731–732: "Ethical deliberation and critical reasoning must not be suspended or ignored in an emergency declaration." So, "must" should be substituted in 810–812 instead of "should."	
846-848	Depopulation, as a method of containment for effective emergency or disaster management and response, should account for	Depopulation, as a method of containment for effective emergency or disaster management and response, must account for human well-being, animals and their welfare, and the importance of specific	The reasons used to justify the drastic measures used in emergency situations requiring depopulation are "human well-being, animals and their welfare, and the importance of specific human-animal bonds and relationships." So, the “should” must be changed to a “must.”	

	human well-being, animals and their welfare, and the importance of specific human-animal bonds and relationships.	human-animal bonds and relationships.		
955-957	The cyclic nature of emergencies and cross-relation of all four phases confirms that planning does not end with the publication of a plan. Disaster preparedness is a continual effort in which the phases of the cycle of emergency management are constantly being anticipated, reviewed, and improved.	The cyclic nature of emergencies and cross-relation of all four phases confirms that planning does not end with the publication of a plan. Disaster preparedness is a continual effort in which the phases of the cycle of emergency management are constantly being anticipated, reviewed, and improved. A written report should be produced, including a review of the plan, process, and outcomes, as well as suggestions for improvement in the future.	Requiring a publicly available written report as part of the “reflect and reimagine” step in animal depopulation disasters ensures transparency and accountability by documenting the rationale behind decisions and outlining strategies to minimize future animal suffering. This report demonstrates a commitment to continuous improvement, ethical responsibility, and the prioritization of more humane (Tier 1) methods.	

910-964	Sound ethical decision-making can be bolstered through the following steps: section	[see "Rationale for Suggested Change"]	The statements in this section must be supported by citations of relevant references. An extensive body of literature exists on ethical decision-making across different fields. The lack of citations in this section outlining discrete steps raises concerns that the *Guidelines* expect veterinarians to engage in ethical decision-making without providing a peer-reviewed framework for ethical decision-making practices (either in veterinary science or in other closely related disciplines like emergency ethics).	-Barry's 2022 chapter vet advocacies & ethical dilemmas - -(2) Vettical, B. S. (2018). An Overview on Ethics and Ethical Decision-Making Process in Veterinary Practice. Journal of Agricultural & Environmental Ethics, 31(6), 739–749. https://doi.org/10.1007/s10806-018-9752-5 - (3) Ashall, V. (2023). Reducing Moral Stress in Veterinary Teams? Evaluating the Use of Ethical Discussion Groups in Charity Veterinary Hospitals. Animals (Basel), 13(10), 1662-. https://doi.org/10.3390/ani13101662
989-990	A poorly executed depopulation may harm patients, and moral distress or residue for emergency responders.	A poorly executed depopulation may harm patients, as well as emergency responders, who may experience moral distress, moral residue, and an increased level of secondary/vicarious trauma.	Moral stress, distress, and residue are some of the types of harm that emergency responders may experience. Secondary trauma (sometimes called vicarious trauma) is another. These harms can be worsened when a depopulation is poorly executed. The suggested sentence is clearer and easier to understand in the suggested language replacement.	2020 Compassion Fatigue in Animal Care Workers 2011 Whiting - perpetration-induced traumatic stress

3324-3330	The goal of commercial swine production is to provide a wholesome, safe, high-quality food for consumers. Because there is little flexibility in the current marketing channel, any eventuality that reduces or eliminates the marketability of swine could have a negative impact on animal welfare in a very short period of time. This puts a high level of importance on the speed at which a decision can be made for swine that cannot be moved or	The goal of commercial swine production is to provide a wholesome, safe, high-quality food for consumers. Because there is currently little flexibility in the current marketing channel, any eventuality that reduces or eliminates the marketability of swine could have a negative impact on animal welfare in a very short period of time. This puts a high level of importance on the speed at which a decision can be made for swine that cannot be moved or marketed because of regulatory issues, natural and manmade disasters, food safety, and other public health issues. In addition, integrators and other large scale animal owners must work to reduce pigs' vulnerability to depopulation by identifying and adopting systemic changes that ensure that depopulation as a response to market	This section takes for granted that "there is little flexibility in the current marketing channel," rather than highlighting this as a crucial moral issue that must be considered and addressed in the mitigation phase of the disaster management cycle.	2022 One Health Animal Disaster Management An Ethics of Care Approach 2021 COVID-19 effects on livestock production - a one welfare issue

	marketed because of regulatory issues, natural and manmade disasters, food safety, and other public health issues.	issues is exceedingly rare.		
3331-3332	Ideally a plan should be developed and tested before an incident requiring depopulation.	Particularly for larger operations, it is imperative that a plan for depopulation be developed and tested, and that farm operators and animal owners have prepared for its implementation prior to any incident requiring depopulation. Adequate preparedness may involve securing equipment, establishing contracts for necessary supplies, training personnel, and performing drills as well as tabletop, functional, and full-scale exercises.	For an industry at high risk of resorting to depopulation for both disease- and non-disease-related purposes, it is insufficient to merely note that developing and testing a plan is "ideal." If one is responsible for large numbers of animals, it is morally required that one is able to reasonably ensure that their lives can be ended without suffering if needed, and adequate planning and preparedness are core to fulfilling this ethical duty. See page 7 (17 of 75) of attached reference for an example of a progressive preparedness approach.	2024 USDA APHIS VS EMERGNECY Preparedness and response training and exercise strategy & plan

3338-3339	Methods used for depopulation should be evaluated on the basis of their ability to achieve the necessary throughput to accomplish work within the allotted time frame.	Methods used for depopulation must be evaluated in terms of their impact on animal welfare, with priority given to methods to avoid negative welfare states including pain, fear, respiratory distress, and other negative affective states. In addition, the ability to achieve the necessary throughput to accomplish work within the allotted time frame must be considered.	Throughput is one consideration, but given the ethical and animal welfare principles espoused by the profession and the numerous different depopulation methods available, methods that prioritize animal welfare must be prioritized, with throughput a secondary consideration.	AVMA animal welfare principles AVMA PVME Joint AVMA – FVE-CVMA statement on the roles of veterinarians in promoting animal welfare
3439-3441	There are certain incidents where the rapid destruction of a population of swine must occur in response to urgent circumstances with as much consideration given to the welfare of the swine as practicable.	There are certain incidents where the rapid destruction of a population of swine must occur in response to urgent circumstances with the least amount of negative welfare impacts to the animals. As depopulation becomes more common, reducing the vulnerability of farms to circumstances that result in depopulation must be considered a core ethical duty of the veterinary profession and veterinarians	This sentence incorporates the definition of depopulation utilized in the 2019 version, which builds lower regard for animal welfare into the definition. It should be updated with the new definition, as well as an acknowledgment that veterinarians and the profession's ethical obligations related to depopulation begin long before the decision to depopulate is made.	Raymond Anthony One Health Care ethics article

		working in animal agriculture.		
3542-3543	If depopulation using a slaughter plant is feasible, the disposition of the carcass must be carefully considered.	If depopulation using a slaughter plant is feasible, the disposition of the carcass must be carefully considered. If the animals are healthy and the decision to depopulate stems from slaughterhouse staffing shortages, processing for the production of carcasses or large cuts is a potential means of increasing slaughterhouse throughput compared to standard post-slaughter processing (Grandin 2021).	This section seems to assume the animals would be processed after slaughter. Commercial slaughter for carcass production is another option, which requires far fewer workers and would be especially fitting in an event such as the COVID-related slaughterhouse closures, where the animals did not pose any kind of health threat.	2021 - Methods to prevent animal welfare problems caused by Covid in pork industry - grandin

3595	Carbon Monoxide	[Carbon Monoxide should be moved to Tier 2 for pigs]	While carbon monoxide (CO) is described as a euthanasia method "with conditions," the conditions described in the 2020 AVMA Euthanasia Guidelines are unlikely to be obtained on a farm. Such conditions include: - "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." These requirements are not met in any of the recent porcine studies on this method: Schwarz 2022, Ramirez 2021, Groth 2022 - "The chamber must be well lighted and must allow personnel direct observation of animals." The two National Pork Board-funded studies on CO as a means of euthanasia/depopulation did not permit visualization of animals (Ramirez 2021, Groth 2022), nor did a study presented at AASV in 2022 (Schwarz 2022). - "Carbon monoxide must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank." Groth (2022), a study	2022 Quantifying a Technique Using Carbon Monoxide for the Depopulation of Swine 1980 euthanasia of young pigs with carbon monoxide 2021 Mass depopulation of swine facilities via on-site generation of carbon monoxide 2022 Assessment of non-catalytic converter gasoline engine exhaust for swine euthanasia

			completed for the National Pork Board, notes that mature sows and feeder pigs were killed "using carbon monoxide (CO) produced by a 1970 gas grain truck. The gas engine system, with no modifications, had been previously used to euthanize [sic] weaned pigs." Other recent studies similarly rely on filtering engine exhaust, rather than utilizing "a precisely regulated and purified form [of CO] 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." Groth (2022) attempted to modify a gas engine system to produce CO by "cooling and filtering particulates out of the exhaust gas prior to contact with the pigs" as did Schwarz (2022); however, none of these modifications result in compliance with the language in the AVMA Euthanasia Guidelines regarding necessary conditions for using CO as a means of euthanasia.	

			In addition, the swine section of the 2020 AVMA Euthanasia Guidelines does not actually describe or discuss use of carbon monoxide beyond noting that CO is one of multiple "[s]tudied gas mixtures" (p 73). In fact, little research has been conducted on pigs regarding the use of CO and none has specifically examined animal welfare impacts. A 1980 study found that "visual signs of severe excitation were observed (sometimes before unconsciousness)" when CO was applied at a high flow rate to piglets. The two recent studies funded by the National Pork Board did not involve any systematic assessment of animal welfare. Ramirez (2021) did not involve live pigs at all while Groth (2022) described a relatively wide range of times until presumed loss of consciousness (cessation of movement): 8–13 minutes. Given the availability of depopulation methods that involve instantaneous or relatively rapid loss of consciousness, combined with minimal negative affective states (considering both duration and intensity), gassing with carbon monoxide should be recategorized as Tier 2. The use of engine exhaust should be described as "not recommended" unless precise limits of temperature and particulate content can be assured and specific levels and rate of carbon monoxide levels adhered to.	

3597-3598	Carbon monoxide is acceptable with conditions for euthanasia of swine5	Carbon monoxide is categorized as "acceptable with conditions" for pigs in the 2020 AVMA Euthanasia Guidelines, although it is not discussed in the text of the swine section. Conditions include being supplied in a regulated, purified form, typically from a commercially supplied cylinder or tank and ensuring animals can be easily visualized. According to the 2020 AVMA Euthanasia Guidelines, unmodified products of combustion are not acceptable due to unreliable composition and displacement rate. A pressure-reducing regulator and flow meter, or equivalent equipment, are critical to ensure the recommended displacement rate. These conditions are unlikely to obtain in an agricultural setting. Very little research has been	It must be made clear that the proposed means of applying CO (via exhaust from an engine) in no way complies with the "conditions" described in the Guidelines on Euthanasia in the general discussion of carbon monoxide (p 27). The "conditions" referred to here should be discussed, especially given the means by which CO is described below as being produced. (See previous comment for list of conditions that are described in the 2020 AVMA Euthanasia Guidelines regarding use of CO, but that are very unlikely to exist on swine farms in the context of depopulation.) It must also be clearly communicated that no research has focused on the welfare impact on pigs when death is achieved through exposure to CO.	AVMA. AVMA guidelines for the euthanasia of animals: 2020 edition.

		done to assess animal welfare when carbon monoxide is used as a method of killing pigs, particularly under field conditions when gas engines are used to generate carbon monoxide.		

3615-3616	Equipment available on farm may be useful for generation of CO and the method has been used successfully on sows and feeder swine	Equipment available on farms, such as gas engine systems, are capable of generating CO. However, in addition to CO, the exhaust gas thus generated contains "carbon particles, nitrates, (hydro) carbonates, various oxides and heat, which cause irritation to the mucous membranes and a considerable degree of excitation" (Lambooy 1980). Therefore, use of unfiltered or uncooled exhaust must not be used for depopulation of pigs. CO gassing has been used to effectively kill sows and feeder swine when means of filtering and cooling exhaust were utilized (Groth 2022); however, welfare assessments have not been performed on pigs killed with this method. In humans, negative affective states such as pain (headache) and nausea have been	It is essential that the information provided in this section conveys that use of exhaust fumes for animal killing is very different from use of purified carbon monoxide in terms of the animal welfare impacts of the method. In addition, the lack of animal welfare science research on this topic needs to be made clear.	2021 Mass depopulation of swine facilities via on-site generation of carbon monoxide 1980 Euthanasia of young pigs using carbon monoxide 2022 Quantifying a Technique Using Carbon Monoxide for the Depopulation of Swine 1980 euthanasia of young pigs with carbon monoxide 2021 Mass depopulation of swine facilities via on-site generation of carbon monoxide 2022 Assessment of non-catalytic converter gasoline engine exhaust for swine euthanasia

		reported (Lambooy 1980). An early study on piglets found that "when carbon monoxide was administered at a fast flow rate, visual signs of severe excitation were observed (sometimes before unconsciousness)"; this included vocalizations during and after the excitation phase (Lambooy 1980). Recent research recommended necessary modifications to the method that are essential to avoid unacceptable welfare outcomes, including cooling the exhaust gas to ambient air temperature with an intercooler, filtering particulates using a Hoover vacuum HEPA filter or MERV 14 V-Bank filter, and ensuring the absence of a catalytic converter. Smaller pigs lost posture starting at 5–6 minutes and continuing until 11 minutes after start of gassing; sows took longer (Groth 2022).		

3641	Hypoxia produced by N2 appears to reduce, but not eliminate, aversive responses in swine.	[Delete sentence or reword to ensure it makes sense]	N2 is nonaversive to pigs, while CO2 is clearly aversive. Hypoxia, by any means, may be aversive, but this section is discussing anoxia (2% or less residual oxygen). Mixtures of N2 and CO2 are aversive because of the CO2 in them.	- 2014 Raj Stunning CO2 and other gases book chapter - 2010 Stunning pigs with different gas mixtures: Aversion in pigs - Raj ABM 1999 Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. Veterinary Record 144: 165-168 - Raj ABM and Gregory NG 1995 Welfare implications of the gas stunning of pigs 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. Animal Welfare 4: 273-280 - Raj ABM and Gregory NG 1996 Welfare implications of the gas stunning of pigs 2. Stress of induction of anaesthesia. Animal Welfare 5: 71-78

3628-3671	Nitrogen.....this can lead to inhalation of foam into the trachea and lungs	[See Rationale for suggested change]	This section is poorly organized and confusing, and a rewrite of this section should be considered. For example: - It must be specified that nitrogen gassing should only be considered as a method of killing when nitrogen is used to achieve anoxic conditions, and "anoxia" must be clearly defined. For example, it is defined by the UK governmental Animal Welfare Committee) as "the absence, or near absence, of oxygen"; the EFSA (2024) defines anoxia as "< 2% by volume of residual oxygen"; and the 2020 AVMA Euthanasia Guidelines specify oxygen levels must be held at sufficiently low levels (2% or 3%) when nitrogen is used for the euthanasia of poultry (p 77). Currently, lines 3532–3633 are correct ("Nitrogen depopulation methods displace air containing 21% oxygen and reduce oxygen levels to < 2%, producing death through anoxia"); however, the term "hypoxia" is then intermittently used (without being defined). - High expansion nitrogen-filled foam is currently discussed both in this section as well as the section below on foams, and contradictory statements are made. High expansion nitrogen foam belongs in "Inhaled methods," given its mechanism of action. It could be included as a subsection of "Nitrogen" or immediately subsequent to this section. - It must be clarified that nitrogen is not directly aversive to animals; aversive responses are only noted when it is	PAMI N2 trailer abstract - 2014 Raj Stunning CO2 and other gases book chapter - 2010 Stunning pigs with different gas mixtures: Aversion in pigs - Raj ABM 1999 Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. Veterinary Record 144: 165-168 - Raj ABM and Gregory NG 1996 Welfare implications of the gas stunning of pigs 2. Stress of induction of anaesthesia. Animal Welfare 5: 71-78

			combined with carbon dioxide or if residual oxygen levels are excessively high, resulting in prolonged hypoxia. References to CO2/N2 mixtures should be relocated to the CO2 section or in a separate section discussing gas mixtures. - There should be an expanded discussion regarding the availability of a nitrogen-gassing trailer, developed by the Prairie Agricultural Machinery Institute (PAMI) and presented at the Humane Endings conference. - Key references have been omitted and should be included, including some of those attached.	
3642-3644	Exposure to 90% Ar, 70% N2/30% CO2, and 85% N2/15% CO2 all resulted in signs of aversion, defined by the authors as escape attempts and	[Delete or relocate statement]	The reason for including this sentence is unclear in a section entitled "Nitrogen," and it creates confusion. If this information is to be included, it must be made clear that the pigs' signs of aversion to the gas mixtures containing CO2 were due to the CO2 itself, not the N2. And the reason for discussing argon in this section is not clear. If argon is to be discussed, the section should be re-titled "inert gasses." If gas mixtures are to be discussed, there should be a separate	

	gasping; however, the proportion of pigs showing these behaviors was lowest with Ar.17		section for gas mixtures, or gas mixtures including CO2 should be included in the CO2 section, since CO2 is well documented to be directly aversive to animals. (See references attached to the comments above.)	
3644-3649	While swine did not show any strong aversive behaviors when exposed to air-filled or nitrogen-filled foams, they seemed to avoid putting their heads and snouts into the foam, and the rate of escape attempts through the lid increased when foam levels became high.18 Escape behaviors were observed and foam residues were noted in the trachea of swine stunned	[Delete or correct and relocate this statement]	Studies in swine show increased escape attempts but no gasping. Newer research suggests that escape attempts occur in response to noise from turning on foam generators or foam levels rising above snout level. Environmental adjustments (driving trailers toward foam generators so the sound increases gradually, and increasing rate of fill) can help mitigate fear and anxiety and result in fewer escape attempts (Campler 2025, a preprint that we believe has been submitted to the Depopulation Panel; Evaluation of High Expansion Nitrogen Foam for Depopulation of Market Swine in Missouri, a report that we believe has been submitted to the Depopulation Panel).	2024 Behavioural response in pigs at gas stunning with foam 2020 Improved pig welfare at slaughter - pigs' responses to air- or nitrogen foam (abstract and presentation)

	with high expansion N2 foam for slaughter, with a high rate of follow-up stunning required (22%) following foam exposure for 3.5 min.19			
3655-3656	Nitrogen gas mixtures do not appear to be directly aversive to swine and appear to reduce, but not eliminate, the behavioral responses to hypoxia.	[Delete or correct and relocate this sentence]	Whether gas mixtures containing N2 are aversive or not depends on what the other gases are in the mixture. N2 itself is nonaversive, while CO2 is—at least to most mammals and to pigs with genotypes commonly found in commercial lines. CO2/N2 mixtures should be discussed in the CO2 section or in a separate section about gas mixtures. The paper cited in the original paragraph states: "Hypoxia, induced by the inhalation of inert gases, such as argon or nitrogen, has also been evaluated to stun pigs under experimental conditions (Raj & Gregory 1995, 1996; Raj 1999). In contrast to hypercapnia, research has shown that hypoxia or anoxia does not cause aversion in pigs and does not induce any signs of respiratory distress prior to loss of consciousness (Raj & Gregory 1995)."	- 2010 Stunning pigs with different gas mixtures: Aversion in pigs - Raj ABM 1999 Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. Veterinary Record 144: 165-168 - Raj ABM and Gregory NG 1995 Welfare implications of the gas stunning of pigs 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. Animal Welfare 4: 273-280 - Raj ABM and Gregory NG 1996 Welfare implications of the gas stunning of pigs 2. Stress of induction of anaesthesia. Animal Welfare 5: 71-78 - 2014 Raj Stunning _ CO2 and Other Gases chapter

3656-3657	Nitrogen is nonflammable, nonexplosive, and readily available as compressed gases.	Nitrogen is nonflammable and nonexplosive. It can be extracted from the atmosphere (for example, for utilization in N2 gassing trailers manufactured by Prairie Agricultural Machinery Institute). It can be purchased as a compressed gas in cylinders. If large volumes are needed, liquid nitrogen is available via nitrogen pumper trunks or LIN tankers. [See "Rationale for suggested change" for additional recommendations on expanding this section.]	If there is to be a discussion of the forms in which it is available, this should include a discussion of the different delivery forms, for example, LIN (liquid nitrogen tankers) v. N2 pumper trucks, and the means by which access to the volumes needed can be arranged during the preparedness stage of the emergency/disaster management cycle. Please also note that the availability of different forms of N2 is already discussed above at lines 3630–3631. A thorough review of this section with an emphasis on improving structure, coherence, and applicability would be beneficial.	2024 Evaluation of nitrogen whole house gassing for the mass depopulation of poultry 2024 Utilizing Nitrogen Foam Euthanasia in Pennsylvania Swine & Poutrly Operations
3658-3660	N2-CO2 gas mixtures are heavier than air and can be contained within an apparatus into which animals and birds can be lowered or immersed.17 N2 containing high-expansion	[Delete or correct and relocate this sentence]	Neither of these sentences belongs in this section. CO2 mixtures should be covered in the CO2 section or in a new section regarding gas mixtures. It is unclear that a depopulation method that relies on lowering animals into a N2-CO2 gas mixtures is currently available, so removal of this reference may be preferred over retaining it. If the ability of gas to be contained "within an apparatus into which animals and birds can be lowered or immersed" is discussed, the discussion should be expanded to discuss the N2 gassing trailer available from PAMI. The	Livetec CGU info Bergen PAMI trailer abstract

	foams appear to be mildly aversive to neonatal and adult swine.18,20,21		words "animals and birds" should be replaced with "pigs," especially as containerized gassing systems that use inert gases are available (reference). High expansion nitrogen foam should be discussed in the section devoted to that topic.	
3727-3728	Specialized electrocution trailers can be built or obtained to improve human safety and throughput of the depopulation process.27	Specialized electrocution trailers can be built or obtained to improve human safety and throughput of the depopulation process. A mobile electrocution trailer was found to have a high throughput. However, due to the specialized nature of this equipment, and extended time for manufacturing of this equipment, specialized electrocution trailers must be obtained preemptively and must be maintained such that it is available for rapid deployment.	This method will only be feasible if the electrocution trailer is secured ahead of time, as it is unlikely it could be rapidly acquired in an emergency.	2020 Validation of a Mobile Electrocution System for Humane Mass Depopulation of Swine – NPB #20-123

3783	The mode of death associated with nitrogen-filled foam is anoxia.	The mode of death associated with nitrogen-filled foam is anoxia. There are two different systems for utilizing high expansion nitrogen foam. One, developed by the company HEFT, relies on utilization of a closed container. After being filled with the high expansion nitrogen foam, a burst of nitrogen gas is used to simultaneously burst the bubbles and rapidly release the nitrogen they contain. The other, utilized by the UK company Livetec and the US-based AES, can be applied in a corral, barn, or open-topped container and relies on building up the foam to a high level and allowing animal movement to burst the foam bubbles.	It is important to make clear that there are two different systems and then to indicate which system was being studied in the research reported in the remainder of the section. While they have an identical mechanism of action, they are appropriate in different contexts and carry different risks. Please see "Evaluation of High Expansion Nitrogen Foam for Depopulation of Market Swine in Missouri," a report that we believe has been submitted to the Depopulation Panel.	HEFT A SYSTEM FOR LARGE-SCALE EUTHANASIA OF LIVESTOCK 2024 Utilizing Nitrogen Foam Euthanasia in Pennsylvania Swine & Poultry Operations 2023 N2 Foam stunning Culhane NAMI 2024 Behavioural response in pigs at gas stunning with foam 2023 The Utilization of Livetec Systems' Nitrogen Foam Delivery System for the Rapid, Large-scale Depopulation of Swine 2023 HEFT Technological Summary 2022 Validation and Demonstration of Utilizing High Expansion Nitrogen Foam for Large Scale Depopulation of Swine 2022 Euthanasia of healthy & non-viable piglets using high-exp n2 foam

3788-3790	Fill time will depend on container size but in one setting in field conditions was accomplished in 100 to 140 seconds with cessation of movement of nursery pigs at 89-100.3 seconds, market pigs at 63.3 seconds and adults at 169.25 seconds.15	[See Rationale for suggested change]	First, it is important to clarify which type of high expansion N2 system is being discussed—closed container v. open container/barn. For example, HEFT's C3 container system (area = 108 sq ft) can be filled within 30 seconds and HEFT reports animals lose consciousness in 10 to 20 seconds. The rate at which a Livetec or AES system can fill a container depends on how many separate foam generators are used and how big the container is. Second, it is also crucial to clarify here and in the section on water-based foam, that cessation of movement may occur at different times relative to loss of consciousness; therefore relying solely on cessation of movement when comparing different foaming methods is problematic. It has been shown that, when pigs are killed with high concentrations of CO2, muscular contractions begin before loss of consciousness (Verhoeven 2016, Rodriguez 2008). In addition, hypercarbia shortens the duration of muscular contractions after loss of consciousness (Lindahl 2022). Although it has not been specifically assessed, hypercarbia is likely to develop with water-based foam due to its mechanism of obstructive asphyxia (airway occlusion), which prevents gas exchange (it is well accepted in anesthesiology that airway occlusion rapidly results in hypercarbia, or elevated CO2 levels in the blood). In contrast, muscular convulsions resulting from	HEFT A SYSTEM FOR LARGE-SCALE EUTHANASIA OF LIVESTOCK https://heftinternational.com/wp-content/uploads/2024/10/C3-Product-Folder.pdf - 2022 Controlled atmosphere stunning of pigs using N2, Ar, CO2 in high-exp foam 2008 Assessment of unconsciousness during carbon dioxide stunning in pigs 2016 Time to loss of consciousness and its relation to Behavior in slaughter Pigs during stunning with 80 or 95% carbon Dioxide 2013 Assessment of unconsciousness in pigs during exposure to nitrogen and carbon dioxide mixtures 1999 Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications

			anoxia alone begin after consciousness is lost and convulsions persist for longer when anoxia is not accompanied by hypercarbia (Raj 1999). Thus, when comparing HENF and water-based foam, it may not be appropriate to equate time to cessation of movement with time to loss of consciousness. A significant percentage of the movement of pigs in the water-based foam is likely due to conscious struggling in response to the inability to expand the lungs in the face of rapidly worsening hypercarbia. In contrast, much of the movement of pigs subjected to HENF is likely to occur after loss of consciousness.	
3793	Some foams may cause dermal and eye irritation.	[This sentence should be deleted.]	Reference is needed or this sentence should be removed. The EFSA scientific opinion entitled “The use of high expansion foam for stunning and killing pigs and poultry” (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2024.8855) specifically examined this issue and noted, "The results provided do not indicate that mucosal irritation occurs due to exposure of animals to the foam. Therefore, it is concluded with a certainty > 50%–100% (more likely than not) that no	UK AWC opinion on the use of high exp N2 foam EFSA scientific opinion use of high exp foam 2023 HEFT Technological Summary

			mucosal irritation due to foam occurs." The UK governmental Animal Welfare Committee noted in their scientific opinion, "A further welfare concern is the possibility of eye and skin irritation from the foam constituents. However, the lack of an "irritant" behavioural response in studies of broilers and laying hens would perhaps suggest that these particular types of bird are not sensitive to the foam formulation used in that specific study. Birds were submerged in air filled foam and stood quietly without signs of irritation until retrieved." At least some high expansion foam products marketed for depopulation/euthanasia specifically note that the foam is non-irritating: https://heftinternational.com/heftfoamagent/	
3794-3795	Have suitable respiration equipment (self-contained breathing apparatus [SCBA], oxygen) and human rescue equipment available.	[This sentence should be deleted.]	High expansion foam is a very light foam that can be blown away rapidly with a leaf blower. Free nitrogen gas will rapidly disperse unless one is in a small, enclosed space. SCBA equipment is not required.	2023 The Utilization of Livetec Systems' Nitrogen Foam Delivery System for the Rapid, Large-scale Depopulation of Swine 2024 Opinion on the Use of High Expansion N2 foam delivery systems for depop of poultry flocks UK AWC 2022 Validation and Demonstration of Utilizing High Expansion Nitrogen Foam for Large Scale Depopulation of Swine

3796	Nitrogen is nonflammable, nonexplosive, and readily available as compressed gases.	Nitrogen is nonflammable, nonexplosive, and readily available as a compressed gas or as liquid nitrogen.	If there is to be a discussion of the forms in which it is available, this should include a discussion of the different delivery forms, for example, LIN tankers v. N2 pumper trucks, and the means by which access to the volumes needed can be arranged during the preparedness stage of the emergency/disaster management cycle. Please also note that the availability of different forms of N2 is already discussed above, at two different locations in the "Nitrogen" section. A thorough review of this section with an emphasis on improving structure, coherence, and applicability would be beneficial.	2024 Evaluation of nitrogen whole house gassing for the mass depopulation of poultry 2024 Utilizing Nitrogen Foam Euthanasia in Pennsylvania Swine & Poultry Operations
3799-3801	In one study to of the animals were determined to have their tracheas occluded by foam but in most studies where necropsy was performed, death was determined to be via anoxia versus occlusion.15	In one study two of the animals were determined to have some foam in their tracheas, but without occlusion. In all of the studies in which necropsy was performed, death was determined to be via anoxia rather than occlusion.	The original statement is false. The study cited (https://porkcheckoff.org/wp-content/uploads/2022/06/21-069-WILLIAMS-final-rpt.pdf) found that in one trial, 50–60% of pigs had high expansion foam present in the airway, however "Of these 60 animals evaluated, none showed signs of occlusion of the trachea." It also states, "In summary, a total of 6 replicates utilizing a total of 551 pigs from wean age to adult were successfully depopulated, with none displaying occlusion of the trachea, utilizing high expansion foam with the Livetec Nitrogen Foam Depopulation System." The EFSA, in its literature review on the use of high expansion foam for poultry and swine slaughter, found no evidence that high expansion nitrogen foam results in occlusion of the airway, but notes that	- EFSA Panel on Animal Health and Welfare. (2024). The use of high expansion foam for stunning and killing pigs and poultry. EFSA Journal. European Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855.

			foam may enter the airway (posing a welfare concern) if bubble size is incorrect. A preprint publication we believe has been submitted to the Swine Working Group (Campler 2025) similarly did not find evidence of airway occlusion in pigs depopulated with high expansion N2 foam. Postmortem lesions were similar between CO2, high expansion N2 foam, and pentobarbital, but very different for WBF.	
3802-3805	Based on the wash-in and wash-out functions, gradual displacement methods using N2 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,	[Delete or relocate this section]	This is applicable to systems that inject gas directly, but not to high expansion N2 foam systems. Part of the benefit of using foam to create a low oxygen environment is that the foam can rapidly displace oxygen and avoid prolonged exposure to inadequately hypoxic conditions. (See attached reference: "An innovative technique dispersing the nitrogen gas in soap foam bubbles resulted in 2.7 times quicker oxygen depletion than when only using free nitrogen gas" (Wallenbeck 2020).)	2020 Improved pig welfare at slaughter –pigs' responses to air- or n2 foam ABSTRACT

	which may be distressing for non-avian species.			
3805-3806	Studies in swine show increased escape attempts but no gasping	Studies in pigs show increased escape attempts but no gasping. Newer research suggests that noise from the foam generators and rising levels of foam are responsible for escape attempts. Provision of anxiolysis via pharmaceutical means, as is mentioned in other species sections in this document and routinely used in companion animal medicine, may be another means of reducing or eliminating negative affective states such as fear and anxiety. Further research is needed.	Given that tissue residues are less of a concern when animals are depopulated rather than slaughtered for consumption, the Panel on Depopulation should seriously consider recommending research into whether anxiolytic medications can be utilized to minimize outcomes indicative of negative welfare, such as escape attempts. Unlike CO2-, airway occlusion- or hyperthermia-based methods, neither high expansion foam nor the nitrogen gas is inherently aversive or painful; rather, pigs seem to react to the noise and buildup of foam past snout level. This suggests that providing anxiolysis prior to depopulation with this method might further improve welfare.	2021 Pharmacokinetics of oral and compounded intravenous gabapentin in Duroc swine (Sus scrofa) Di Cesare, F., Negro, V., Ravasio, G., Villa, R., Draghi, S., & Cagnardi, P. (2023). Gabapentin: Clinical Use and Pharmacokinetics in Dogs, Cats, and Horses. *Animals*, *13*(12), 2045. https://doi.org/10.3390/ani13122045 Erickson, A., Harbin, K., MacPherson, J., Rundle, K., & Overall, K. L. (2021). A review of pre-appointment medications to reduce fear and anxiety in dogs and cats at veterinary visits. *The Canadian veterinary journal = La revue veterinaire canadienne*, *62*(9), 952–960. Rørvang, M. V., Blad, M., Lindahl, C., & Wallenbeck, A. (2024). An added aroma changes the

				behaviour of domestic pigs in a novel situation aimed for stunning. *Applied Animal Behaviour Science*, *270*, 106145-. https://doi.org/10.1016/j.applanim.2023.106145
3806-3807	The Nitrogen is expensive and specialized equipment is needed to get the gas in the proper form and mixed with the foam.	Nitrogen is less costly and more widely available than carbon dioxide, and costs continue to decrease as nitrogen separation systems improve. One of the primary benefits of nitrogen is that it is produced throughout the country via air separation units that are owned and operated by gas supply companies, unlike CO2, suppliers of which are reliant on third party manufacturing plants for which CO2 is not their primary product. For these reasons, N2 is far less prone to significant supply chain interruption. In addition, because the liquid form of nitrogen is much denser than CO2, a greater amount of	The claim that "nitrogen is expensive" lacks context and is false when compared to the cost of CO2 and, in some parts of the country, the cost of the large volumes of water required for use of water-based foam. The source of this information is Livestock Welfare Strategies, which can be consulted regarding procurement and costs of nitrogen and carbon dioxide in the context of depopulation. Specialized equipment is necessary, but is currently readily available from three different distributors: HEFT (https://heftinternational.com/), Livetec (https://www.livetecsystems.co.uk/), and AES Inc. (https://www.agemergency.com/).	2024 Evaluation of nitrogen whole house gassing for the mass depopulation of poultry

		nitrogen can be transported per trailer. Thus, transport costs are generally much lower for nitrogen than for CO2. Specialized equipment is needed for the utilization of high expansion nitrogen foam, therefore it is necessary to purchase and stockpile such equipment in the preparedness stage, prior to any actual emergency.		
3807	Travel costs for nitrogen tanks may be prohibitive.	[Delete this sentence or provide substantiation]	Reference is required. Per attached reference, liquid nitrogen (LIN) is "readily available throughout North America,...low cost... and cost effective to transport."	2024 Evaluation of nitrogen whole house gassing for the mass depopulation of poultry

4519	6.1 General Considerations	[Consider adding the following language somewhere within the General Considerations section] Historically, the need for depopulation has been rare and intermittent, however, in recent years, depopulation due to highly pathogenic avian influenza (HPAI) has become a regular occurrence. The current outbreak began 3 years ago in the United States and, due to the virus having become endemic in wild birds on numerous continents, including North America, HPAI infections of commercial flocks are likely to be a common occurrence for the foreseeable future. For this reason, it is essential that adequate attention be given to other parts of the emergency management cycle, particularly in terms of mitigating risk.	This section does not mention the fact that intensive poultry production has contributed to HPAI, via increased frequency of LPAI-HPAI conversion events (Dhingra 2018) or that research suggests larger flocks are likely to become infected with HPAI. This is important, given that risk mitigation is an important step in the disaster management cycle that is referenced in the introduction. The draft Guidelines emphasize exposure to wild birds as increasing disease risk, but do not address issues like stocking density, air quality, house/farm population size, and stress level, which can also affect disease risk. Biosecurity and minimizing exposure to wild birds who may be carrying HPAI is one component of this. In addition, approaches including vaccination, reducing flock sizes, and improving animal resilience via minimizing housing-related stressors, such as poor air quality, have been proposed (Granger et al. 2024, World Organization for Animal Health 2023, OHHLEP 2023, UNEP CMS 2024).	- https://www.woah.org/app/uploads/2023/12/en-woah-policybrief-avianinfluenzavaccinationandtrade.pdf - 2018 Geographical &Historical Patterns in the Emergences of Novel Highly Pathogenic Avian Influenza (HPAI) H5 &H7 Viruses in Poultry - The Panzootic Spread of Highly Pathogenic Avian Influenza H5N1 Sublineage 2.3.4.4b: A Critical Appraisal of One Health Preparedness and Prevention." - https://awionline.org/sites/default/files/uploads/documents/Comments-APHIS-HPAI-Response-Activities-Draft-Programmatic-EIS.pdf - Swayne, D.E. & Sims, L. (2023). Vaccine Usage to Control Highly Pathogenic Avian Influenza in Poultry and Other Domestic Birds: Setting the Scene, available at: https://rr-americas.woah.org/app/uploads/2023/05/0206-engswayne-hpai-vax-setting-stage.pdf - Resolution 14.18, UN ENVIRONMENT PROGRAMME, CONVENTION ON MIGRATORY SPECIES, (February 2024), https://www.cms.int/sites/defaul

				t/files/document/cms_cop14_res.14.18_avian-influenza_e.pdf

4707-4710	More recently, during the HPAI outbreak starting in 2022, there was a significant decrease in the time to depopulation. This change has markedly decreased the lateral spread of the virus from farm to farm.	More recently, during the HPAI outbreak starting in 2022, there was a significant decrease in the time to depopulation. This change is theorized to have decreased the lateral spread of the virus from farm to farm, though this has not been confirmed (APHIS 2024) and the current HPAI has resulted in more than twice the number of birds destroyed as during the previous outbreak.	This statement requires substantiation. It equates correlation with causation. The USDA/CEAH analysis that investigated this was unable to draw any firm conclusions. For example, it states, "While one intent of the 24–48 hour depopulation goal is to prevent lateral spread, it is difficult to objectively define and measure the extent to which depopulation methods or timing prevent or contribute to lateral spread. Phylogenetic data helps identify premises where lateral spread likely occurred, and may provide insight into directionality; however, the epidemiological and production data is needed to better understand the timing and mechanisms of virus movement. Conclusive evidence indicating the exact day of transmission is rare, and this makes it challenging to confidently separate cases of lateral spread that occurred before detection from cases of lateral spread that occurred after detection due to the timing of depopulation.... For commercial turkey and duck premises, this report suggests that premises involved in a CSLT [common source or lateral transmission] cluster started depopulation sooner than premises categorized as IND [independent point source introduction]. However, similar median times were observed for commercial table egg and commercial broiler premises. This association is counterintuitive to what CEAH analysts expected and is likely due	- https://awionline.org/sites/default/files/uploads/documents/Comments-APHIS-HPAI-Response-Activities-Draft-Programmatic-EIS.pdf

			to State-level influence, given that the time to start depopulation for premises categorized as CSLT varied by State." Given research that flock size on a given operation is inversely correlated with the speed at which it can be depopulated, please consider expanding this section to discuss other means of reducing lateral spread (see pages 11-20 of the attached document, which include an analysis of depopulation records showing that excessively large farm populations make it impossible to meet APHIS's 48-hour depopulation goal, which presumably increases the risk of these operations spreading HPAI to other flocks, wildlife, etc.).	
4802-4803	Inert gases cause death by hypoxia, and therefore have an animal welfare advantage compared to carbon dioxide which can potentially be aversive to avian species.19-21	Inert gases cause death by anoxia, and therefore have an animal welfare advantage compared to carbon dioxide which can potentially be aversive to avian species.	Given the extremely low levels of oxygen in Nitrogen Whole House Gassing (NWHG), it appears that "extreme hypoxia" or "anoxia" would be more precise/correct terms than "hypoxia." Since anoxia is referenced above in the Swine sections on nitrogen and nitrogen foam (lines 3800, 3783, 3633), it seems this would be preferred. For example, the UK governmental Animal Welfare Committee defines *anoxia* as "the absence, or near absence, of oxygen." The EFSA (2024) defines anoxia as "< 2% by volume of residual oxygen." The 2020 AVMA Euthanasia Guidelines specify	- EFSA Panel on Animal Health and Welfare. (2024). The use of high expansion foam for stunning and killing pigs and poultry. EFSA Journal. European Food Safety Authority, 22(7), e8855. https://doi.org/10.2903/j.efsa.2024.8855. - 2024 UK AWC Opinion on the Use of High Expansion N2 foam delivery systems for depop of poultry flocks

			oxygen levels must be held at sufficiently low levels (2% or 3%) when nitrogen is used for euthanasia of poultry (p 77).	AVMA. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf. Page 77.
4843-4844	In situations where it is possible to maintain gas concentrations at a high level, an inert gas such as nitrogen or argon can also be used in a containerized system.	In situations where it is possible to maintain gas concentrations at a high level, an inert gas such as nitrogen or argon can also be used in a containerized system. For example, a nitrogen gassing trailer, with a built-in nitrogen generator, has been developed by the Prairie Agricultural Machinery Institute. Although it was developed for pigs, it can be utilized for poultry species as well. In addition, Livetec has developed containerized gassing units for poultry that can utilize both argon and nitrogen.	Consider expanding discussion so that readers will be able to access necessary equipment.	PAMI N2 trailer abstract Livetec CGU product information

4858-4860	Field research has demonstrated that LAPS is an effective and humane method of depopulation of poultry, however there remain significant technical hurdles, (such as handling modules/crates, removing birds, etc.), hindering its widespread adoption.	Field research has demonstrated that LAPS is an effective and humane method of depopulation of poultry, however there remain significant technical hurdles, (such as handling modules/crates, removing birds, etc.), hindering its widespread adoption. However, mobile units that can be transported via trailer are available	It should be clearly communicated that mobile LAPS units are available in the U.S. from TechnoCatch.	http://www.lapsinfo.com/press-release

4973-4984	At slaughter houses, electrical stunning in the United States involves pulsed direct current with low current (25 to 45 mA/ bird),103 low voltage (10 to 25 V),55-57 and high frequency (approx. 500 Hz).55-57 This type of system became possible with advances in electrical circuitry and changes to the length of the water bath cabinet that increase dwell time of the birds and decrease the total resistance in the water bath.55 In a survey of 329	A mobile device called the H2H Euthanizer can be utilized for depopulation of small flocks, or survivors of other depopulation methods. When utilized per manufacturer's instructions, it is reported to lead to instantaneous and irreversible loss of consciousness, followed by death.	This current section appears very out of place, as it discusses electrical stunning with water baths in slaughterhouses, failing to mention the controversy regarding whether the type of electrical stunning performed in the US actually renders birds unconscious rather than merely immobilizing them (McKeegan, 2020, p 10-12). Moreover, the mobile individual bird electrical stunning systems most applicable to a depopulation scenario do not use a waterbath at all. This section should be deleted and replaced with information relevant to on-farm euthanasia and depopulation.	2020 Improving welfare in poultry slaughter 2018 Effectiveness and method H2H Euthanizer 9 page report 2019 Top Equipment_Operators Manual_H2H Euthanizer_2019-12-30 H2H_Euthanizer - Chicken electrocution

	US poultry plants, 92.1% reported using electrical stunning and 77.4% of those plants used low-voltage (10 to 25 V), high-frequency (500 Hz) systems.58 Efficacy of these systems is influenced by the species, number, and size of the birds passing through the water bath because with increasing size and number of birds in the bath at one time the resistance increases and because parallel paths of current arise with increasing numbers of birds. Variable resistance can			

	result in insufficient current to produce immediate unconsciousness. Constant-current stunners may alleviate this problem.59			
5001-5002	High expansion foams with large bubble sizes (>15mm; 0.6in) are less likely to result in occlusion of the airway	High expansion foams with large bubble sizes (> 15mm; 0.6 in) are less likely to result in occlusion of the airway compared to smaller bubbles, instead causing death due to severe hypoxia or anoxia	Given the extremely low levels of oxygen in HENF, it appears that "extreme hypoxia" or "anoxia" would be more precise/correct terms than "hypoxia." Since anoxia is referenced above in the Swine sections on nitrogen and nitrogen foam (lines 3800, 3783, 3633), it seems this would be preferred. For example, the UK governmental Animal Welfare Committee	EFSA HEFT opinion AWC HEFT opinion Culhane presentation

	compared to smaller bubbles, instead causing death due to hypoxia.60,61		defines anoxia as "the absence, or near absence, of oxygen." The EFSA (2024) defines anoxia as "<2% by volume of residual oxygen." The 2020 AVMA Euthanasia Guidelines specify oxygen levels must be held at sufficiently low levels (2% or 3%) when nitrogen is used for euthanasia of poultry (p 77).	
5188-5244	Virtually any sealable container could be used for containerized gassing, from trash cans with lids to purpose-built units with automated gas delivery systems. The container size and number need to be appropriate for the flock size and equipment available to handle the containers.	The container size and number need to be appropriate for the flock size and equipment available to handle the containers. Virtually any sealable container could be used for containerized gassing, from trash cans with lids to purpose-built units with automated gas delivery systems, however, the animal welfare impacts of different systems vary widely depending on factors such as (1) whether gas flow and temperature can be controlled, (2) whether the design of the container results in piling of birds such that some birds die via mechanical asphyxia (pressure on their coelom preventing	At present, several different methods of containerized gassing are described and presented as relatively equivalent. However, some containers offer very little control over gas flow and temperature, factors that impact animal welfare. In addition, the design of the container and the gas(es) used also significantly impact animal welfare. In order to ensure readers understand the welfare implications of different types of containerized gassing, a more careful and nuanced discussion is needed.	McKeegan, DEF. 2018. Mass depopulation. In: Advances in Poultry Welfare (Mench Ed.). 5726 Woodhead Publishing, Kidlington, United Kingdom. Livetec CGU product information

		breathing), (3) what type of gas is used and at what concentration, and (4) whether birds require extensive handling or can be walking into the enclosure used for gassing (McKeegan 2018). If containerized gassing is to be used, every effort should be made to utilize a system that maximizes animal welfare. To achieve this, advanced planning and preparation are required, including obtaining the necessary equipment and enacting contracts to enable rapid acquisition of needed gases.		
5294-5296	It is important to differentiate between medium/low-expansion foams and high expansion foams as they have very different animal welfare impacts (see other foam sections).	It is important to differentiate between medium/low-expansion foams and high expansion foams as they have very different animal welfare impacts. [Reiterate the difference between the two different types of foam in terms of the mechanism of killing, associated affective states, and time to loss of	The welfare implications of low- and medium-expansion foams should be articulated here. To avoid confusion, the terms "expansion," rather than "density" should be used. High expansion foams will necessarily be low density, and vice-versa.	

		consciousness, and impact on animal welfare, rather than merely referring to another section]		
5311	The method of death is mechanical hypoxia.61,63	The method of death is airway occlusion (obstructive asphyxia), which prevents respiration and the exchange of both oxygen and carbon dioxide.	"Mechanical hypoxia" is an ambiguous and inaccurate term for describing airway occlusion. Per the attached reference (Ewen, B.J. 2016, Nondrowning Asphyxia in Veterinary Forensic Pathology: Suffocation, Strangulation), and Mechanical Asphyxia, mechanical asphyxiation is defined as one of the following: positional asphyxia (= position of the animal compromises the ability to breathe) or traumatic asphyxia (= external chest or abdominal compression by a heavy object preventing respiration). Furthermore, airway occlusion results not only in hypoxia but also in hypercarbia (Murray 2022). In a conscious animal, increase in PaCO2 increases drive to breathe and, based on our understanding of types of dyspnea in humans, increases the intensity of the unpleasant sensation of air hunger when coupled with inability to bring in more air. The terms "airway occlusion" or "obstructive asphyxia" are more accurate and precise descriptors for the mechanism of death of both compressed air foam and low- or medium-expansion water-based foam.	2016 Nondrowning asphyxia in veterinary forensic pathology_ suffocation, strangulation & mech asphyxia 2014 Mellor Introducing Breathlessness 2022 Complete tracheal obstruction during anaesthesia for ventral slot decompression

5436-5439	In addition to the challenges described above for use of oral drugs to decrease negative affective states at the time of killing, another challenge in this context is individual consumption of sublethal doses and differentiating sedated animals from deceased individuals. Confirmation of death prior to disposal is crucial with this method.	Depending on the medication utilized, it is possible that, in some cases, differentiating sedated animals from deceased animals may be challenging. In such cases, consideration should be given to delaying disposal of carcasses beyond the time point at which sedated birds who survived the depopulation method would have recovered.	Anesthetic, analgesics, and anxiolytics are not being proposed as a killing (lethal) method. Therefore, discussion of sublethal doses is inappropriate.	
5446-5447	Because of the anxiety associated with extreme hypovolemia, exsanguination as a sole method of killing should	Because of the pain associated with laceration of the cervical region and anxiety associated with extreme hypovolemia, exsanguination as a sole method of killing must	It is accepted that birds feel pain immediately in response to trauma, such as deep laceration of the skin and soft tissues in the cervical region (McKeegan 2020 p. 33, Aghwan 2016, p. 424-425). Therefore, both pain and anxiety are potential concerns with this method of killing.	2016 Efficient halal bleeding, animal handling, and welfare- A holistic approach for meat quality 2020 Improving welfare of poultry at slaughter

	be used only on unconscious animals.14	not be used on conscious animals.		