Animal Welfare Institute

900 PENNSYLVANIA AVENUE, SE, WASHINGTON, DC 20003 · 202-337-2332 · AWIONLINE.ORG

October 22, 2024

Submitted via Online Public Comment Portal

Planning Committee
National Academies of Sciences, Engineering, and Medicine
500 Fifth Street NW
Washington, DC 20001

Re: AWI Comments Regarding Potential Research Priorities to Inform Readiness and Response to Highly Pathogenic Avian Influenza A (H5N1)

Dear Members of the Planning Committee,

Thank you for hosting the important workshop entitled "Potential Research Priorities to Inform Readiness and Response to Highly Pathogenic Avian Influenza A (H5N1)."[1] The Animal Welfare Institute (AWI) appreciates the opportunity to submit comments. AWI is a nonprofit organization founded in 1951 whose mission is to reduce animal suffering caused by people. The ongoing outbreak of Highly Pathogenic Avian Influenza (HPAI) has become one of the most dire animal welfare crises of our time; therefore, our organization has been extensively engaged in efforts to mitigate its impacts. The following suggestions address research gaps related to both HPAI prevention and response. Specifically, we recommend prioritizing further research into three areas: 1) the development of HPAI vaccines for poultry; 2) the role that larger flock sizes play in HPAI spread; and 3) the need for higher-welfare depopulation methods.

I. Research Needs: HPAI Prevention

Given the enormous impact that the current HPAI outbreak has had on animals (especially poultry, dairy cattle, and wildlife) and the people responsible for caring for or studying them, as well as the virus's pandemic potential, AWI agrees with One Health experts that more emphasis

[1] National Academies of Sciences, Potential Research Priorities to Inform Readiness and Response to Highly Pathogenic Avian Influenza A (H5N1): A Workshop (Oct. 22-23), https://www.nationalacademies.org/event/43695_10-2024_potential-research-priorities-to-inform-readiness-and-response-to-highly-pathogenic-avian-influenza-a-h5n1-a-workshop.

must be placed on preventing HPAI infections.[2] To this end, we encourage prioritizing research on HPAI vaccines and vaccination programs for poultry, as well as further study of the effect of very large flock sizes on HPAI risk. These are issues we also recently encouraged the U.S. Department of Agriculture (USDA) to consider in comments we submitted on its draft Programmatic Environmental Impact Statement on HPAI response.[3]

A. HPAI Vaccination

Vaccination against HPAI of U.S. poultry, particularly egg-laying hens and turkeys in high-risk areas, appears to be a very promising way to decrease the risk this disease poses to animal welfare, wildlife, and public health.[4] HPAI vaccination greatly decreases birds' risk of becoming infected and ensures that any birds who do become infected shed far lower amounts of virus than they would otherwise,[5] decreasing environmental contamination and the risk of spread to other farms, animals, and people, including responders.[6] In light of France's successful experience

---

[2] *The Panzootic Spread of Highly Pathogenic Avian Influenza H5N1 Sublineage 2.3.4.4b, A Critical Appraisal of One Health Preparedness and Prevention*, THE ONE HEALTH HIGH-LEVEL EXPERT PANEL, (2023), https://cdn.who.int/media/docs/default-source/one-health/ohhlep/the-panzootic-spread-of-highly-pathogenic-avian-influenza.pdf?sfvrsn=205b68bd_16&download=true, at 1 (hereinafter *A Critical Appraisal of One Health Preparedness and Prevention)*; Resolution 14.18, UN ENVIRONMENT PROGRAMME, CONVENTION ON MIGRATORY SPECIES, (February 2024), https://www.cms.int/sites/default/files/document/cms_cop14_res.14.18_avian-influenza_e.pdf; CMS Resolution 12.6. Resolution 12.6, UN ENVIRONMENT PROGRAMME, CONVENTION ON MIGRATORY SPECIES, (February 2024), https://www.cms.int/sites/default/files/document/cms_cop14_res.12.6_rev.cop14_wildlife-health-and-migratory-species_e.pdf.

[3] AWI. (2024). Comments on APHIS HPAI Response Activities Draft Programmatic EIS. Available at: https://awionline.org/sites/default/files/uploads/documents/Comments-APHIS-HPAI-Response-Activities-Draft-Programmatic-EIS.pdf. Accessed October 15, 2024. (hereinafter *AWI comments on HPAI Response EIS*)

[4] *Avian influenza vaccination: why it should not be a barrier to safe trade*, WORLD ORGANISATION FOR ANIMAL HEALTH, (December 28, 2023), https://www.woah.org/en/avian-influenza-vaccination-why-it-should-not-be-a-barrier-to-safe-trade/.

[5] Germeraad, E.A., Bouwman, K.M., Jansen, C.A., et al. (2024). Progress report: Transmission study testing HVT based H5 vaccine against highly pathogenic avian influenza (HPAI) H5N1 virus (clade 2.3.4.4b) First report, 8-weeks post vaccination with VAXXITEK. First Report, Project No. BO-43-111-083, Wageningen Bioveterinary Research. Available at: https://edepot.wur.nl/656515. Accessed September 23, 2024; Germeraad, E.A., Bouwman, K.M., Jansen, C.A., et al. (2024). Progress report: Transmission study testing HVT-based H5 vaccine against highly pathogenic avian influenza (HPAI) H5N1 virus (clade 2.3.4.4b) First report, 8-weeks post vaccination with Vectorimmune ® AI. First Report, Project No. BO-43-111-083, Wageningen Bioveterinary Research. Available at: https://edepot.wur.nl/656515. Accessed September 23, 2024; Hasan, N. H., Ignjatovic, J., Peaston, A., & Hemmatzadeh, F. (2016). Avian Influenza Virus and DIVA Strategies. Viral Immunology, 29(4), 198–211. https://doi.org/10.1089/vim.2015.0127; Capua, I., Terregino, C., Cattoli, G., & Toffan, A. (2004). Increased resistance of vaccinated turkeys to experimental infection with an H7N3 low-pathogenicity avian influenza virus. Avian pathology : journal of the W.V.P.A, 33(2), 158–163. https://doi.org/10.1080/03079450310001652077

[6] Mo, J., Spackman, E., & Swayne, D. E. (2023). Prediction of highly pathogenic avian influenza vaccine efficacy in chickens by comparison of in vitro and in vivo data: A meta-analysis and systematic review. Vaccine, 41(38), 5507–5517. https://doi.org/10.1016/j.vaccine.2023.07.076; Lee, J., Lee, C. W., Suarez, D. L., et al. (2024). Efficacy of commercial recombinant HVT vaccines against a North American clade 2.3.4.4b H5N1 highly pathogenic avian influenza virus in chickens. PloS one, 19(7), e0307100. https://doi.org/10.1371/journal.pone.0307100; Palya, V., Tatár-Kis, T., Walkóné Kovács, E., et al. (2018). Efficacy of a Recombinant Turkey Herpesvirus AI (H5) Vaccine in

with HPAI vaccination,[7] it appears likely that initiating HPAI vaccination of high-risk U.S. flocks would decrease by orders of magnitude the number of birds lost annually (to the disease and to depopulation) due to the HPAI virus.

Many of the challenges previously associated with HPAI vaccination, such as differentiating vaccinated and not-infected animals from vaccinated and infected animals (i.e., the DIVA problem)[8] and adapting HPAI surveillance systems[9] have been addressed. However, there are still areas ripe for research, such as the development of rapid HPAI tests that can provide results on farms, and commercialization of vaccines that provide a long duration of immunity when given in ovo or in the hatchery or that can be administered via water or spray.[10] In addition, because HPAI vaccinations can only be used if approved by the USDA, research is needed to ensure that all relevant impacts are considered and incorporated into decision-making on this issue. For example, the potential for negative impacts on international trade are currently a chief reason why HPAI vaccinations have not been approved in the U.S., while potential benefits to animal welfare are not considered by USDA at all.[11] Basic research on incorporating animal welfare into cost-benefit analysis has recently been initiated,[12] however, more specific research is

---

Preventing Transmission of Heterologous Highly Pathogenic H5N8 Clade 2.3.4.4b Challenge Virus in Commercial Broilers and Layer Pullets. Journal of immunology research, 2018, 3143189. https://doi.org/10.1155/2018/3143189

[7] Gruber, P. (2024). France Sees Success Vaccinating Ducks Against Avian Flu. Available at: https://www.lancasterfarming.com/farming-news/poultry/france-sees-success-vaccinating-ducks-against-avian-flu/article_c88adc58-75e3-11ef-a0d1-87928be5869d.html?itm_source=parsely-api&utm_source=article&utm_medium=summary&utm_campaign=What%20To%20Read%20Next. Accessed September 23, 2024.

[8] Hasan, N. H., et al. (2016); Mirzaei, S. G., Shoushtari, A., & Noori, A. (2020). Development and evaluation of real-time reverse transcription polymerase chain reaction test for quantitative and qualitative recognition of H5 subtype of avian influenza viruses. *Archives of Razi Institute*, 75(1), 17- 22. https://doi.org/10.22092/ari.2019.120821.1201; Suarez, D. L. (2012). DIVA Vaccination Strategies for Avian Influenza Virus. *Avian Diseases*, *56*(4s1), 836–844. https://doi.org/10.1637/10207-041512-review.1.

[9] 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-eng-swayne-hpai-vax-setting-stage.pdf.; EFSA Panel on Animal Health and Animal Welfare (AHAW), European Union Reference Laboratory for Avian Influenza, et al. (2024). Vaccination of poultry against highly pathogenic avian influenza - Part 2. Surveillance and mitigation measures. *EFSA journal. European Food Safety Authority*, *22*(4), e8755. https://doi.org/10.2903/j.efsa.2024.8755; *High Pathogenicity Avian Influenza in Layers: Considerations and Essential Components for Vaccination and Surveillance*, International Egg Commission Avian Influenza Global Expert Group (2023), https://www.internationalegg.com/resource/considerations-and-essential-components-for-hpai-vaccination-and-surveillance/.

[10] EFSA Panel on Animal Health and Animal Welfare (AHAW), European Union Reference Laboratory for Avian Influenza, et al. (2023). Vaccination of poultry against highly pathogenic avian influenza - part 1. Available vaccines and vaccination strategies. *EFSA journal. European Food Safety Authority*, *21*(10), e08271, https://doi.org/10.2903/j.efsa.2023.8271

[11] USDA APHIS. (2016). Policy and Approach to HPAI Vaccination. Available at: https://www.aphis.usda.gov/media/document/306/file. Accessed October 14, 2024.

[12] Budolfson, M., Espinosa, R., Fischer, B., & Treich, N. (2024). Monetizing Animal Welfare Impacts for Benefit–Cost Analysis. *Journal of Benefit-Cost Analysis*, 1–18. doi:10.1017/bca.2024.19

needed regarding how to incorporate animal welfare and other important considerations into USDA decisions about HPAI vaccination.

B. Impact of Flock Size on Infection and Transmission Risk

The U.S. differs from most other countries in that it has many extremely large flocks. While specific numbers are rarely made publicly available, the National Agricultural Statistics Service's Census on Agriculture indicates that, in 2022, there were 7,406 farms that sold 500,000 or more broilers and 834 farms that sold 100,000 or more turkeys.[13] There were 347 commercial egg farms housing 100,000 or more hens at one time. Data from the current outbreak indicates that many HPAI-affected egg farms housed over a million hens in one location – some operations had over 5 million hens – with individual barns at times containing upwards of 400,000 birds.[14]

AWI's review of the available data suggests that larger operations are at greater risk of contracting HPAI and, once infected, they are almost never able to depopulate in the time period recommended by USDA's Animal and Plant Health Inspection Service (24-48 hours) to curtail further spread.[15] For example, on operations confining a million or more hens, the average time between diagnosis and depopulation is 9.54 days, with a range of 3 to 22 days.[16] Research on infectious diseases suggests that larger farm sizes "have the potential to facilitate larger livestock epidemics."[17] Specifically with regard to HPAI, research (both in the U.S. and internationally) suggests that the probability of becoming infected with HPAI is higher for larger flocks.[18]

---

[13] *2022 Census of Agriculture, United States Summary and State Data, Volume 1, Geographic Area Series, Part 51* U.S. DEP'T OF AGRIC., NATIONAL AGRICULTURAL STATISTICS SERV, (February 2024), https://www.nass.usda.gov/Publications/AgCensus/2022/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf. (hereinafter *2022 NASS Census of Agriculture*)

[14] *2022–2023 Highly Pathogenic Avian Influenza Outbreak: Summary of Depopulation Methods and the Impact on Lateral Spread*, U.S. DEP'T OF AGRIC., ANIMAL AND PLANT HEALTH INSPECTION SERV, https://www.aphis.usda.gov/sites/default/files/hpai-2022-2023-summary-depop-analysis.pdf, at 13, Table 2, (hereinafter *Summary of Depopulation Methods*); *Confirmations of Highly Pathogenic Avian Influenza in Commercial and Backyard Flocks, List of Detections by Day*, U.S. DEP'T AGRIC., ANIMAL AND PLANT HEALTH INSPECTION SERV., https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/commercial-backyard-flocks. (hereinafter *USDA Confirmations of HPAI in Commercial & Backyard Flocks*)

[15] *AWI comments on HPAI Response EIS* at 11-20.

[16] *Id.* at 18-19.

[17] Meadows, A. J., Mundt, C. C., Keeling, M. J., & Tildesley, M. J. (2018). Disentangling the influence of livestock vs. farm density on livestock disease epidemics. *Ecosphere (Washington, D.C)*, *9*(7). https://doi.org/10.1002/ecs2.2294

[18] Zhao, Y., Richardson, B., Takle, E., et al. (2019). Airborne transmission may have played a role in the spread of 2015 highly pathogenic avian influenza outbreaks in the United States. *Scientific reports*, *9*(1), 11755. https://doi.org/10.1038/s41598-019-47788-z; Nguyen, X. D., Zhao, Y., Lin, J., Purswell, J. L., et al. (2023). Modeling long-distance airborne transmission of highly pathogenic avian influenza carried by dust particles. *Scientific reports*, *13*(1), 16255. https://doi.org/10.1038/s41598-023-42897-2; Mannelli, A., Ferrè, N., & Marangon, S. (2006). Analysis of the 1999–2000 highly pathogenic avian influenza (H7N1) epidemic in the main poultry-production area in northern Italy. *Preventive Veterinary Medicine*, *73*(4), 273–285. https://doi.org/10.1016/j.prevetmed.2005.09.005; Leibler, J. H., Otte, J., Roland-Holst, D., et al. (2009). Industrial

However, research specifically examining flock size as a risk factor for becoming infected with HPAI has thus far only considered farms and barns whose poultry populations are orders of magnitude smaller than those in the U.S.[19] To our knowledge, no research has examined the role of extremely large farm sizes, as seen in the U.S., on risk of becoming infected with HPAI or spreading infection to other farms, humans, or wildlife. Such research is essential for informing policy, such as whether caps on farm population size are warranted.

II. Research Needs: Higher-Welfare Depopulation Methods

A. The Need for Higher-Welfare Depopulation Methods

To date, over 100 million birds have been depopulated (killed en masse) in the U.S. due to HPAI detection on the premises where they were kept.[20] As mentioned above, poultry farms in the U.S. are often very large, housing hundreds of thousands or even millions of birds in a single location.[21] Commercial egg production operations frequently house 100,000 to 425,000 birds in a single barn (house) and operations may have dozens of houses.[22] When HPAI is detected on a commercial premises, USDA policy requires that all birds on site be depopulated, with a goal of completing the killing within 48 hours in order to curb the spread of disease.

At present, the primary methods of depopulation on commercial premises are ventilation shutdown plus heat (VSD+) and low- or medium-expansion water-based foam (also known as airway-occluding foam).[23] AWI and other animal welfare advocates criticize both methods because of the level of suffering they cause. Because of their negative impact on animal welfare, neither method is recognized by or meets the requirements of the World Organization for Animal Health's (WOAH) Terrestrial Animal Health Code, as described in its section on "Killing of Animals for Disease Control Purposes."[24] In addition, because of their negative implications for

---

food animal production and global health risks: exploring the ecosystems and economics of avian influenza. *EcoHealth*, *6*(1), 58–70. https://doi.org/10.1007/s10393-009-0226-0; Thomas, M. E., Bouma, A., Ekker, H. M., et al. (2005). Risk factors for the introduction of high pathogenicity Avian Influenza virus into poultry farms during the epidemic in the Netherlands in 2003. *Preventive Veterinary Medicine*, *69*(1), 1–11. https://doi.org/10.1016/j.prevetmed.2004.12.001.

[19] Ibid: Thomas, M.E., et al (2005); Mannelli, A. et al (2006).

[20] *USDA Confirmations of HPAI in Commercial & Backyard Flocks*

[21] *2022 NASS Census of Agriculture*

[22] *Summary of Depopulation Methods* at 13, Table 2; Delaware Department of Agriculture Public Records related to HPAI 2022 obtained via Public Records Request by Animal Outlook. Available at: https://awionline.org/sites/default/files/uploads/documents/DE-Public-Records-re-HPAI-Depop-2022.pdf. Accessed on October 15, 2024. See page 4.

[23] *Summary of Depopulation Methods,* at 1; Reyes-Illg, G., Martin, J. E., Mani, I., Reynolds, J., & Kipperman, B. (2022). The Rise of Heatstroke as a Method of Depopulating Pigs and Poultry: Implications for the US Veterinary Profession. *Animals: an open access journal from MDPI*, *13*(1), 140. https://doi.org/10.3390/ani13010140. (hereinafter *The Rise of Heatstroke as a Method of Depopulating)*

[24] WOAH. (2016). Chapter 7.6 Killing of Animals for Disease Control Purposes. Terrestrial Animal Health Code. Available at: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-

animal welfare, expert animal welfare bodies convened by the European Union (EU) and United Kingdom (UK) governments oppose the routine use of these methods to kill poultry for disease control purposes.[25] Neither VSD+ nor airway-occluding foam can legally be used in the EU or UK.[26]

VSD+ involves sealing a poultry house, turning off the ventilation system, and turning on heaters to raise the temperature in the house.[27] (Recently, there have also been proposals to add steam instead of or in addition to heat.[28]) VSD+ causes death via heatstroke, or hyperthermia.[29] Severe overheating causes damage to numerous body systems before the birds lose consciousness, including sloughing of the gastrointestinal tract, muscle breakdown, and fluid congestion of the lungs, and the temperatures reached exceed chickens' nociceptive (pain) threshold.[30] It often takes several hours for birds to die from VSD+ and they remain conscious for most of this period.[31] As a result, in the words of the UK's Animal Welfare Committee: "[VSD+] may be assumed to represent a profoundly negative experience for the bird, and potential welfare harms are likely to include anxiety, fear, pain, malaise, and breathlessness."[32]

---

access/?id=169&L=1&htmfile=chapitre_aw_killing.htm. Accessed October 11, 2024. (hereinafter *WOAH Chapter Killing of Animals for Disease Control*).

[25] EFSA Panel on Animal Health and Welfare (AHAW). (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. (hereinafter *EFSA - Use of high expansion foam for stunning and killing*); Department for Environment, Food and Rural Affairs (Defra), Animal Welfare Committee, Advice on emergency culling for the depopulation of poultry affected by high pathogenic avian influenza (HPAI) – consideration of ventilation shutdown (VSD), (June 2023) available at: https://assets.publishing.service.gov.uk/media/65eae0965b652445f6f21a98/Advice_on_emergency_culling_for_the_depopulation_of_poultry_affected_by_high_pathogenic_avian_influenza__HPAI____consideration_of_ventilation_shutdown__VSD_.pdf (hereinafter *AWC Advice on emergency culling – consideration of VSD)*; EFSA Panel on Animal Health and Welfare (AHAW). (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; *Opinion on the use of High Expansion Nitrogen Foam Delivery Systems for depopulation of poultry flocks affected by notifiable disease in the UK*, DEPARTMENT FOR ENVIRONMENT FOOD AND RURAL AFFAIRS, ANIMAL WELFARE COMMITTEE, (2024), https://www.gov.uk/government/publications/awc-opinion-on-high-expansion-nitrogen-foam-for-culling-poultry/awc-opinion-on-the-use-of-high-expansion-nitrogen-foam-for-culling-poultry (hereinafter *AWC Opinion on the use of High Expansion Nitrogen Foam).*

[26] *The Rise of Heatstroke as a Method of Depopulating*.

[27] USDA APHIS. (2022). HPAI Response: Ventilation Shutdown Plus (+) Policy. Available at: https://www.aphis.usda.gov/sites/default/files/ventilationshutdownpolicy.pdf. Accessed October 14, 2024. (hereinafter *USDA VSD+ policy*)

[28] Mendoza, A. V., Weimer, S., & Williams, Z. (2024). Can steam be usable as a "plus" for ventilation shutdown? *Journal of Applied Poultry Research*, *33*(1), 100381-. https://doi.org/10.1016/j.japr.2023.100381.

[29] *The Rise of Heatstroke as a Method of Depopulating*.

[30] *Ibid.*

[31] *Ibid;* Anderson, K.E., Eberle-Krish, K.N., Malheiros, R.D., et al. (2019). Evaluating the environmental and physiological effects of ventilation shutdown, with or without the addition of heat or carbon dioxide, on turkeys and broiler chicken. Available at https://www.uspoultry.org/programs/research/search-abstracts/repository/BRF008%20Final%20Report.pdf. Accessed September 17, 2024.

[32] *AWC Advice on emergency culling – consideration of VSD.*

Not all birds die when subjected to heatstroke-based depopulation methods. The USDA reports that, in most cases when VSD+ is used to depopulate a poultry house, there are numerous survivors, and a secondary method is required to kill animals who survive severe overheating.[33] In fact, the USDA reports that, in 74% of commercial egg houses in which VSD+ was used, a secondary method was required due to the existence of survivors.[34] USDA records indicate that, in some cases, it took up to 5 days to kill VSD+ survivors.[35] Due to the damage heatstroke does to the body, survivors of VSD+ would be expected to suffer severely until their deaths.[36]

Although USDA policy is that VSD+ should only be used if other methods, deemed "preferred' by the American Veterinary Medical Association, are not available,[37] AWI's analysis of USDA records obtained under FOIA have found that, from the start of the HPAI outbreak through July 2024, over 82% of poultry have been killed in depopulations that used VSD+ as their sole method or one of multiple methods employed on the premises.[38] A USDA analysis confirms the widespread reliance on VSD+, particularly in the egg industry; hit hardest by HPAI, it has depopulated 68% of its layer hen houses with VSD+.[39]

Low- or medium-expansion water-based foam results in a faster death than VSD+. However, based on its mechanism of killing, it is likely that the birds experience poor welfare prior to losing consciousness. The mechanism of killing for this method is airway occlusion leading to suffocation (defined here as *physical separation of the upper respiratory tract from atmospheric air*).[40] Available research suggests that, prior to loss of consciousness, the physiological changes that result from airway occlusion cause a subjective experience of severe air hunger, the type of breathlessness or dyspnea reported by humans to be the most unpleasant.[41] The UK government's animal welfare committee notes, "Welfare concerns arise from this mode of action which is equivalent to drowning or suffocation … neither of which are recognised as humane

---

[33] *Summary of Depopulation Methods* at 31 (Table 5).
[34] *Id.*
[35] *Id.* at 32 (Figure 12).
[36] *The Rise of Heatstroke as a Method of Depopulating*.
[37] *USDA VSD+ policy.*
[38] The records analyzed by AWI cover the period from February 2022-July 2024 and were obtained from the USDA via the following Freedom of Information Act requests: 2022-APHIS-02403-F, 2022-APHIS-02981-F, 2022-APHIS-03427-F, 2022-APHIS-03876-F, 2022-APHIS-04627-F, 2022-APHIS-04840-F, 2022-APHIS-05330-F, 2022-APHIS-05448-F, 2023-APHIS-00166-F, 2023-APHIS-00497-F, 2023-APHIS-00924-F, 2023-APHIS-01406-F, 2023-APHIS-01858-F, 2023-APHIS-01860-F, 2023-APHIS-02441-F, 2023-APHIS-02997, 2023-APHIS-03591-F, 2023-APHIS-04122-F, 2023-APHIS-05202-F, 2023-APHIS-05712-F, 2024-APHIS-00644-F, 2024-APHIS-01212-F, 2024-APHIS-01737-F, 2024-APHIS-02606-F, 2024-APHIS-02997-F, 2024-APHIS-04031-F, 2024-APHIS-04533-F, 2024-APHIS-05202-F, & 2024-APHIS-06286-F.
[39] *Summary of Depopulation Methods* at 13 (Table 2).
[40] Benson, E. R., Alphin, R. L., Dawson, M. D., & Malone, G. W. (2009). Use of water-based foam to depopulate ducks and other species. *Poultry science*, *88*(5), 904–910. https://doi.org/10.3382/ps.2008-00268
[41] 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.

under European legislation."[42] Similarly, the EU's Panel on Animal Health and Welfare says of water-based foam, "death due to drowning in fluids or suffocation by occlusion of the airways is not accepted as a humane method for killing animals, including poultry."[43]

The third widely used method of depopulation in the US, gassing with carbon dioxide (CO_2), is approved by WOAH and used in countries that eschew VSD+ and water-based foam on animal welfare grounds.[44] As a depopulation method, gassing with CO_2 (40% or higher for chickens) can be done in carts, containers, or whole barns.[45] CO_2 makes up approximately 0.04% of the atmosphere and poultry can detect CO_2 when concentrations reach between 3.1-10%.[46] Research has found that, starting at concentrations of around 10-20%, chickens display signs that it is aversive or noxious.[47] Exposure to CO_2 can be uncomfortable because: (1) it is an acidic gas that forms carbonic acid when it reacts with moist mucous membranes; (2) it can stimulate pain receptors at concentrations of 40%; and (3) it can trigger respiratory distress as blood levels increase.[48] However, higher CO_2 concentrations induce anesthesia, leading to rapid loss of consciousness, and it is theorized that building up levels gradually may allow birds to lose consciousness prior to experiencing severe discomfort.

WOAH encourages the use of whole house gassing (introduction of the gas into the barn) because "it eliminates welfare issues resulting from the need to manually remove live birds" into containers or carts.[49] Whole house gassing (WHG) with CO_2 has been the subject of numerous research studies and is used in other countries.[50] As of August 2022, the National Veterinary

---

[42] *AWC Opinion on the use of High Expansion Nitrogen Foam.*
[43] EFSA Panel on Animal Health and Welfare (AHAW). (2019). Killing for purposes other than slaughter: poultry. *EFSA journal. European Food Safety Authority*, *17*(11), e05850; EFSA Panel on Animal Health and Welfare (AHAW). (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
[44] *WOAH Chapter Killing of Animals for Disease Control*; *The Rise of Heatstroke as a Method of Depopulating* (See Table A1).
[45] McKeegan, D. (2018). Mass Depopulation. In J.A. Mench (Ed.), *Advances in Poultry Welfare* (pp. 351–372). Woodhead Publishing. ISBN 978-0-08-100915-4.
[46] Gent, T. C., Gebhardt-Henrich, S., Schild, S. A., Rahman, A. A., & Toscano, M. J. (2020). Evaluation of Poultry Stunning with Low Atmospheric Pressure, Carbon Dioxide or Nitrogen Using a Single Aversion Testing Paradigm. *Animals: an open access journal from MDPI*, *10*(8), 1308. https://doi.org/10.3390/ani10081308; McKeegan, D. E. F., McIntyre, J., Demmers, T. G. M., Wathes, C. M., & Jones, R. B. (2006). Behavioural responses of broiler chickens during acute exposure to gaseous stimulation. *Applied Animal Behaviour Science*, *99*(3), 271–286. https://doi.org/10.1016/j.applanim.2005.11.002; McKeegan, D. E., Smith, F. S., Demmers, T. G., Wathes, C. M., & Jones, R. B. (2005). Behavioral correlates of olfactory and trigeminal gaseous stimulation in chickens, Gallus domesticus. *Physiology & behavior*, *84*(5), 761–768. https://doi.org/10.1016/j.physbeh.2005.03.005
[47] *Ibid*.
[48] Baker, B. I., Torrey, S., Widowski, T. M., Turner, P. V., Knezacek, T. D., Nicholds, J., Crowe, T. G., & Schwean-Lardner, K. (2020). Defining characteristics of immersion carbon dioxide gas for successful euthanasia of neonatal and young broilers. *Poultry science*, *99*(9), 4408–4416. https://doi.org/10.1016/j.psj.2020.05.039
[49] *WOAH Chapter Killing of Animals for Disease Control.*
[50] Sparks, N. H., Sandilands, V., Raj, A. B., Turney, E., Pennycott, T., & Voas, A. (2010). Use of liquid carbon dioxide for whole-house gassing of poultry and implications for the welfare of the birds. *The Veterinary record*, *167*(11), 403–407. https://doi.org/10.1136/vr.c3813; Beutelschies, S. (2016). *NVS Development of CO2*

Stockpiles contained 18 whole house gassing (WHG) units;[51] however, the limited data available suggests that, in the U.S., depopulation by WHG with CO_2 is only rarely used, with CO_2 gassing in carts or containers being far more common.[52] Depopulating large operations with CO_2 carts and containers can take days or weeks – significantly longer than WHG, which requires just a few hours per house, including sealing of the building and set-up and removal of equipment. However, CO_2 gas is produced as a by-product of several industries and is thus subject to shortages, therefore acquiring it in amounts sufficient for WHG of large layer barns can be challenging.[53] Lack of planning for emergency access to sufficient volumes of CO_2 and lack of training in CO_2 WHG also appear to be obstacles to the use of this method.[54] Research needs to encourage a shift to this method include: 1) identifying and modeling CO_2 supply in poultry production regions to help ensure ready access to a quantity sufficient for WHG; 2) assessing different protocols for introduction of CO_2 into different types of poultry houses, with the goal of optimizing animal welfare; and 3) development of training programs to improve the comfort level of producers, animal health officials, and emergency responders with this method.

B. Current Status of Novel Higher-Welfare Poultry Depopulation Methods in the U.S.

Use of low-welfare depopulation methods, especially VSD+, is often defended by noting (1) their ability to destroy a large population of animals relatively rapidly, sparing them suffering due to HPAI and preventing the spread of disease to other flocks, and (2) the lack of availability of other higher-welfare methods. However, there are two higher-welfare methods of depopulation that are not being utilized, despite potentially being as fast or faster than the depopulation methods currently used in the U.S.: (1) high-expansion nitrogen foam (HENF); and

---

Whole House Gassing for Emergency Depopulation of Poultry. YouTube. https://www.youtube.com/watch?v=TKM_yKFovj4; McKeegan, D. E., Sparks, N. H., Sandilands, V., Demmers, T. G., Boulcott, P., & Wathes, C. M. (2011). Physiological responses of laying hens during whole-house killing with carbon dioxide. *British poultry science*, *52*(6), 645–657. https://doi.org/10.1080/00071668.2011.640307. Turner, P. V., Kloeze, H., Dam, A., Ward, D., Leung, N., Brown, E. E., Whiteman, A., Chiappetta, M. E., & Hunter, D. B. (2012). Mass depopulation of laying hens in whole barns with liquid carbon dioxide: evaluation of welfare impact. *Poultry science*, *91*(7), 1558–1568. https://doi.org/10.3382/ps.2012-02139. Livetec. (n.d.). CO2 Whole-House Gassing. Available at: https://www.livetecsystems.co.uk/resource-hub/co2-whole-house-gassing-whg-product-guide/.

[51] Sifford, R.B. (Deputy Administrator, USDA APHIS Veterinary Services). (2022, August 12). Letter to Gwendolen Reyes-Illg, (Veterinary Advisor, Animal Welfare Institute).

[52] *Summary of Depopulation Methods*; also see *supra* note 37 regarding information received by AWI from USDA via FOIA requests.

[53] Newman, J. (2022, Aug. 26). There's a Carbon Dioxide Shortage, and Food and Drink Makers are Scrambling. *Wall Street Journal*.

[54] Delaware Department of Agriculture. (2022). Delaware Department of Agriculture Public Records related to HPAI 2022 obtained via Public Records Request by Animal Outlook. Available online: https://awionline.org/sites/default/files/uploads/documents/DE-Public-Records-re-HPAI-Depop-2022.pdf (accessed October 13, 2024). Page 8-9. "[W]e have never tried to access CO2 trucks of manifolds until this moment. We do not have safety personnel available….Co2 requires personnel knowledgeable about setting up a manifold system and we do not have anyone in the state with that type of training."

(2) nitrogen whole house gassing (NWHG). Both are being used in other countries and are available in the U.S.[55]

For these methods, the mechanism of killing is the creation of anoxic conditions (i.e., a local environment that is oxygen-free or has an extremely low oxygen concentration, typically less than 2%) by displacing oxygen with nitrogen gas (N_2).[56] Anoxic conditions rapidly lead to loss of consciousness in poultry, typically within several seconds to less than one minute.[57] Because nitrogen gas is non-aversive and undetectable by poultry, nitrogen gassing is considered acceptable as a form of euthanasia.[58] Most of the atmosphere (78%) is comprised of nitrogen and the gas can be easily extracted from the atmosphere, therefore it is not subject to shortages.[59]

1. High-expansion Nitrogen Foam

HENF was developed in the UK, with the first research being published in 2008.[60] The foam concentrate utilized has a very high expansion ratio, meaning it is light and relatively dry, compared to the water-based foam widely utilized in the United States. Large bubble size ensures it does not occlude the airway, but rather works as a carrier for the gas with which it is filled to

---

[55] HENF is utilized in the UK (https://www.livetecsystems.co.uk/our-solutions/on-farm-products/nitrogen-foam-delivery-system/) and South Korea https://www.stiecosys.com/eng/business/animal-euthanasia-system (and available in the US through Agricultural Emergency services (agemergency.com/). WHG is used in Italy and the Netherlands, can be used for commercial depopulations with the approval of the provincial veterinary officer in Canada, and is available in the US through Livestock Welfare Strategies (https://livestockwelfarestrategies.com/).

[56] Hill, J. (2024), Evaluation of Nitrogen Whole House Gassing for the Mass Depopulation of Poultry. Presentation for Poultry Innovation Partnership, https://poultryinnovationpartnership.ca/presentation/evaluation-for-adopting-nitrogen-in-whole-barn-gassing-during-the-mass-depopulation-of-poultry/. (hereinafter *Hill - Evaluation of Nitrogen Whole House Gassing*); Raj, A. B., Smith, C., & Hickman, G. (2008). Novel method for killing poultry in houses with dry foam created using nitrogen. *The Veterinary record*, *162*(22), 722–723. https://doi.org/10.1136/vr.162.22.722

[57] AVMA. (2020). Guidelines for the Euthanasia of Animals. Available at: https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf. Accessed October 13, 2024. P. 27-28

[58] *Ibid.*

[59] Intratec Solutions. (2021). Technology Profile: Production of Nitrogen from Air. Chemical Engineering. Available at: https://www.chemengonline.com/technology-profile-production-of-nitrogen-from-air/.

[60] University of Glasgow; Royal Veterinary College; Livetec; LST International. (2008). Welfare Assessment of Anoxic Gas-Foam as an Agent for Emergency Killing of Poultry; Research Project Final Report MH0143; United Kingdom Department for Environment, Food and Rural Affairs. https://randd.defra.gov.uk/ProjectDetails?ProjectId=15445; Raj, A. B., Smith, C., & Hickman, G. (2008). Novel method for killing poultry in houses with dry foam created using nitrogen. *The Veterinary record*, *162*(22), 722–723. https://doi.org/10.1136/vr.162.22.722; Gerritzen, M.A., Reimert, H.G.M., Hindle, V.A., McKeegan, D.E.F., & Sparrey, J.M. (2010). Welfare assessment of gas filled foam as an agent for killing poultry, Report 399. Livestock Research Wageningen UR. https://www.researchgate.net/publication/275647993_welfare_assessment_of_gas_filled_foam_as_an_agent_for_killing_poultry

create an anoxic environment.[61] Early research explored filling the foam with CO_2,[62] but current systems use N_2 because it is non-aversive and produces better foam quality.[63]

At present, there are two different types of systems for delivery of HENF. One involves placing animals into a closed container, rapidly filling it with the HENF, and releasing a burst of N_2 gas to help pop the bubbles.[64] The other involves introducing the HENF directly into a poultry house, in a manner similar to that by which water-based foam is applied, or into a large open container or corral.[65] To date, there have been several research studies carried out in the U.S., including at the University of Minnesota and by several state departments of agriculture, with funding from the National Animal Disease Preparedness and Response Program (NADPRP) or the National Pork Board.[66]

2. Nitrogen Whole House Gassing

NWHG was initially dismissed as a possible method of depopulation because, unlike CO_2, N_2 is not heavier than ambient air and it was expected that it would dissipate too rapidly in a poultry house to produce anoxic conditions.[67] However, scarcity of CO_2 led to investigation of WHG with N_2 in Italy and the Netherlands and it was found that, when N_2 is vaporized from its liquid

---

[61] McKeegan, D. E.F., Reimert, H. G., Hindle, V. A., Boulcott, P., Sparrey, J. M., Wathes, C. M., Demmers, T. G., & Gerritzen, M. A. (2013). Physiological and behavioral responses of poultry exposed to gas-filled high expansion foam. *Poultry science*, *92*(5), 1145–1154. https://doi.org/10.3382/ps.2012-02587

[62] Gerritzen, M.A. & Sparrey, J. (2008). A pilot study to assess whether high expansion CO2-enriched foam is acceptable for on-farm emergency killing of poultry. *Animal Welfare*, 17(3), 285–288.

[63] McKeegan, D.E.F., Gerrizen, M.A., Sparrey, J.M. (2012, August). High expansion gas filled foam-a humane agent for emergency killing. *British Poultry Abstracts*, pp.2-4.

[64] Culhane, M. (2023). Effective use of inert gas (nitrogen) for poultry depopulation requires methods that consider bird species, age, size, and sex-specific factors. Presented at AVMA Humane Endings Conference, Chicago, IL, Jan. 26-29, 2023. (hereinafter *Culhane – effective use of nitrogen gas for poultry depopulation*); HEFT. (n.d.). High Expansion Foam Technology. Available at: https://heftinternational.com/technology/. Accessed October 14, 2024.; *EFSA - Use of high expansion foam for stunning and killing.*

[65] *AWC Opinion on the use of High Expansion Nitrogen Foam*; Livetec. (n.d.). Nitrogen Foam Delivery System. Available at: https://www.livetecsystems.co.uk/our-solutions/on-farm-products/nitrogen-foam-delivery-system/. Accessed October 14, 2024.; Livetec Systems Ltd. (2013). *Development of an On-Farm Anoxic Gas Foam Delivery System*; Research Project Final Report MH0151; United Kingdom Department for Environment, Food and Rural Affairs. https://randd.defra.gov.uk/ProjectDetails?ProjectId=18404

[66] *Culhane – effective use of nitrogen gas for poultry depopulation*; Hunt, L. (2022) Using Nitrogen Foam for Swine Depopulation. Presented at Seventh International Symposium on Animal Mortality Management, Raleigh, NC, USA, 27–30 June 2022; Sparrey, J. (2021). *Depopulation of Swine by Inert Gassing Utilizing the Livetec Systems Nitrogen Foam Delivery System*; NPB #20-099. https://porkcheckoff.org/wp-content/uploads/2022/07/20-099-SPARREY-final-rpt.pdf; Williams, T. (2022, March 30). Validation and Demonstration of Utilizing High Expansion Nitrogen Foam for Large Scale Depopulation of Swine, NPB Project #21-069. Available at: https://porkcheckoff.org/research/validation-and-demonstration-of-utilizing-high-expansion-nitrogen-foam-for-large-scale-depopulation-of-swine/

[67] Gerrtizen, M. (2006). Acceptable methods for large scale on-farm killing of poultry for disease control. Ph.D. Dissertation, Utrecht University, Faculty of Veterinary Medicine. Available at: https://www.researchgate.net/publication/275647690_Acceptable_methods_for_large_scale_on-farm_killing_of_poultry_for_disease_control. Accessed October 14, 2024.

state, it is heavier and colder than atmospheric air and can be utilized for WHG.[68] In addition to being used for HPAI-related depopulations in Italy and the Netherlands, this method has been refined through on-going research in Canada, particularly in commercial egg operations, and NADPRP has funded a project in Minnesota.[69]

Higher-welfare nitrogen-based depopulation methods are complementary to each other, because they are applicable in different environments. For NWHG, buildings must be enclosed and relatively well sealed. HENF, on the other hand, can be applied when these conditions aren't present.

C. Research Needs for Nitrogen-Based Depopulation Methods

The USDA recently acknowledged that it considers NWHG acceptable for caged poultry and HENF to be acceptable for confined, floor-reared poultry. However, neither method has, to date, been used for HPAI depopulation. There are several research gaps that could help these higher-welfare methods replace methods such as VSD+ and airway-occluding foam.

1. Logistical Research Needs

For both N_2-based depopulation methods, research focused on the logistics of N_2 supply would be extremely helpful. While N_2 is easily sourced in all parts of the country, in order for N_2 depopulation methods to be practical in an emergency, it is necessary to identify and model liquid N_2 supply sources for egg and poultry production regions.

Research focused on modelling commercial-scale applications is also necessary. For example, for a given operation, it can be determined well in advance of an emergency how many nitrogen delivery trucks would be needed, where they would need to be stationed on the premises, and what the throughput (number of birds or poultry houses depopulated per day) would be. This would aid in preparedness by proactively ensuring there are adequate equipment and supplies available. Development of standard operating procedures (SOPs) and calculation tools for these methods would also help accelerate their adoption.

2. Method Optimization Research Needs

For ethical reasons, it is essential that any live animal research utilizes animals who have already been designated for killing, such as "end-of-lay" hens.[70]

---

[68] *Hill - Evaluation of Nitrogen Whole House Gassing*; Orlando, C., Galuppo, F., & Soranzo, E. (2022). Operative Instruction for Poultry Killing and Related Operations (Report). ULSS6 Euganea. (Translated from Italian.); Galuppo, F. (2023). The 2021-2022 H5N1 AI Epidemic in Italy: Using Nitrogen for Outbreak Depopulation. (Presentation) ULSS6 Euganea. (Translated from Italian).
[69] *Ibid.*
[70] In the US, the majority of egg-laying hens are killed on-farm using CO_2 carts.

To date, most of the research on HENF has focused on its use for depopulation of confined, floor-reared poultry and birds who have been moved into specially designed containers. In the U.S. egg industry, most hens are raised either in battery cages, which are typically stacked atop one another, creating a tall structure, or in multi-tiered aviaries. A small trial has demonstrated that HENF can penetrate battery cages to a height of at least 5 meters and results in an oxygen concentration within the cages of less than 1%.[71] Another trial by the Pennsylvania Department of Agriculture documented that HENF could reach a height of 30 ft.[72] However, additional research is needed to determine the efficacy and animal welfare implications of this method in cage or aviary systems. Unfortunately, the U.S. egg and poultry industry has so far shown little interest in funding such research.

Another HENF research need is development of a vaporizer that would increase practicality of the method.[73] At present, there are two ways of delivering liquid N_2 (LIN): LIN transport tankers and nitrogen pumper trucks. Due to truck weight restrictions, nitrogen pumper trucks can carry less than ¼ of the N_2 compared to transport tankers, as they are fitted with a large, heavy vaporizer, the weight of which restricts the size of their nitrogen load. Because the HENF delivery systems currently available lack a built-in N_2 vaporizer, they require N_2 to be delivered by pumper trucks. However, if a small, high flow-low pressure vaporizer were integrated into the HENF delivery system, then LIN transport tankers could also be used. This would improve the practicality of this method because LIN transport trailers are ubiquitous in the U.S. and far more available than nitrogen pumper trucks.

With regard to NWHG, trials to date have been done primarily on poultry houses that are relatively small by U.S. standards: 40,000 or fewer birds per barn in comparison to U.S. egg operations, which often house over 100,000 birds per barn.[74] Research on optimizing the use of this method in larger poultry houses is necessary if it is to replace VSD+, the method currently used to depopulate most large poultry houses in the U.S.[75] In addition, given that hens often experience poor welfare during routine "end-of-lay" killing, another promising area of research would be the construction or modification of hen houses to permit use of NWHG for routine as well as emergency on-farm killing.

Finally, there is a need for published, peer-reviewed open access research on N_2-based depopulation methods. At present, much of the research, especially on NWHG, has been

---

[71] Personal communication. Email from Julian Sparrey (Livetec) to Gwendolen Reyes-Illg (AWI), May 27, 2022.
[72] Kalley, A. (2024, April 21-24). Utilizing Nitrogen Foam Euthanasia in Pennsylvania Swine and Poultry Operations [Conference presentation]. Northeast US Animal Health Association (USAHA) 2024 Annual Meeting, Philadelphia, PA, United States. https://www.usaha.org/upload/District/NEUSAHA/2024/2024_NEUSAHA_AAVLD_program_FULL_.pdf
[73] Personal communication. Jeff Hill (Livestock Welfare Strategies) to Gwendolen Reyes-Illg (AWI). October 14, 2024.
[74] *Summary of Depopulation Methods* at 13.
[75] *Id.* at 12.

performed by private companies or poultry associations that have chosen not to publish this research. Adoption of higher-welfare depopulation methods is likely to be hindered if this pattern continues.

III. Conclusion

Thank you for considering these comments on research priorities to inform readiness and response to HPAI. To summarize, for HPAI prevention, AWI encourages research into: (1) development of rapid, on-farm HPAI tests; (2) commercialization of HPAI vaccinations that can be administered to poultry in ovo, at the hatchery, or in water or spray; and (3) methods for incorporating animal welfare, public health, and other important impacts into USDA decision-making about approval of HPAI vaccination programs for poultry. We also recommend research evaluating the impact of large flock sizes on probability of HPAI infection and spread of infection. In terms of response, we encourage research that will result in a shift toward use of higher-welfare depopulation methods, including whole house gassing with CO_2 or N_2 and high-expansion N_2 foam. This includes both logistical research and research to optimize these methods in terms of animal welfare, practicality, and accessibility.

The importance of using a One Health framework – and a One Welfare framework as well – for addressing the challenge of HPAI cannot be overstated. Human physical and psychological health and welfare have already been impacted by HPAI and by the need to carry out massive poultry depopulations, particularly when low-welfare methods are used.[76] The potential impact of HPAI on public health is so great, prioritizing prevention is the only rational approach. Please do not hesitate to reach out if you have any questions or concerns, or if you require access to any of the references cited herein.

Sincerely,

Gwendy Reyes-Illg, DVM, MA
Scientist and Veterinary Medicine Consultant

Zack Strong
Acting Director and Senior Attorney

Farmed Animal Program
Animal Welfare Institute

[76] Baldwin, M. (2022). Ventilation Shutdown and VSD+. Presentation to US Animal Health Association Committee on Animal Welfare, Oct. 11, 2022, available at: https://usaha.org/upload/Committee/2022Reports/2022_Animal_Welfare.docx.