Project Title and NPB project identification number: Use of water-based foam to achieve rapid depopulation of swine: Assess physiological and behavioral effects in swine exposed to 5 different water-based foam products (NPB 22-084)
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Industry Summary:

Swine herds in the United States are susceptible to introductions of catastrophic diseases that may require mass depopulation for timely disease containment. Current options for depopulating swine, as outlined in the AVMA Guidelines for the Depopulation of Animals face logistic constraints, pose significant worker safety risks, and may not be practical at large scales. Medium-expansion Class A water-based firefighting foam (WBF) is approved and used for poultry depopulation, given its expedient onset of death post-application and relative ease of deployment. Although WBF is not currently approved for swine, The Ohio State University has conducted and published several foundational trials using WBF for depopulating pigs of various production phases. Our results show that WBF results in rapid unconsciousness followed by death with effectivity comparable to CO_2 but without the safety hazards and production bottlenecks of inert gases. We established a timeline for cessation of movement, cardiac arrest, and brain death during foam immersion in nursery pigs and cull sows under field conditions. Using those results, we established a 7.5-minute dwell-time to ensure non-recoverability post-foaming. We have shown that Class A foam is a strong candidate as a rapid and scalable option for swine depopulation, with regard for swine welfare considerations, across all production types in the U.S. swine industry.

As we started to transfer WBF technology from academia to end users in the swine industry, we identified gaps in knowledge that must be addressed. We had conducted foundational trials using a single WBF concentrate, Phos-Chek WD881, given its availability through the National Veterinary Stockpile and because it is commonly used to depopulate poultry. Nevertheless, through National Pork Board-sponsored full-scale depopulation exercises, we learned that acquiring Phos-Chek WD881 can be difficult due to regional differences in availability. We also learned that while there are more than twenty Class A foam concentrates available in the U.S., not all will produce foam suitable for swine depopulation under conditions previously described. As an example, the foam concentrate used in one state exercise, Chemguard Class A Plus, had to be mixed at a 2.5% concentration to produce adequate foam instead of the 1% we routinely use for Phos-Chek WD881. This led to a shortage of foam concentrate, increased cost, and potentially decreased swine welfare because the stronger concentration may have been more irritating to the pigs. Therefore, in this proposal, we seek to investigate and evaluate Class A foam concentrates currently available on the market to ensure nationwide availability of foam options in case depopulation becomes necessary.

This project expands on options for water-based foam depopulation, which will improve the availability of the method nationally and reduce supply chain bottlenecks and intra-industry competition for resources during simultaneous multi-site depopulation events. To achieve this, we will: 1) Evaluate varying concentrations of commonly available Class A foam concentrates for their suitability as depopulation foaming agents, 2) Confirm the efficacy of the top performing foam candidates and investigate their effects on swine welfare, and 3) Develop deployment guides for end-users detailing operational parameters of foam depopulation of swine.

Expectations, in the wake of foreign animal disease introduction, will involve timely containment to limit disease spread across populations. Depopulation of infected sites, regardless of size, will require means that provide for depopulation in an efficient and scalable manner. Failure to quickly contain the spread of foreign animal disease has the potential to devastate large regions of swine production. Results obtained through this study will provide the industry with a list of options that have been validated prior to the depopulation event. Decisions based on scientific evidence will likely diminish damage to the US pork industry, and this project will allow producers and animal health officials to make appropriate, rational, and scientific decisions.

Key Findings:

- Multiple Class A foams on the US market may be suitable for large scale swine depopulation.
- Short-term exposure to tested Class-A foams did not cause any significant physiological reactions in pigs.
- The overall assessment by swine industry stakeholders showed a slight preference for WBF and N_2F over CO_2.

Keywords: Depopulation, Water-based foam, Carbon dioxide, Nitrogen-gas foam, Swine, Welfare

Scientific Abstract:

Prevention of outbreaks and transmission of foreign animal diseases (FAD) is a high priority for the U.S. swine industry. Using existing depopulation guidelines for future contingency plans may be challenging due to limited scaling opportunities as most methodologies are deployed on an individual level. Water-based foam (WBF) has previously been approved for herd-level depopulation of floor-raised poultry. Previous studies by this research group have determined WBF using Phos-Chek WD881 Class A foam to be a promising candidate for rapid depopulation of large populations of swine. However, there are currently more than two dozen Class-A foams on the U.S. market that remain untested. In addition, novel nitrogen-gas foam- (N_2F) and CO_2 depopulation methods are undergoing evaluation and may be suitable alternatives to WBF. Thus, the objectives of this study were to 1) evaluate currently available Class A foam concentrates for swine depopulation suitability, 2) assess short-listed Class A foam concentrates from Objective 1 for depopulation efficacy and potential negative impacts on swine welfare, 3) describe and calculate the operational parameters of Class A foam depopulation by animal throughput and cost estimates for swine depopulation of different herd sizes and production types, 4) describe and compare method efficacy and behavioral outcomes between WBF, N_2F, and CO_2-gas depopulation methods ***(will be presented elsewhere in report by Dr. Williams)***, and 5) describe attitudes and perspectives of swine industry stakeholders on WBF and CO_2-gas depopulation methods.

Sixteen Class A foams were sourced and tested for suitability using previously established foam generating systems with gas-driven water pumps and dual expansion foam nozzles in 15-cubic yard containers. Fill time and decay rates were measured for 0.5, 1, and 3% foam-water concentrations. Out of 16 obtained Class A foams for assessment, four foams (BioEX BioFor N Class A Foam, FireIce Polar Class A EcoFoam, Buckeye Platinum Class A Foam, and National Foams Knockdown Class A Foam) beside Phos-Chek WD881, showed promising properties for additional field testing using live pigs in a modified 84.4 cubic-yard hydraulic rendering trailer. The average price for these foams was $26.4 USD/gallon with an average fill time of 54.9, 55.4 and 51.5 seconds and a decay rate of 0.26, 0.22 and 0.12 inches/minute, for 0.5%, 1.0% and 3.0% foam-water concentrations, respectively.

To assess any short-term physiological reactions to the shortlisted foams, 75 nursery pigs were divided into five replicates of three, with each triplet of pigs lowered into a polyethylene bulk container. Foam was filled up to the shoulder height (approximate 12-18 inches). Pigs were allowed to move around in the foam for 15 minutes while being observed for any visible reactions to the foam. After 15 minutes, additional foam was applied, covering the animals, and filling the container with a dwell-time of 7.5 minutes. After a 7.5-minute dwell-time pigs were removed from the container. Overall, the degree of pulmonary hemorrhages was significantly different between foam groups (P=0.04), with Phos-Chek WD811 inducing a higher number of pulmonary hemorrhages scores of three and four compared to Biofor N (P=0.043). No other physiological differences were observed.

For the field trial, 16-17 pigs across three replicates per short-listed foam, were loaded into the trailer, which was filled up until overflowing and left for a 7.5-minute dwell time. The average fill time (±SD) and time to last recorded movement or sound

coming from the trailer post-foaming was 81.2 (21.5) seconds and 123.1 (50.7) seconds, respectively. All foams performed well during the depopulation process. No pigs showed any signs of regaining consciousness after the 7-5-minute dwell time and all pigs were declared deceased upon inspection.

Using the data generated from the foam generating setup, foam specifications and associated costs, a self-populating excel spreadsheet for calculating depopulation costs and timelines were constructed for swine producers wanting to estimate preparedness costs for different foam options.

To determine the attitudes on the use of WBF, N_2F, and CO_2-gas depopulation methods, swine industry stakeholders of different backgrounds were invited for a live demonstration of these methods. They were also recruited to participate in a survey covering 11 key assessments and a final overall impression per demonstrated method. In total, 32 participants reviewed the three depopulation methods, and the overall highest scored and ranked method was WBF followed by N_2F and CO_2.

The findings from this study revealed four new possible Class A foam products suitable for swine depopulation that could be added to a future stockpile to avoid bottlenecks in times of FAD emergencies, if WBF depopulation is approved. This study also revealed important attitudes and perspectives from swine industry stakeholders on WBF, N_2F and CO_2-depopulation. These results will help with future considerations and refinements of method protocols to ensure stakeholders the best possible outcome for both staff and animals during emergency scenarios.

Introduction: *An overview of the researchable question and its importance to producers.*

The incursion of foreign pathogens or emerging diseases for the US swine industry is a constant threat, as exposure and transmission from rapid movements of people, goods and animals increase the risk for outbreaks to occur far from the originating source (Gauderault et al., 2020). Disease outbreaks cause negative impacts on animal health and welfare, public health, the environment, and supply disruptions within the agri-food economy. To minimize such effects, disease control contingency plans are needed. As an example, African Swine Fever (ASF) is a severe viral disease affecting domestic and feral pigs, for which currently there is no commercially available vaccine. Although the US swine herd is free of ASF, there is current and ongoing transmission throughout China, parts of Europe, and the Dominican Republic (OIE, 2020). Because the ASF virus and other virulent pathogens can be transmitted by numerous modes of transmission, preparation of contingency plans remains a high priority for the US swine industry. Thus, any contingency plans must include detailed steps for depopulation of large numbers of swine in a short timeframe.

Existing recommended guidelines for depopulation and euthanasia developed by the American Veterinary Medical Association (AVMA, 2019; AVMA 2020) are informative and thoroughly developed, however, their deployment during large outbreaks under field conditions may be challenging. As recommended by the AVMA, developing, and testing a plan before an incident occurs becomes imperative. The goal of best practice depopulation systems is to minimize or eliminate animal anxiety, pain, and distress before the loss of consciousness. Therefore, when evaluating depopulation systems, both the induction of unconsciousness and handling/restraint processes must be considered (AVMA 2019).

Currently recommended depopulation methods for swine include manual blunt force trauma, gunshot, nonpenetrating and penetrating captive bolt, electrocution, movement to slaughter and inhaled methods (carbon dioxide and anesthetic overdose) (AVMA, 2019), which may be suitable for individual pigs or smaller groups but currently lack the scalability and promptness needed to prevent additional spread while ensuring minimal animal distress and pain during a rapid and humane destruction (AVMA, 2019). Recommended methods such as gunshot, electrocution may present dangers to the physical health of depopulation personnel due to equipment failure; and fatigue during long-term exposure to any of the recommended depopulation procedures may increase risks of mental health disorders (Baysinger & Kogan, 2022).

A depopulation alternative currently approved for floor-raised poultry is the use of water-based foam (WBF) (Gurung et al., 2018). Although not currently approved by AVMA, recent research has shown a universal and reliant potential for the use of the biodegradable PHOS-CHEK WD881 Class A foam for swine (Lorbach et al., 2021; Kieffer et al., 2022; Arruda et al., 2022; Campler et al., 2023), sheep and goats (Park et al., unpublished), and cattle (Capria et al., 2023) depopulation. Current evidence shows that the use of WBF is equally efficient to the use of carbon dioxide (CO_2) and compressed nitrogen foam ($CAFN_2$) (Lorbach et al., 2021). However, one solution does not fit all situations in the U.S. pork industry. Thus, it is important to assess risks and available options to ensure that a good balance between rapid destruction and minimized suffering is upheld (Sawyer and Huertas, 2018). There is currently a range of different Class A foams on the US market that may be suitable for WBF depopulation, but that are currently untested for that purpose. Having multiple foams or methods alternatives within the depopulation framework would open up additional options for swine producers in different parts of the country and reduce potential bottlenecks in sourcing equipment and associated agents. Furthermore, additional testing and comparison of different depopulation methods is valuable to the swine industry to further evaluate efficacy, logistical frameworks for implementation and animal welfare implications. Previous studies have reported high efficacy for CO_2 and nitrogen-gas (N_2) during small-scale testing in swine but concerns regarding the potential aversiveness of the inhalants have been raised (Meyer et al., 2005; Stikeleather et al., 2013; Rice et al., 2014; Kinsey et al., 2016; Sutherland et al., 2017). The effect of N_2-gas and N_2/argon gas mixtures has been investigated for swine euthanasia during laboratory conditions (Llonch et al., 2013; Sadler et al., 2014) but its foam equivalent potential for large scale swine depopulation remains largely unknown. Since then, novel large-scale swine depopulation options for both CO_2 (Pepin et al., 2022) and nitrogen-gas foam (N_2F) have recently been developed and tested during field conditions (Park et al., unpublished; Cheng et al., 2023) but any published literature remains limited. A recent survey study reported that the overall perception of WBF depopulation seems to be positive among swine industry stakeholders (Cheng et al., 2023), but additional feedback from key industry stakeholder regarding their attitudes and perception of different large-scale depopulation options are important to gauge interest and concerns.

Thus, the proposed study aimed to 1) evaluate the suitability of currently available off-the-shelf Class-A foam concentrates to be used in swine depopulation, 2) test the best performing Class A foams on swine during field conditions, 3) Describe and calculate the operational parameters of Class A foam depopulation by animal throughput and cost estimates for swine depopulation of different herd sizes and production types, 4) describe and compare method efficacy and behavioral outcomes between water-based foam, nitrogen-gas (N_2) foam, and carbon dioxide (CO_2)-gas depopulation methods, and 5) describe attitudes and perspectives of swine industry stakeholders on water-based foam, nitrogen-gas foam (N_2F), and carbon dioxide (CO_2)-gas depopulation methods.

Objectives:

- **Objective 1:** Evaluate currently available Class A foam concentrates for swine depopulation suitability.
- **Objective 2:** Assess short-listed Class A foam concentrates from Objective 1 for depopulation efficacy and potential negative impacts on swine welfare.
- **Objective 3:** Describe and calculate the operational parameters of Class A foam depopulation by animal throughput and cost estimates for swine depopulation of different herd sizes and production types.
- **Objective 4:** *Describe and compare method efficacy and behavioral outcomes between water-based foam, nitrogen-gas foam (N_2F), and carbon dioxide (CO_2)-gas depopulation methods (see Dr. Todd Williams, NPB project)*
- **Objective 5:** Describe attitudes and perspectives of swine industry stakeholders on water-based foam, nitrogen-gas foam (N_2F), and carbon dioxide (CO_2)-gas depopulation methods.

Materials & Methods: *This section should include experimental design, methods and procedures used, number of animals, etc.*

Objective 1. Evaluate currently available Class A foam concentrates for swine depopulation suitability.

Regional and national U.S manufacturers of firefighting foam were approached regarding their availability of polyfluoroalkyl and perfluorooctanesulfonic-acid (PFAS/PFOS)-free Class-A foam products to use for the study. All obtained foams were evaluated in a low- and high-pressure pump foam production system for their expansion rate (fill time), ability to fill the container (fill success rate) and decay rate post fill three different foam-water solution concentrations (0.5%, 1.0% and 3.0%) across three replicates per foam and concentration level. Obtained foams at the time of trial can be found in Table 2 and 3.

Foam generation and application:

For the low-pressure pump system, foam was generated using a gas-powered trash pump with 5.1 cm hose ports (BravePro, BRP500TP2, Honda, Tokyo, Japan) and a medium-expansion foam handline nozzle (AWG, Model M2 16712-3, AWG Fittings GmbH, Ballendorf, Germany).

For the high-pressure pump system, foam was generated using a two-stage high pressure water pump system composed of gasoline powered water pumps (2MP13HR, AMT Pump Company, Royersford, PA, USA).

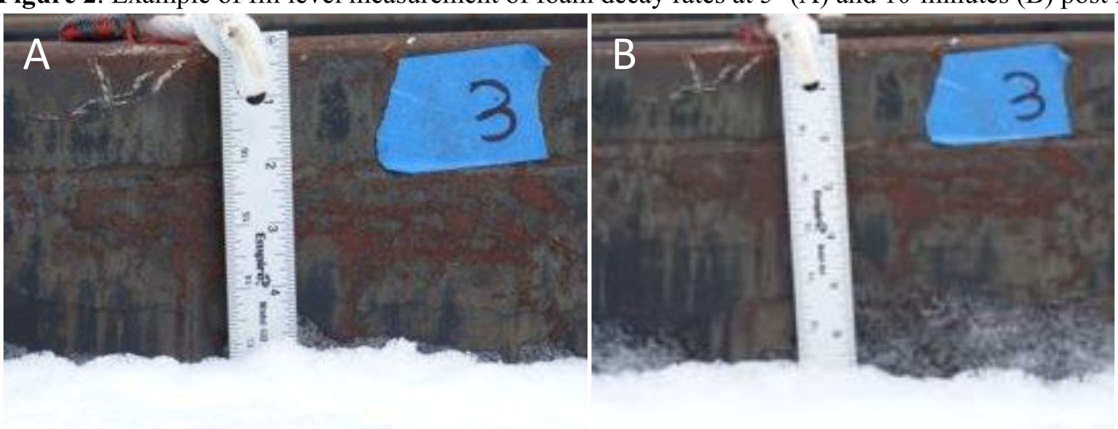
Both the low- and high-pressure systems were connected to a 300-gallon freshwater vessel with a pre-mixed foam-water solution via 2.0-inch-diameter suction hoses connected to the pump inlet. The pump was in turn connected to dual-expansion handline nozzles (KR-M4, ANSUL, Marinette, WI, USA) via a 1.5-inch diameter firehose, generating a 40-50:1 foam to water-expansion ratio. Each foam was allocated 100 gallons of water-foam concentrate to fill the container.

The low-pressure pump system was able to generate approximately 450 cubic feet of foam per minute while the high-pressure pump system was able to generate approximately 800 cubic feet of foam per minute at max capacity. Foam was delivered until either a visible overflow of the container was observed or until the allocated 100-gallon foam-water solution was gone. When the foam production seized, a 10-minute dwell time was timed to assess decay rate post-fill. Personnel involved in the operation wore personal protective equipment including hats, gloves, glasses, boots and appropriate waterproof clothing at all times. Two research team members were assigned to each foaming unit, operating one pump and one aspirated nozzle, respectively. Foam production initiation was considered as time zero, from which foam fill completion and all other time measurements were calculated. Dwell-time was defined as the time after foam generation ceased.

Six empty standard 15-cubic yard roll-off containers (16.0 x 7.5 x 4.5 feet, length, width, height) were used to test foam fill properties during a time span of ten minutes (Figure 1). Each foam concentration was carefully measured and pre-mixed, and thoroughly stirred in a 300-gallon fresh-water tank and three containers were assigned and filled consecutively, creating three replicates of the same foam concentration while enabling the fill and assessment of two foams simultaneously. The fill success rate was evaluated if the foam would be able to fill the entire container with the use of 100 gallons of pre-mixed foam-water solution at each concentrate level. When 100 gallons of pre-mixed foam-water solution was used, the pumps were shut off and the missing quantity of foam was measured by measuring the space between the foam surface and the top of the container. All foams were initially tested with the recommended 1.0% foam-water solution. If the 1.0% foam-water solution was unable to fill the container, the 0.5%-foam solution was not tested, and the 3.0% water-foam solution was tested instead. The decay rate for each of the three fill replicates of each foam was photographed and measured at 0-, 5- and 10-minutes post-fill completion to assess foam fill levels and the average decay rate for each foam and foam-water solution concentration (Figure 2A+B). When ten minutes had passed and the final decay rate was recorded, each container was emptied, and power washed between foams.

Figure 1. Roll-off containers (15-cubic yard) used in the assessment of Class A foams.

Figure 2. Example of fill level measurement of foam decay rates at 5- (A) and 10-minutes (B) post fill, respectively.



Objective 2: Assess short-listed Class A foam concentrates from Objective 1 for depopulation efficacy and potential negative impacts on swine welfare.

Foam exposure trial in nursery pigs

The goal of the exposure trial experiments was to further evaluate the shortlisted WBF concentrates selected from Objective 1 as well as Phos-Chek WD881. Phos-Check WD881 was utilized as a control for efficacy in nursery pigs based on previously reported data (Kieffer et al., 2022). Seventy-five nursery pigs were enrolled into the trial. Each foam concentrate was tested across five replicates with three pigs used for each replicate (15 pigs per foam concentrate, total n=75). Three non-porous, commercial-grade polyethylene bulk containers (Uline, Inc., Pleasant Prairie, WI, USA) were used to contain pigs during the foam exposure trial. A single set of foaming equipment was set up as previously described for the foam trials. A custom-cut section of 0.25 in (0.64cm) plywood was fitted into the bottom of the container to provide a flat surface for the pigs to stand. Foam was pumped into the container until it was at the approximate shoulder height of the pigs (12-18 inches deep). Three randomly selected pigs were lowered by hand into the foam. The pigs were released once they gained purchase on the bottom of the container and were able to stand unassisted. The pigs were allowed to move around in the foam for 15 minutes. During this time, the pigs were observed for abnormal physiologic changes such as appearance of skin lesions. After 15 minutes, additional foam was applied, covering the animals, and filling the container. The pigs were immersed for a minimum of 7.5 minutes. The time to last audible movement was recorded for each replicate. A forklift was used to empty the container and animals were retrieved for physical and pathological examination. Death was confirmed by lack of chest excursions or spontaneous movement, and absence of corneal reflex.

Immediately following depopulation of each replicate, partial necropsies were performed, focused on the cranial, cervical, and thoracic regions. Animals were observed for potentially negative reaction from exposure to the foam, as demonstrated by abnormal findings on necropsy or histology. Tissue samples from each pig were collected from the pinna, inguinal skin, palpebral conjunctiva, nasal turbinate, proximal trachea, and right middle lung. If gross changes were evident, samples representative of the most affected region were also collected. The samples were immersed in 10% neutral buffered formalin for a fixation period of at least 72 hours and processed by The Ohio State University's Comparative Pathology & Digital Imaging Shared Resource. Histologic slides were stained using hematoxylin and eosin. All slides were reviewed and scored by an anatomic pathologist resident according to the established rubric (Figure 3), under the guidance of two board-certified comparative anatomic pathologists with contribution of a third anatomic pathologist.

Field trial

Animals and biologger implantation

A subset of two-hundred market-ready pigs of mixed sex from a wean-to-finish facility (average weight 280 lbs) were enrolled in the study as they unintentionally had to be prematurely removed from the food chain. This event allowed for the depopulation and associated data collection to be conducted during field condition. Pigs were divided into four groups of 50 and further assigned into three replicates per foam (replicate 1: N=17, replicate 2: N=17, and replicate 3: N=16). For each replicate, a subset of five pigs was randomly selected for biologger (DST-Centi-HRT,Star-Oddi,Garðabær,Iceland) implantation one to two hours prior to foam exposure. The animals were briefly restrained via hog snare by experienced personnel. Aseptic technique was not utilized as the animals were to be depopulated within two hours following implantation. Local anesthesia was administered in the form of lidocaine HCL 1%, buffered with 8.4% sodium bicarbonate in a 1:10 ratio. Approximately 20-30ml of the buffered solution (2-3 ml lidocaine) was injected into the subcutaneous region of the body wall at the level of the elbow, close to the heart. A stab incision was made into the blocked tissue utilizing a #10 scalpel blade and extended 2-3 cm ventrally. The subcutaneous tissues were bluntly dissected either digitally or utilizing a straight forceps, creating a pocket for the device. The biologger was inserted and seated into the pocket and the incision was closed using surgical skin staples. The devices were programed to collect biological data including movement, heart rate, and electrocardiogram readings before and during the depopulation process.

Depopulation trailer

A modified 84.4-cubic yard rendering trailer (dimensions 40.0 × 7.75 × 7.35 ft; length, width and height) was used in the field trial part of this objective (Figure 3A+B). The rendering trailer was customized with a hydraulic lift system and a top-hinged side to side half width/whole gate at the back of the trailer to enable additional loading access via a ramp and to facilitate easy carcass unloading post-depopulation. A cut gate was located at mid-length, allowing for added compartmentalization within the trailer during loading and foaming to accommodate smaller or larger groups of animals. An elastomeric polyurethane coating was added to the trailer floor for increased traction and to reduce potential animal slipping during the foaming procedure. The trailer walls consisted of smooth steel without any protruding seems, bolts, hinges or other hardware to reduce animal injuries during pig escape attempts or movements during foaming. a two-stage high pressure water pump system. All pigs were directly loaded from their home pens into the modified rendering trailer using a single file loading ramp.

Foam generation and post-depopulation logistics

The four best performing foams (BioEX BioFor N Class A Foam, FireIce Polar Class A EcoFoam, Buckeye Platinum Class A Foam, and National Foams Knockdown Class A Foam) from Objective 1 were used in this field-trial scenario. Foam was generated by simultaneously using three two-stage high pressure water pump systems described in Objective 1. For this objective, pigs were placed in the front half of the trailer and foam was delivered through the open canopy until a visible overflow of the trailer was observed. Personnel involved in the operation wore personal protective equipment including hats, gloves, glasses, boots and appropriate waterproof clothing at all times. Two research team members were assigned to each foaming unit, operating one pump and one aspirated nozzle, respectively. To prevent falling accidents off or into the trailer during the foaming procedure, staff wore fall protection harnesses equipped with snap hook lanyards attached to a guide wire on top of the trailer.

Foam production initiation was considered as time zero, from which foam fill completion and all other time measurements were calculated. Dwell-time was defined as the time after foam generation ceased. After a dwell-time of 7.5 min, the trailer was driven to a designated dumping area, hydraulically lifted, and all pigs and foam was removed. After the pigs had been removed, a team of trained investigators evaluated individual pigs for any signs of consciousness. Immediate euthanasia was applied if any signs consistent with the onset of regained consciousness were observed.

Behavioral observations and data

A trained welfare specialist observed and timed the entire depopulation process for each foam and replicate through the open canopy on top of the trailer using the ethogram described in Table 1.

The time to the first observed pig reactions to foam initiation, time to foam reaching shoulder height, head height (full immersion), and final fill height were recorded. Adverse pig responses (escape attempts, vocalizations, and resurfacing pigs during the fill process) were tallied until no longer observed. The behavioral assessment continued until all observable signs of pig movements ceased (including subjective signs such as sudden foam irregularities or disruptions caused by pig movement beneath the foam), at which time a final time was recorded. Biologgers were extracted during a post-mortem dissection prior to pigs being moved to a landfill location and all data recorded was downloaded to a field laptop immediately after extraction.

Figure 3. Exterior (A) and interior view (B) of the 84.4-cubic yard modified rendering trailer with a halfwidth/whole gate attached loading ramp and internal center cut-gate for compartmentalization.

Table 1. Behavioral assessment for pigs during a Class-A Foam depopulation method.

Variable	Definition	Unit
Vocalization	Pig is vocalizing	Count (no.)
Escape attempt	Pig is climbing or jumping at side of trailer or other pig	Count (no.)
Resurfacing	Any visible body part (back, top of head, snout) breaking the foam surface after fill completion (filled trailer).	Count (no.)
Animal struggle	Summary of combined number of pigs performing vocalizations, escape attempts or resurfacing	Count (no.)
First response	Time to first aversive response to the foam	Seconds (s)
Fill time to shoulder	Time for the foam to reach shoulder height of the pig	Seconds (s)
Fill time above head	Time for the foam to reach above the head of the pig	Seconds (s)
Time to last struggle	Time to the last observation/detection of either vocalization, resurfacing, or movement post-filling.	Seconds (s)
Post-dwell time consciousness	Number of pigs showing any signs of regaining consciousness after the foaming procedure dwell-time.	Count (no.)

Objective 3. Describe and calculate the operational parameters of Class A foam depopulation by animal throughput and cost estimates for swine depopulation of different herd sizes and production types.

The aim of Objective 3 was to estimate costs and throughput calculations specifically for Class A foam depopulation options using our already established two-stage high pressure water pump system during different scenarios, including small and large-scale depopulation for pigs of different sizes. Data was obtained from the foam specifications obtained in Objective 1 and used in the logistical throughput and cost calculations to estimate a conservative depopulation timeline for smaller subset of herds to full scale whole herd depopulation scenarios. In addition, the aim was to calculate a per-head cost for each of the final Class A foam candidates for pigs in different production stages. This step is important to establish a precautionary investment cost for all foaming equipment and foam concentrations as well as water costs for rapid and successful depopulation of a herd.

The main deliverable of this objective was an overall conservative cost/time estimate that can be successfully extrapolated across herd sizes and production systems to help swine producers to make an informed choice on water-based foam depopulation options. Data from the calculated cost scenarios for each shortlisted Class A foam was be pre-programmed into an Excel spreadsheet with macro functionality to quickly establish estimated costs for specific production systems and herd sizes using a minimal number of manual inputs. This cost-basis spreadsheet was created for decision-makers to be able to use and update as needed.

Future application: If the framework of the cost basis excel spreadsheet is received well, a future proposal and plan would be to increase the number of Class A foams, foaming equipment and container options for cost analysis, and to organize an official producer evaluation of the usefulness a cost basis software for depopulation of different species. The end goal would be to develop readily functional software, available online on the National Pork Board homepage or elsewhere, for producers to access and calculate costs based on their individual circumstances during a depopulation emergency.

Objective 4: Describe and compare method efficacy and behavioral outcomes between water-based foam, nitrogen-gas foam (N_2F), and carbon dioxide (CO_2)-gas depopulation methods.

Please see Dr. Todd Williams report for this specific section.

Objective 5. Describe attitudes and perspectives of swine industry stakeholders on water-based foam, nitrogen-gas foam (N_2F) and carbon dioxide (CO_2)- based depopulation methods.

Survey development

The survey tool was developed and reviewed by the research team and the final survey was reviewed by The Ohio State University Institutional Review Board prior to the study. The pre-and post-survey assessing attitudes and perspectives, which were identical for each depopulation method, used a scoring system (1-5, higher is better), is presented in Figure 4. The secondary post-survey where each depopulation method was ranked against each other (1-3, lower is better) is presented in Figure 5.

Figure 4. Pre- and post-survey for swine industry stakeholder's attitudes and perspectives on water-based foam, nitrogen-gas foam (N_2F), and carbon dioxide (CO_2)- based depopulation methods.

For each question, check one box corresponding to the score that best reflects your thoughts.
1 = very poor, 2 = poor, 3 = fair, 4= good, 5 = excellent

	1	2	3	4	5	Not Enough Information
How well did this method minimize the pigs' distress (ex., vocalizations, excessive movements) once the foam was applied?						
How well did this method maintain the safety of operating personnel?						
How well did this method maintain the safety of surrounding bystanders?						
How well did this method maintain the safety of surrounding animals?						
How well did this method maintain the safety of the surrounding environment?						
How well does this method protect the emotional or psychological health of involved personnel (e.g. reducing visibility, sounds, etc.)?						
How well would this method mitigate negative public sentiments in response to depopulation?						
How well does this method fulfill the AVMA's criterion of considering potential legal and religious requirements?						
How available are this method's supplies (N_2 gas, foam concentrate) and generating equipment (ex., vaporizer) needed for depopulating large numbers of pigs?						
How easy is it to maintain this method's equipment in proper working order for use in emergency situations?						
How compatible is this depopulation method with current carcass disposal strategies?						
What is your overall impression of this method for swine depopulation?						

***Key points that influenced your scores*:**

Figure 5. Post-survey for swine industry stakeholder's ranking (1-3, 1 lower is better) of water-based foam, nitrogen-gas foam (N_2F), and carbon dioxide (CO_2)- based depopulation methods.

Please complete after you have observed ALL 3 methods.

1. Please choose the option that best describes your current organization.
   - A. State regulatory agency
   - B. Federal regulatory agency
   - C. Veterinary medical association
   - D. Pork organization
   - E. Academic institution
   - F. Other: ____________________

2. What is your highest level of education?
   - A. High school
   - B. Bachelor's
   - C. Master's
   - D. Professional degree (e.g. DVM) - Please specify:____________________
   - E. PhD

4. If any, what board-certified specializations have you completed?

   ____

5. How much experience do you have working in the swine industry? ________ years

6. Have you been involved in or observed depopulation procedures before?

   ☐ Yes ☐ No

   If yes, which species? ______________________

7. Please select which **swine depopulation** method(s) you have previously performed or observed (select all that apply).

   ☐ Blunt force trauma ☐ Non-penetrating captive bolt ☐ Penetrating captive bolt

   ☐ Gunshot ☐ Electrocution ☐ Ventilation shut down plus

   ☐ Carbon dioxide (CO_2) ☐ Sodium nitrite overdose ☐ Anesthetic overdose

   ☐ None

8. Please rank the methods based on each criterion (1 = best, 3 = worst).
   ***If multiple methods rank equally, please assign them the same score.**

	Rank		
	Water-Based Foam	Nitrogen Foam	Carbon Dioxide
Ability to minimize the distress of depopulated pigs			
Ability to maintain safety of operating personnel			
Ability to maintain safety of around bystanders			
Ability to maintain safety of surrounding animals			
Ability to maintain safety of surrounding environment			
Protection of emotional or psychological health of involved personnel (e.g. reducing visibility, sounds, etc.)			
Ability to mitigate negative public sentiments in response to method			
Consideration for potential legal or religious requirements			
Availability of supplies and generating equipment needed to depopulate large numbers of pigs			
Ease of maintaining equipment in proper working order for emergency situations			
Compatibility with current carcass disposal strategies			
Rank based on overall preference after seeing today's demonstrations: (1 = most preferred, 3 = least preferred)			

9. Please provide any additional comments or thoughts on your experience today:

Participant Recruitment

Participants for this study were recruited by invitation by the National Pork Board (NPB). Key swine industry stakeholders such as AVMA depopulation task force members, animal health officials, national pork board members, and animal welfare specialists were invited to participate in a demonstration of three depopulation methods: 1) water-based foam, 2) nitrogen-gas foam (N_2F), and 3) carbon-dioxide (CO_2). All participants could participate in the depopulation demonstration without having to participate in the survey. Participants that wanted to participate in the survey were informed to volunteer during the orientation session and to give oral or written consent to members of the research group. Each participant was tasked with giving their opinions for each demonstrated depopulation method prior and after the event (i.e., a pre- and post-survey). The survey did not ask for contact- or personal identifying information.

Results: Report your research results by objective.

Objective 1:

The aim was to obtain 20 different Class-A foam for assessment. In the end we were able to procure 15 Class-A foams from 13 manufacturers (Table 2 and Table 3) in addition to Dawn dish soap which was tested due to producer interest as an alternative. We initially intended to use an inline foam eductor to be able to regulate water-foam solution concentrations directly. However, due to poor performance during pilot-testing, the inline foam eductor had to be omitted from the research protocol, and the adjustment to pre-mixed solutions and 3 consecutive replicates was implemented instead of randomization of foams and concentrations.

High-Pressure Foam Evaluation

Out of the foam concentrates tested, only four WBF's (BFN, BP, KD, and PEF) besides WD881 successfully filled the container for all concentration levels tested (Table 2). Therefore, as this was our definition for shortlisting, only results for these four foams will be presented in comparison to WD881 below. At 0.5% concentration, the mean (SD) fill times for BFN, BP, KD and PEP were slower compared to WD881. PEF and BFN filled the container significantly slower compared to WD881 ($P < 0.01$) while KD tended to have a faster fill-time compared to WD881 ($P = 0.09$). At 1.0% concentration, no significant differences in fill times were observed between the foams, but BFN had a numerically faster fill time compared to WD881 (Table 2).

For the 3.0% concentration the mean fill times for BP, KD and PEP were significantly slower compared to WD881 ($P < 0.01$) while no difference was observed between BFN and WD881 (Table 2). However, BFN was numerically faster compared to WD881 (Table 2). The average decay rates for BFN, BP, KD, and PEF were 0.86 (± 0.6), 0.56 (± 0.7) and 0.3 (±0.3) cm/minute compared to 0.4 (±0.2), 0.1 (±0.2) and 0.1 (± 0.2) cm/minute for WD881 for 0.5%, 1.0% and 3.0%, respectively (Table 3). The decay rate of PEF was faster compared to WD881 at 0.5% (1.2 ± 0.3 vs. 0.36 ± 0.2 cm/minute, $P = 0.01$) and 1.0% (1.5 ± 0.4 vs. 0.1 ±0.2 cm/minute, $P < 0.01$). The decay rate of BP was faster compared to WD881 at 3.0% concentration (0.5 ± 0.1 vs. 0.1 ± 0.2 cm/minute, $P = 0.02$) (Table 3). No other significant differences in decay rate were observed.

Beyond the short-listed foam concentrates capable of working at all concentrations, an additional three products successfully filled the container at 1.0% concentration with a mean fill time of 57.1 s (± 8.6) and a decay rate of 1.1 (± 0.6) cm/minute. At 3.0% concentration, an additional nine foam concentrates successfully filled the container with a mean fill time of 52.5 s (± 6.1) and a decay rate of 0.5 (± 0.3) cm/minute. Dawn® dish soap was unable to generate enough foam to fill the container at any concentration level and was not further considered. Mean fill times and fill successes for all individual foams and concentrations for the high-pressure trial are listed in Table 3.

Table 2. Summary of mean fill time (seconds) and measure of success (Y=yes, N=no) for 100 gallons of Class A foam concentrates at 0.5, 1.0, and 3.0% concentrations using a high-pressure pump system.

Class A Foam Concentrate	**Mean Fill Time (s±SD)[a]**			**Container Fill Success (Y/N)**		
	0.5%	**1.0%**	**3.0%**	**0.5%**	**1.0%**	**3.0%**
PHOS-CHEK WD881[b]	43.3 (3.2)	50.0 (3.5)	46.0 (1.0)	Y	Y	Y
Ansul Silv-ex Plus	-	-	45.0 (2.6)	-	N	Y
BioFor N[b]	78.7 (2.5)	49.3 (2.3)	43.0 (4.5)	Y	Y	Y
Buckeye Platinum[b]	51.7 (2.5)	51.3 (3.1)	50.3 (1.2)	Y	Y	Y
Chemguard Chemattack	-	64.7 (4.2)	56.7 (3.1)	N	Y	Y
Chemguard Class A Foam	-	45.7 (1.5)	52.0 (3.5)	N	Y	Y
Crestar	-	-	44.7 (2.5)	-	N	Y
Enforcer Firebull	-	-	46.0 (1.0)	-	N	Y
Fireade Class	-	-	47.3 (2.3)	-	N	Y
Firestopper AB 40002 FFC[c]	-	-	-	-	N	N
Fire-Trol 103[c]	-	-	56.0 (3.0)	N	N	Y
Knockdown[b]	48.0 (1.7)	52.7 (4.2)	52.3 (5.1)	Y	Y	Y
Pinnacle Class	-	-	-	-	N	N
Phos-Chek First response	-	-	52.0 (2.0)	-	N	Y
Polar Ecofoam[b]	71.0 (2.6)	50.3 (4.7)	56.7 (2.1)	Y	Y	Y
Solberg Fire-Brake	-	59.3 (6.1)	62.3 (0.6)	N	Y	Y
Non-Class A Foam Concentrate						
Dawn® dish soap	-	-	-	N	N	N

[a] If 1% concentration did not fill the container; the 0.5% concentration was omitted from testing.
[b] Short-listed foam concentrates for full assessment.
[c] Foams no longer in production.
Missing data (-) indicates that data was not collected due to filling failure.

Table 3. Total foam decay (inches/minute) for three foam-water solution concentrations (0.5%, 1.0% and 3.0%) for 16 Class A Foam produced in a high-pressure pump system. N/A represent unsuccessful foam fill attempts. Bold WBF indicate short-listed foams.

		Average foam decay (cm ± SD, per min)		
Company	**Concentrate**	**0.5%**	**1.0%**	**3.0%**
A.J Stone Company Ltd	Enforcer Firebull Class A Foam	n/a	n/a	0.69 ± 0.08
Ansul	Ansul Silv-ex Plus Class A Foam	n/a	n/a	0.18 ± 0.08
BioEx	**Bio For N Class A Foam**	**0.51 ± 0.25**	**0.25 ± 0.17**	**0.0 ± 0.0**
Buckeye	**Buckeye Platinum Class A Foam**	**0.46 ± 0.17**	**0.33 ± 0.35**	**0.18 ± 0.07**
Chemguard	Chemguard Chemattack Class A Foam	n/a	0.23 ± 0.28	0.64 ± 0.25
Chemguard	Chemguard Class A Plus Foam	n/a	1.70 ± 0.13	0.84 ± 0.33
Chemonics	Fire-Trol 103 Class A Foam	0.69	0.18 ± 0.28	0.36 ± 0.53
Crestar Fire	Crestar Class A Foam	n/a	n/a	0.33 ± 0.25
Dawn Dish Soap	Dawn detergent	n/a	n/a	n/a
Fireade	Fireade Class A Foam	n/a	n/a	0.38 ± 0.23
FireStopper, USA	Firestopper AB 40002 FFC Class A Foam	n/a	n/a	3.43 ± 2.92
FireIce	**Polar Ecofoam Class A Foam**	**1.14 ± 0.28**	**1.50 ± 0.43**	**0.56 ± 0.41**
Hazard Control Tech	Pinnacle Class A Foam	n/a	n/a	n/a
National Foams	**Knockdown Class A Foam**	**0.56 ± 0.08**	**0.08 ± 0.06**	**0.18 ± 0.06**
Perimeter Solutions	Phos-Chek First response Class A Foam	n/a	0.58 ± 0.53	0.15 ± 0.10
Perimeter Solutions	**Phos-Chek WD881 Class A Foam**	**0.36 ± 0.15**	**0.08 ± 0.15**	**0.08 ± 0.15**
Perimeter Solutions	Solberg Fire-Brake Class A Foam	n/a	0.74 ± 0.23	0.18 ± 0.03

Low-Pressure Foam Evaluation

Fewer foam concentrates were used for the low-pressure foam evaluation as some foams were no longer in production at the time of testing. Due to the results obtained for Dawn® dish soap in the high-pressure trial, this product was left out for further testing. Out of the foam concentrates tested, only two WBF's (BP and KD) besides WD881 successfully filled the container for all concentration levels tested (0.5%, 1.0%, and 3%) (Table 4). Therefore, as this was our definition for shortlisting, only results for these three foams will be presented in comparison to WD881 below. At 0.5% concentration, the mean (SD) fill times for BP and KD were 93.3s (±1.5) and 113.0s (14.7) respectively, compared to 149.3s (±27.8) for WD881 (Table 4). At 1.0% concentration, the mean fill time for BP and KD, were 99.7s (±3.8) and 106.7s (6.5) respectively, compared to 99.0s (± 1.7) for WD881 (Table 4). For the 0.5% concentration the fill time for BP was significantly faster compared to WD881 (P=0.03) and tended to be faster compared to KD (P = 0.08). No difference in fill time between KD and WD881 was observed. For the 3% concentration the mean fill time for BP and KD was 100.3s (±0.6) and 111.0s (8.9) respectively, compared to 108.0s (± 1.7) for WD881 (Table 4). Regarding individual WBF fill times, BP filled the container significantly faster compared to WD881 (P <0.01) while no difference was observed between KD and WD881 (P = 0.11) at the 3.0% concentration (Table 4). No significant differences in fill times were observed at 1.0% concentration.

The average decay rates for BP and KD were 0.84 (± 0.47), 0.38 (± 0.1) and 0.59 (±0.1) cm/minute compared to 1.78 (±1.5), 0.97 (± 0.5) and 0.42 (± 0.1) cm/minute for WD881 for 0.5%, 1.0% and 3.0% respectively (Table 5). The decay rate of BP was faster compared to WD881 at 0.5% (1.2 ± 0.3 vs. 0.36 ± 0.2 cm/minute, P = 0.01) and 1.0% (1.5 ± 0.4 vs. 0.1 ±0.2 cm/minute, P < 0.01). BP tended to have a slower decay rate compared to WD881 at 1.0% concentration (0.42 ± 0.1 vs. 1.78 ± 1.5 cm/minute, P =0.09) and tended to have a slower decay rate compared KD at 3.0% concentration (0.34 ± 0.1 vs. 0.84 cm/minute, P = 0.06) (Table 5). No other differences were observed.

Beyond the short-listed WBFs capable of performing well at all concentrations, an additional four foam concentrates (Chemguard Chemattack, BFN, Phos-Chek First Response and PEF) successfully filled the container at 1.0% and 3.0% concentrations (Table 5). The mean fill times for these foams were 126.8 s (± 16.2) and 112.4 s (± 9.0) with a decay rate of 1.0 (± 0.8) and 0.9 (± 0.4) cm/minute for 1.0% and 3.0% concentrations, respectively (Table 5). Mean fill times and fill successes for all individual foams and concentrations for the high-pressure trial are listed in Table 4. As BP and KD also were successful in the high-pressure pump system trial, only high-pressure pump systems foams were carried over to field-trials due to the faster fill times.

The average cost (±SD) across all tested foams was $25.0 (±14.5) USD/gallon, (min = 14.9, max = 72.3) (Table 6). Out of the 16 foams tested using a high-pressure foam pump system, four foams (BioEX BioFor N Class A Foam, FireIce Polar Class A EcoFoam, Buckeye Platinum Class A Foam, and National Foams Knockdown Class A Foam) were shortlisted for additional testing using live animals during field conditions. These average price for these foams were $26.4 (±8.0) USD/gallon (min = 20.5, max = 37.4) and were able to fill the test container at all concentrations using the allocated 100 gallons, with an average fill time of 54.9, 55.4 and 51.5 seconds and a decay rate of 0.26, 0.22 and 0.12 inches/minute, for 0.5%, 1.0% and 3.0% foam-water concentrations, respectively (Table 6).

Table 4. Summary of mean fill time (seconds) and measure of success (Y=yes, N=no) for 100 gallon of high-pressure Class A foam concentrates at 0.5, 1.0, and 3.0% concentrations using a low-pressure pump system.

Class A Foam Concentrate	**Mean Fill Time (s±SD)[a]**			**Container Fill Success (Y/N)**		
	0.5%	**1.0%**	**3.0%**	**0.5%**	**1.0%**	**3.0%**
PHOS-CHEK WD881	152.7 (22.5)	99.0 (6.1)	113.7 (9.8)	Y	Y	Y
Ansul Silv-ex Plus	-	-	112.3 (11.8)	-	N	Y
BioFor N	-	109.7 (13.8)	108.0 (12.2)	N	Y	Y
Buckeye Platinum[b]	46.3 (0.6)	103.0 (4.6)	100.3 (0.6)	Y	Y	Y
Chemguard Chemattack	-	125.7 (15.5)	108.7 (9.0)	N	Y	Y
Chemguard Class A Foam	-	102.0 (8.3)	111.3 (9.7)	N	Y	Y
Crestar	-	-	108.7 (3.0)	-	N	Y
Enforcer Firebull	-	-	-	-	N	N
Knockdown[b]	113.0 (14.7)	106.7 (6.5)	111.0 (8.9)	Y	Y	Y

Pinnacle Class	-	-	-	-	N	N
Phos-Chek First response	-	144.0 (12.6)	112.3 (10.9)	N	Y	Y
Polar Ecofoam	-	128.0 (7.8)	120.7 (14.3)	N	Y	Y
Solberg Fire-Brake	-	-	110.0 (8.0)	N	N	Y

[a]If the 1% concentration did not fill the container; the 0.5% concentration was omitted from testing.
[b]Shortlisted foams
Missing data (-) indicates that data was not collected due to fill failure.

Table 5. Source company and cost of a selected number of commercially available and tested Class A foam concentrates and a commercial dish soap in the United States.

Class A Foam Concentrate	**Source Company**	**Cost ($USD)/gallon[a]** **(July/2023)**
PHOS-CHEK WD881	Perimeter Solutions	34.8
Ansul Silv-ex Plus	Ansul	20.8
BioFor N	BioEx	20.6
Buckeye Platinum	Buckeye	37.4
Chemattack Class A Foam	Chemguard	20.0
Chemguard Class A Plus+	Chemguard	20.0
Crestar	Crestar Fire	17.8
Enforcer Firebull	A.J. Stone Company, Ltd.	14.9
FireAde	FireAde	24.8
FireStopper AB 40002 FFC[b]	FireStopper, USA	72.3
Fire-Trol 103[b]	Chemtronics	24.8
Knockdown	National Foams	20.5
Pinnacle	Hazard Control Tech	23
Phos-Chek First Response	Perimeter Solutions	27.3
Polar Ecofoam	FireIce	27.1
Solberg Fire-Brake	Perimeter Solutions	19.1
Non-Class A Foam Concentrate		
Dawn® dish soap	Procter & Gamble	25.0

[a]shipping costs are not included and may vary geographically
[b]foams no longer in production at the time of publication

Table 6. Average foam decay (inches/minute) for three foam-water solution concentrations (0.5%, 1.0% and 3.0%) for 16 Class A Foam produced in a low-pressure pump system. N/A represents unsuccessful foam-fill attempts. Bold WBF indicates short-listed foams.

		Total foam decay (cm per minute)		
Company	**Concentrate**	**0.5%**	**1.0%**	**3.0%**
A.J Stone Company Ltd	Enforcer Firebull Class A Foam	n/a	n/a	n/a

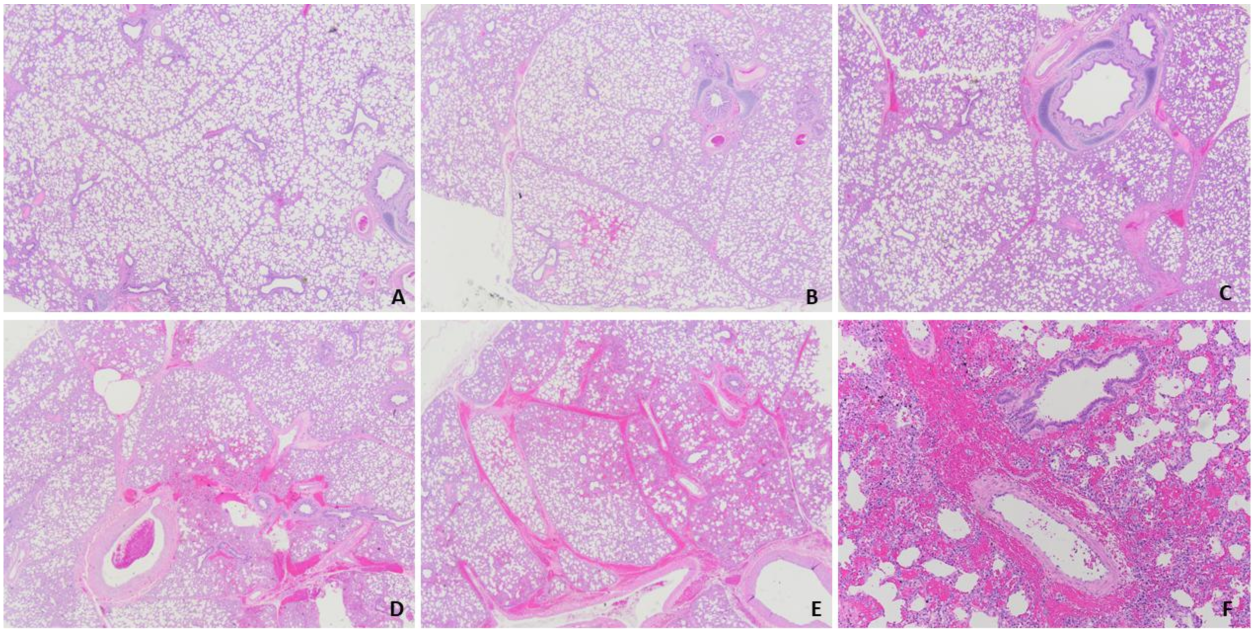
Ansul	Ansul Silv-ex Plus Class A Foam	n/a	n/a	0.51 ± 0.25
BioEx	Bio For N Class A Foam	n/a	0.0 ± 0.0	0.08 ± 0.15
Buckeye	**Buckeye Platinum Class A Foam**	**0.58 ± 0.15**	**0.43 ± 0.15**	**0.33 ± 0.15**
Chemguard	Chemguard Chemattack Class A Foam	n/a	1.19 ± 0.89	1.19 ± 0.53
Chemguard	Chemguard Class A Plus Foam	n/a	3.30 ± 0.66	2.54 ± 0.25
Crestar Fire	Crestar Class A Foam	n/a	n/a	0.84 ± 0.56
FireIce	Polar Ecofoam Class A Foam	n/a	1.57 ± 0.46	2.08 ± 0.08
Hazard Control Tech	Pinnacle Class A Foam	n/a	n/a	n/a
National Foams	**Knockdown Class A Foam**	**1.09 ± 0.56**	**0.33 ± 0.15**	**0.84 ± 0.28**
Perimeter Solutions	Phos-Chek First Response Class A Foam	n/a	1.27 ± 0.25	0.25 ± 0.43
Perimeter Solutions	**Phos-Chek WD881 Class A Foam**	**1.78 ± 1.55**	**0.97 ± 0.48**	**0.43 ± 0.28**
Perimeter Solutions	Solberg Fire-Brake Class A Foam	n/a	n/a	0.25 ± 0.43

Objective 2: Assess short-listed Class A foam concentrates from Objective 1 for depopulation efficacy and potential negative impacts on swine welfare.

Foam exposure trial in nursery pigs

The major microscopic finding was pulmonary hemorrhage, which was observed in at least one pig within all treatment groups. The distribution of these hemorrhages was predominantly perivascular within the minimally to mildly affected pigs, with extension into peribronchiolar regions and alveolar spaces with increasing severity (Figure 6). Using a pair-wise Fishers' exact test with pair-wise Bonferroni corrections, a significant difference between the degree of pulmonary hemorrhages and foam groups was found (P=0.04). The pair-wise comparison revealed that Phos-Chek WD881 induced a higher number of observed pulmonary hemorrhages scores of three to four (Figure 6) compared to Biofor N (P=0.043*)*. No other effects on hemorrhages were observed between the different foams.

Figure 6. Microscopic appearances of pulmonary hemorrhages by score (H&E).



A) Score 0, where no hemorrhage is present within pulmonary parenchyma. **B)** Score 1, where hemorrhages affect <10% of parenchyma. **C)** Score 2, where hemorrhages affect >10-25% of parenchyma. **D)** Score 3, where hemorrhages affect >25-50% of parenchyma. E) Score 4, where hemorrhages are present >50% of parenchyma. **F)** The distribution of hemorrhages within the most severely affected lungs consists of a perivascular, peribronchiolar, and intraalveolar pattern.

Within nasal turbinates in all treatment groups were inflammatory infiltrates consisting of either a predominantly lymphoplasmacytic population, or a neutrophilic and eosinophilic response accompanied by fewer lymphocytes and plasma cells. Segmental epithelial deciliation was concurrently present. These changes were not significantly different across all treatment groups (*P*=0.75). Within the trachea, varying degrees of infiltrating populations of lymphocytes and plasma cells, along with fewer neutrophils, were within the tracheal lamina propria and as deep as the superficial submucosa. Like within the nasal turbinates, segmental respiratory epithelial deciliation was observed, and some regions were suggestive of epithelial attenuation. These changes were not significantly different across treatment groups (*P*=0.52).

Conjunctival tissue lesions were less frequently observed and were limited to the presence of scattered lymphoplasmacytic +/- eosinophilic inflammatory cell populations within the conjunctival stroma, and this was not significantly different across treatment groups (*P*=0.39). The skin of the pinna and inguinal region displayed the occasional change of scattered aggregates of infiltrating eosinophils and neutrophils, and/or rare subcorneal pustules. However, these dermal changes were overall minimal to mild, and there were no significant differences across treatment groups for both pinna (*P*=0.343) and inguinal skin (*P*=0.96).

Field trial

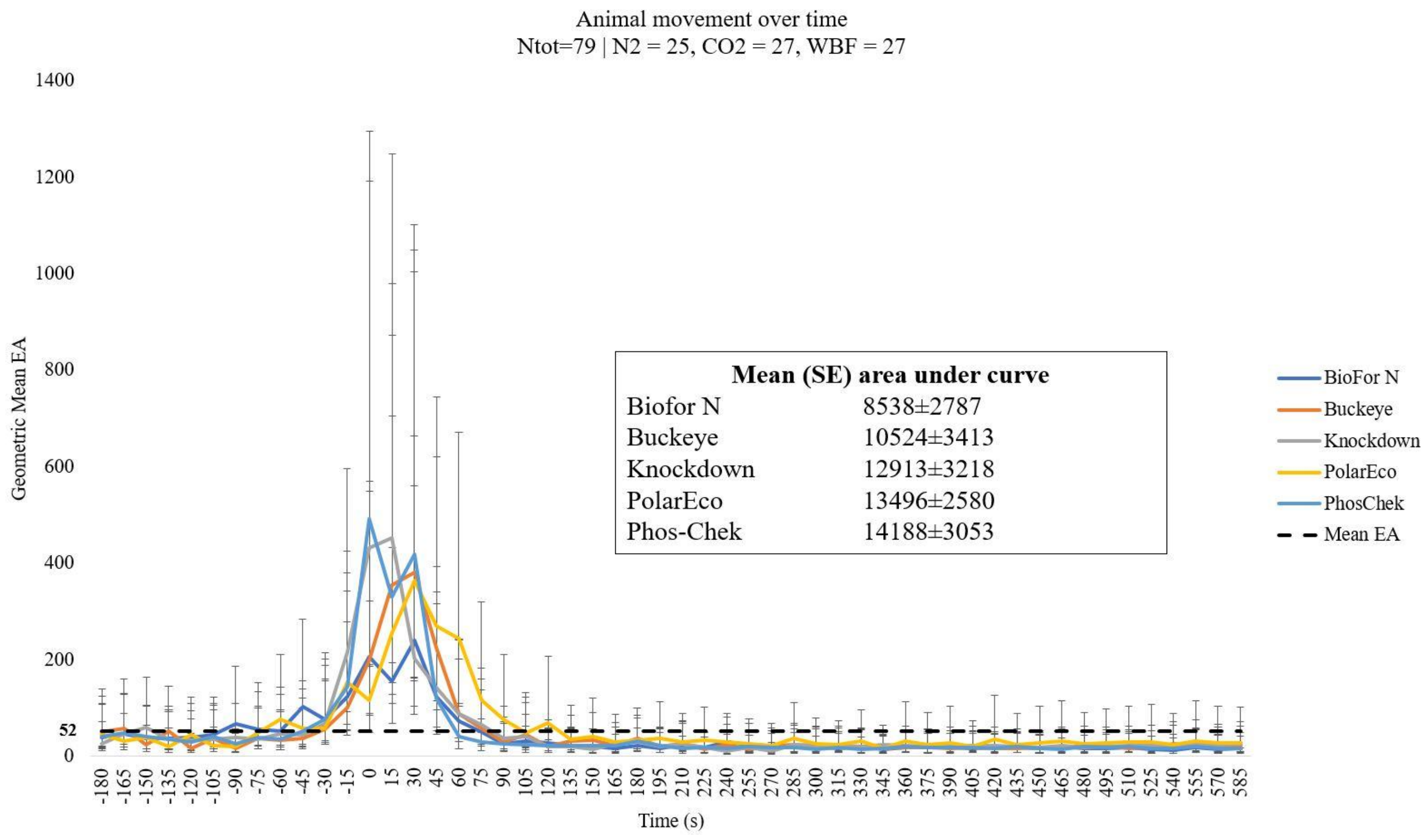
For the short-listed foams from Objective 1 (BioEX BioFor N Class A Foam, FireIce Polar Class A EcoFoam, Buckeye Platinum Class A Foam, and National Foams Knockdown Class A Foam), the average fill time (s ± SD) across the 4 foams was 81.2 (21.5) seconds (Table 7). The average time to the first response to the foam, which was mainly initiated by the foam fill level reaching shoulder level, was 21.1 (10.5) seconds while the average time to the foam reaching the shoulder level was 21.3 (7.1) seconds. The average time for the foam to reach above the head of the pigs was 28.4 (11.0) seconds while the last time of any recorded movement or sound coming from the trailer post-foaming was 123.1 (50.7) seconds. The average fill time, time to first response of the pigs to the foam, time to when the foam reached pigs' shoulder level, time to when the foam reached above the pigs' head while standing on all four legs, the time to last reported activity post-foam fill, as well vocalizations, successful resurfacing attempts, and the total number of pigs struggling (vocalization, resurfacing, or escape attempts) for each foam can be found in Table 7.

Table 7. Average fill time measurements across three replicates and the total number of pig behaviors for four tested medium-expansion class-A foams at a 1.0% foam-water concentration during a field depopulation scenario. Each replicate contained 16-17 pigs for a total of 50 pigs per foam.

Measurement (mean)	**BioFor N (N=50)**	**Polar Ecofoam (N=50)**	**Buckeye (N=50)**	**Knockdown (N=50)**
Fill Time (s)	75	107	85	58
Time to first response (s)	28	24	20	n/a
Fill time to shoulder (s)	29	20	18	16
Fill time above head(s)	34	28	34	24
Time to last activity (s)	123	110	136	117
Measurement (count)				
Total vocalization	11	0	1	0
Total resurfacings	0	0	2	0
Escape Attempts	8	4	3	1
Animal Struggle	8	4	5	1

All foams performed satisfactorily based on the initial properties observed before the field trial. All pigs for all trials and foams were declared deceased post the 7.5-minute dwell time. No conscious pigs or any signs of pigs regaining consciousness was observed. The mean activity levels were calculated across all pigs and replicates for each foam and are presented as geometric means with 95% confidence intervals in Figure 7. No statistically significant differences in pig activity were observed between the foams, although individual variation in activity levels were observed between pigs, as indicated by the confidence intervals. Overall, the activity levels of the pigs were heightened from about 30 seconds prior to foam start up until two minutes post foam initiation for all foams. Area under the curve determined as the area from the first peak above to the last peak above the geometric mean did not show any statistical differences $P>0.05$. Numerically, Biofor N had the lowest over all activity levels, followed by Buckeye, Knockdown, PolarEco and Phos-Chek WD881 in that order (Figure 7).

Figure 7. Mean [95% confidence interval] pig activity (EA) for pigs depopulated with medium-expansion water-based Class-A foams (BioFor N, Buckeye, Knockdown, PolarEco, and Phos-Chek WD881). Foam initiation occurred at time point zero. Mean (SE) area under the curve represents the average total activity performed for all animals across all replicated for each foam. Mean geometric EA across all foams and replicates is indicated by the dotted black line.



Objective 3: **Describe and calculate the operational parameters of Class A foam depopulation by animal throughput and cost estimates for swine depopulation of different herd sizes and production types.**

A self-populating working cost and throughput excel sheet has been created using the product specifics of all the 16 initially tested Class-A foams. The excel sheet allows the user to input a multitude of factors associated with their specific circumstances. The user can input the foam of choice, the size of the container used, the number of containers used, the average pig age, average pig size, herd size number of pump systems used, local water cost per gallon, local fuel cost per gallon, the average loading time of pigs into the container, and the estimated time for disposal and return trip.

Using this user provided data, the excel sheet will return an output including the container volume, the average space requirement per pig, the practical number of pigs that could be loaded into the container, the number of fills required to complete the depopulation event, foam cost per gallon, the amount and cost of water and fuel needed for the completion of the depopulation event, the water and fuel cost per fill, estimated time per fill, the estimated number of batches that can be completed per hour, the total time needed to complete the depopulation event. The total cost per fill and pig, and finally the total cost of the entire depopulation event, excluding staff costs.

In addition, a quick matrix sheet will provide an idea of how your container and number of pump systems will relate to other standard sized containers in terms of fill time. This matrix will indicate the appropriateness of the fill time by three levels (good, improvement recommended, and unacceptable fill time) indicated by a green, yellow, and red icon, respectively. Based on the group's previous research we would recommend a fill time, regardless of container size, to be below 60 seconds. This would be indicated by the green light, a yellow icon would place the fill time between 60 and 90 seconds, and anything about 90 seconds would yield a red icon. An example of the calculation sheet output is shown in Figure 8.

Figure 8. Screenshot of a cost and throughput calculation sheet for a hypothetical medium-expansion water-based Class A foam depopulation event.

OUTPUT			
Container Volume (cubic yard)	84.4	Container Volume (cubic ft)	2278.5
Container Volume (cubic meter)			64.5
Average pig weight (lbs)	300	Space requirement per pig (ft2)	4.8
Average pig weight (kgs)	136.1	Space requirement per pig (m2)	0.45
Practical number of heads per fill	64.6	Number of fills required	15.5
Floor Area (square feet)	310	Floor Area (square meter)	28.8
Foam *Phos-Chek WD881 Class A Foam (1%)*		Cost per gallon ($ USD)	34.8
Foam Needed (gallons) per fill	3.79	Total foam needed (gallons)	58.8
Water Needed per fill (gallons)	303.0	Water Needed (gallons)	4692
Fuel cost per fill ($ USD)			4.70
Total foam cost ($ USD)			2046.24
Total fuel cost for pump(s) ($USD)	72.85	Total water cost ($ USD)	46.9
Time per fill (min)	1.0	Batches per hour	2.1
Fill time should ideally be below 1 minute. Green, yellow or red symbol represent good, improvement recommended, unacceptable fill time, respectively		*Batch per hour includes a 7.5 minute dwell time for the foam to work to ensure animals do no recover or regain consciousess*	
Total depopulation time (hours)			7.4
Total depopulation time (hours) - *Multiple containers working simultanolusly*			n/a
Cost per fill ($ USD)			139.5
Cost per pig ($ USD)			2.16
Total cost of depopulation (Approx. $ USD)			2160.7

Quick sheet for fill times across multiple pumps and standard container sizes

Container size (cubic yard)	PUMPS 1	2	3
10	0.4	0.2	0.1
12	0.4	0.2	0.1
15	0.5	0.3	0.2
20	0.7	0.4	0.2
30	1.1	0.5	0.4
40	1.4	0.7	0.5
User container size	3.0	1.5	1.0

Objective 4: Describe and compare method efficacy and behavioral outcomes between water-based foam, nitrogen-gas foam (N_2F), and carbon dioxide (CO_2)-gas depopulation methods.

Please see Dr. Todd Williams report, NPB project number:)

Objective 5. Describe the attitudes and perspectives of swine industry stakeholders on water-based foam (WBF), nitrogen-gas foam (N_2F), and carbon dioxide (CO_2) based depopulation methods.

The morning of an organized demonstration event for swine industry stakeholders, participants were recruited to complete post-demonstration surveys. Surveys collectively included three method-specific surveys, along with an overall final survey across all three methods. The content of surveys was tailored to reflect each respondent's immediate perception on how well a method satisfied each criterion that the AVMA considers during the process of approving depopulation methods. Paper copies of the informed consent process and surveys were provided to each person who expressed interest in the survey.

In total, 32 participants from a variety of organizations (State regulatory agencies, N=4; Federal regulatory agencies, N=2; Veterinary medical organization, N=3; Pork organization, N=7; Academia, N=9; Other (producers, retail, private practice, N=7), and with various educational backgrounds (PhD, N=2; PhD + professional degree, N=4; Professional degree, N=10, MSc, N=5; BS, N=10; High school education, N=1) volunteered to participate in the survey. Average (±SD) swine industry work experience was 14.4 (±12.3) years (range: 0-40 years). Additionally, 40.6% (13/32) of participants had previously been involved in or had observed animal depopulations other than the events relating to this survey.

Recruited participants had a 100% (32/32) completion rate of the survey. Participants were allocated into three observation groups (Group 1, N=10; Group 2, N=11; Group 3, N=10) that rotated between simultaneously on-going depopulation method demonstrations. While each group began by observing a different method, the order in which methods were viewed remained consistent in all groups. Water-based foam scored (Figure 9) and ranked (Figure 10) the highest in terms of overall impression of the method, followed by N_2F and CO_2. However, all treatments had perceived strengths and weaknesses when it came to specific survey statements. The mean scores for each survey statement (1-5, higher being better) for each depopulation method (WBF, N_2F, and CO_2) are presented in Figure 9. The mean post-survey comparative ranking scores (1-3, lower is better) for each depopulation method are shown in Figure 10.

Figure 9. Mean scores (±SD) for each survey statement (1-5, higher is better) answered by swine industry stakeholders (N=32) for observed depopulation methods (WBF, N_2F, and CO_2).

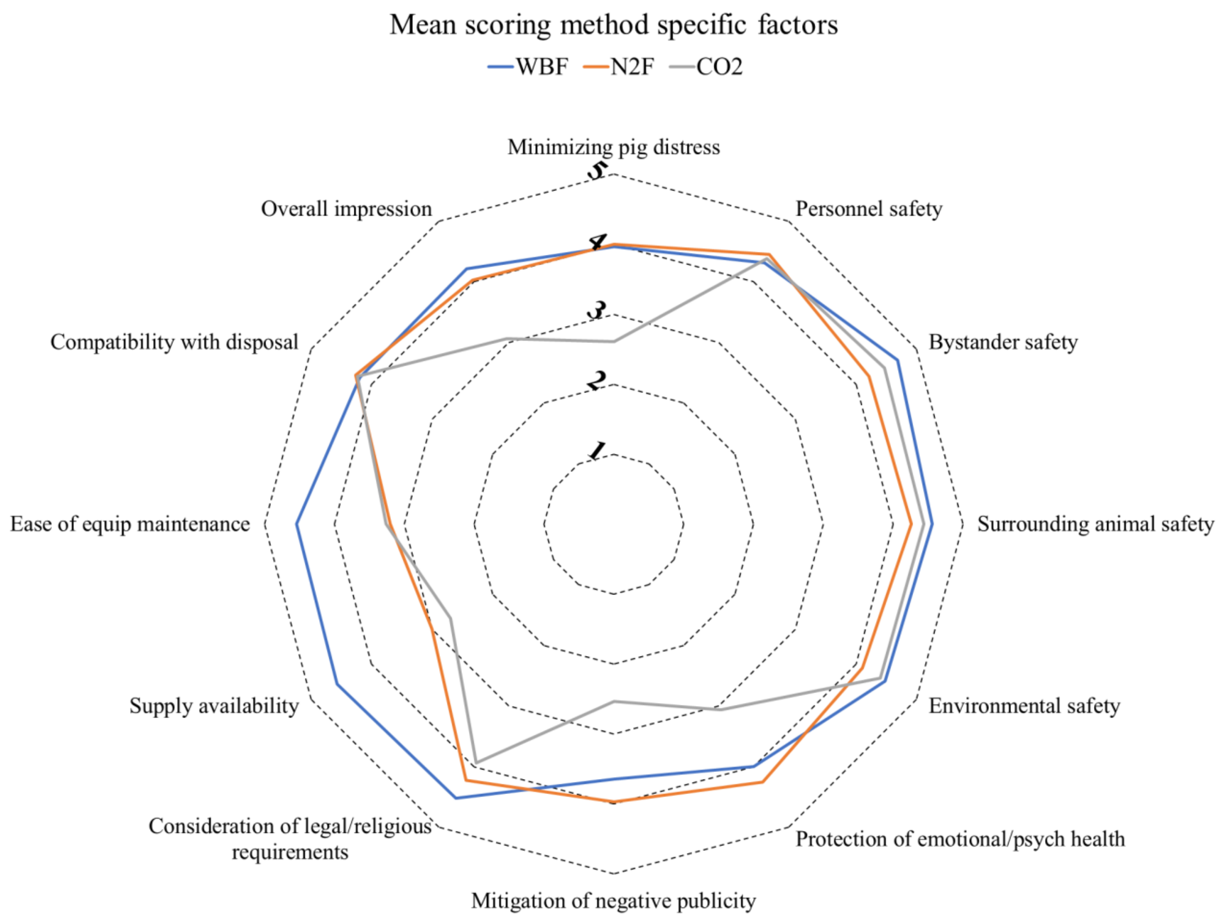
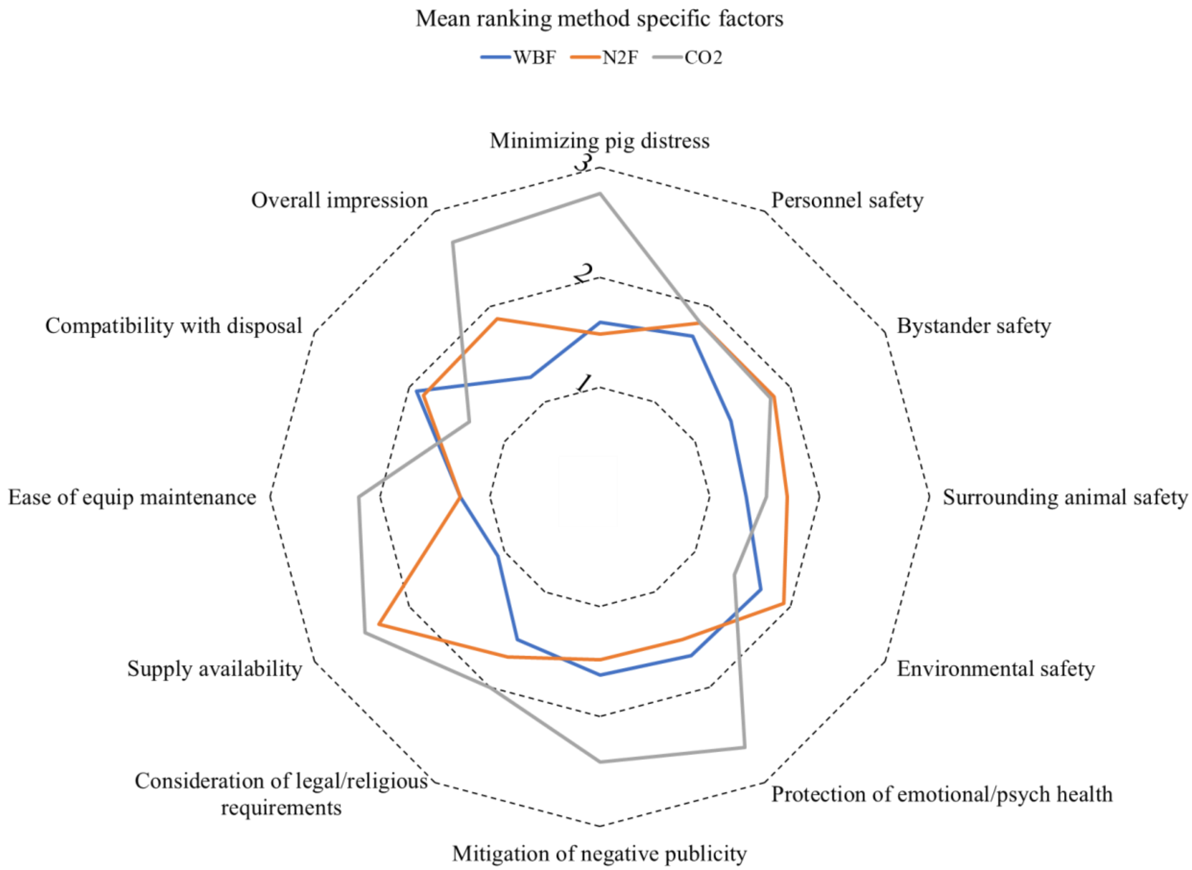


Figure 10. Mean ranking (1-3, lower being better) scores for swine industry stakeholders (N=32) on survey statements regarding the use of water-based foam (WBF), nitrogen-gas foam (N_2F), and carbon dioxide (CO_2) for swine depopulation.



Discussion: Explain your research results and include a summary of the results that is of immediate or future benefit to pork producers.

The U.S is currently ill-equipped to handle large scale incursions of FADs in the swine industry. Depopulation methods that are currently approved by the AVMA are thoroughly evaluated and implemented on a small scale, but not designed for rapid destruction of whole herds in a timely manner to prevent additional transmission during an emergency scenario. Having well-established contingency plans in place is paramount to safeguard the US swine industry, animal health and welfare as well as public health during times of pathogen introductions to the swine population. Recently, mobile WBF methods for large-scale depopulation of pigs during field conditions, based on the previously AVMA-approved approach for poultry, have been refined and used with promising results such high efficacy, low user-risk and expedient loss of consciousness. Moreover, additional options using novel mobile field-setups for gas-infused foam alternatives such as N_2F or gradual oxygen-displacement by CO_2 are also being tested to date. Evaluating these methods is important to minimize animal pain and suffering throughout the depopulation methods. In the current study we aimed to assess suitable foam options for use in WBF depopulation, assess the most suitable foams during field conditions with live pigs, and calculate operational parameters and logistics for consideration for swine producers interested in the WBF depopulation method. Finally, we aimed to describe the attitudes and perceptions of swine industry stakeholders on WBF, N_2F and CO_2 depopulation during field conditions.

Objective 1. Evaluate currently available Class A foam concentrates for swine depopulation suitability.

In this study we were able to obtain 16 available PFOS/PFAS free Class A foams from the US market to test during field conditions. Additional products exist but sourcing these specific products requires either special licensing (such as being a retailer of firefighting agents and equipment) or is only accessible for organizations or entities actively involved in firefighting. Therefore, we settled with the 16 non-contingent foams that are readily available either through homepages or off the shelf by individual producers.

Out of the 16 foams, four foams (BioEX BioFor N Class A Foam, FireIce Polar Class A EcoFoam, Buckeye Platinum Class A Foam, and National Foams Knockdown Class A Foam) had properties suitable for our WBF-setup such as low water usage, limited decay rate post filling, and good foam to water expansion ratio. These foams were shortlisted for additional testing using pigs during field conditions. Having additional foam options is important as it limits local or regional supply issues that may occur during a large FAD outbreak. In addition, the shortlisted foams have long shelf-lives at normal room temperatures of at least 10 years or more, (with Polar Class A Ecofoam and Phos-Chek WD881 Class A Foam, being stable even after freeze and thaw cycles) meaning that they are suitable for stockpiling in case of future emergencies. Cost was taken into consideration when assessing the foam products, but with varying shipping costs across the country some foams may be considerably cheaper regionally compared to others, therefore shipping was not included. However, with an average purchase cost of $26.6 USD/gallon, only one foam (Firestopper AB 40002 FFC Class A Foam) was significantly more expensive compared to the others at $72.3 USD/gallon but did not perform well during the test phase. This shortlist of recommended foams is only based on our current setup and should not be interpreted as a blanket recommendation for different types of foam-generating equipment.

Objective 2: Assess short-listed Class A foam concentrates from Objective 1 for depopulation efficacy and potential negative impacts on swine welfare.

Nursey-aged pigs were immersed in each of the four shortlisted foams to shoulder-height for 15 minutes prior to terminal foam application. During this prolonged exposure time, none of the pigs displayed signs of distress or discomfort. Adverse physiological effects or contact irritation causing tearing of the eyes, skin reddening, or sneezing were not apparent in any of the tested animals. After a 15-minute observation period, the final application of foam was applied. A dwell time of 7.5 minutes, which has been previously validated by our group, was permitted. Utilizing this protocol, all the tested foams were effective for producing rapid unconsciousness followed by death. Necropsy was performed on each of the depopulated animals and samples of mucous membrane and skin tissues were submitted for histology. The primary pathological finding was pulmonary hemorrhage, which was consistent in gross examination and histological evaluation. These lesions were present in at least one pig all treatment groups (all shortlisted foams) and are thought to be associated with perimortem dyspnea following application of WBF. However, the severity of hemorrhage was significantly different between treatment groups with more severe hemorrhaging (scores 3-4) observed in Phos-Chek WD881 compared to BioFor N. It is important to know the severity of pulmonary hemorrhage associated with each of the foam products as this could indicate that one foam is more caustic to the animals than another. In cases where multiple products are available, given equivalent efficacy, this knowledge may lead to choosing a foam associated with less severe pulmonary lesions. Other histological findings included inflammation within the nasal turbinates, conjunctiva, and skin. These observations were inconsistent, and we were unable to determine if these lesions resulted from exposure to WBF or were indicative of pre-existing allergy or chronic upper respiratory infection. We concluded that additional investigation is warranted to further discriminate the causation of the reported inflammation. We determined that any of the shortlisted foams we tested, when used with current foaming equipment, has minimally negative impact on swine welfare and can be used successfully for emergency depopulation.

Field trial

All shortlisted and tested foams performed well in filling the larger 84.4-cubic yard rendering trailer. The foam retained its level during the 7.5-minute dwell time and did not seem to be affected by any pig movements in the trailer. The foam fill times spanned between 58 seconds and 1 minute 47 seconds. Using Phos-Chek WD881 Class A foam as a baseline from previous studies (Arruda et al., 2022; Campler et al., 2023) we established that a recommended fill time of any container or vessel used for depopulation using WBF should be around 1 minute or less. Additionally, we suggest that improvements should be implemented for any fill times between 1 minute and 1 minute 30 seconds, and that any systems in need of more than 1 minute and 30 seconds to fill a container should be improved to minimize the time pigs would experience partial coverage of foam or possible ability to resurface. Based on this, BioEX BioFor N Class A Foam, Buckeye Platinum Class A

Foam, and National Foams Knockdown Class A Foam all met this criterion while FireIce Polar Class A EcoFoam did exceed 1 minute 30 seconds to fill when using our specific equipment. Despite this, FireIce Polar Class A EcoFoam performed well in all other aspects of the testing, and slower fill times can always be mitigated by adding additional pumping systems.

The pigs reacted similarly to all four foams as the foam reached shoulder level and above the pigs' heads. A numerically larger number of stress indicators such as vocalizations and escape attempts were observed in the BioEX BioFor N Class A Foam replicates but was likely due to individual variation between replicates rather than an effect of the specific foam. One of the main limitations of the WBF methodology is that visible cues of the animals if limited. The foam levels increase rapidly and is above the head levels around the 30-second mark, which is preferable for expediting unconsciousness of the animals, but an obstacle in properly recording any visible stress or discomfort until the onset of unconsciousness. The time to the last registered activity in the trailer, used here as an indication for when pigs stopped moving by auditory cues post-fill, was similar between the four foams, and occurred around two minutes post fill completion. The 2-minute time period to the last registered activity was around a minute faster compared to the previously established cessation-of-movement approximations of three minutes in our previous study using implanted Star-Oddi biologgers with tri-axial accelerometer technology (Campler et al., 2023) but similar to the 2-minute time to unconsciousness derived from electroencephalographic during similar conditions using Phos-Chek WD881 in piglets (Korenyi-Both et al., 2022). Although additional research would reinforce the current findings, the results of this study indicate that at least five Class-A foam options on the US market are suitable for swine depopulation using our setup. These findings add data to a very limited number of tested Class-A foam options and if AVMA approved, would significantly ease any preparation and stockpiling off Class-A foams either centrally such as the U.S National Veterinary Stockpile or by individual swine producers.

Objective 3: **Describe and calculate the operational parameters of Class A foam depopulation by animal throughput and cost estimates for swine depopulation of different herd sizes and production types.**

An important goal of assessing depopulation methods is identifying perceived logistical and operational parameters that may either boost or hinder successful deployment. Based on our WBF testing scenarios, foam producing capacity and foam specifications, we created a self-populating Excel spreadsheet that, based on user input, will calculate, and approximate a range of informative parameters regarding the estimated cost and time needed to conduct a depopulation procedure from start to finish. This spreadsheet currently includes all the 16 initially examined and tested Class A foams obtained on the U.S. market as well the option for adding new products in the future. Users are also able to customize container sizes, number of pumps used, as well as water, and gas costs to take into account fluctuating market prices to establish a cost per pig for different scenarios. The user is also able to quickly get an overview for how their specific container size would fare compared to industry standard 10 to 40-cubic yard containers. We believe that this spreadsheet will be valuable for swine producers to establish equipment needed for their site(s) and estimate future investment costs and direct costs, besides labor costs, that will be needed either to prepare for a depopulation event for a range of different production sites and herd sizes. There is great value in being able to create your own scenarios using a range of different container sizes or customized vessels of their choosing without having to field test each one prior to a live event. However, we still emphasize the importance of field testing any final equipment without animals to estimate the true fill times and foam properties prior to any depopulation event.

Objective 4: Describe and compare method efficacy and behavioral outcomes between water-based foam, nitrogen-gas foam **(N_2F)*, and carbon dioxide (CO_2)-gas depopulation methods*.**

Please refer to Dr. Todd Williams's report, NPB project

Objective 5. Describe attitudes and perspectives of swine industry stakeholders on water-based foam, nitrogen-gas foam (N_2F), and carbon dioxide (CO_2)- based depopulation methods.

The attitudes and perspectives from swine industry stakeholders upon viewing demonstrations of WBF, N_2F and CO_2 depopulation methods are key. Specifically, obtained responses are likely to convey opinions from those closely familiar with the industry based on work life experience and will either be in active- or supporting roles during a depopulation event. The current survey for this project served as a comparative follow-up to a previously published survey by Cheng et al., (2023) where 87.7% of swine industry stakeholders that observed a WBF depopulation event, considered it a better alternative for swine depopulation than currently existing and approved AVMA methods.

The survey in this study reported that study participants overall scored WBF higher than N_2F, followed by CO_2 immediately after viewing each of these depopulation methods. However, and as expected, perceptions on the three depopulation methods varied between stakeholders regarding specific statements. For instance, N_2F ranked the highest for minimizing pig distress, protection of emotional or psychological health of caretakers, and as a better method for mitigating negative views from the general public. This was an interesting observation, as WBF and N_2F operate very similarly except for the type of gas used during foam generation. It is likely, due to previous research showing aversiveness (Kinsey et al., 2016; Sutherland et al., 2017) and historically negative connotations, that the gas component to foam is viewed in a more positive light compared to CO_2 and thus regarded as more effective than WBF. On the other hand, multiple comments were made that N_2F "*equipment [was] too loud*", "*very noisy*", and "*very noisy, lots of pig movement before foam applied*" which is an area needing to be addressed to reduce distress for pigs. For WBF, comments ranged between "*animals not in distress*", "*animals were extremely calm*", or "*pigs did not appear stressed [from] foam...*" to noted concern that it "*takes a while for pigs to stop moving*". The protective element of safeguarding the caretaker, the trailer and physical and visual barrier of the foam, is identical for WBF and N_2F but even more so for the CO_2 method, where pigs were loaded into a fully covered trailer, shielding observers from viewing the depopulation process. However, the main difference, besides possible negative connotation, between the foam-based and CO_2 methods is the occlusion of airways caused by the foam. Such occlusion may eliminate vocalizations post-head coverage, whereas they can be produced and heard until loss of consciousness during CO_2 depopulation. This was one element frequently mentioned in survey responses, as participants could hear "*many vocalizations for several minutes*" after the onset of the gas. Additionally, a few observers expressed concern that "*foam was flying away*" due to the lightness of N_2F foam specifically. As seen for the ranking scores for the protection of emotional/psychological health for caretakers and mitigation for negative publicity, the CO_2 method score worse compared to WBF and N_2F. It is possible that the views of swine industry stakeholders, which are likely to be on the conservative side, would be amplified in surveys done with the general public which has limited insight regarding available depopulation options and decision-making during FAD incursions.

Carbon dioxide depopulation scored higher on environmental safety and disposal but lower on ease of equipment maintenance, publicity, emotional health of caretakers and minimizing animal distress. On the other hand, WBF scored higher on personnel safety, supply availability, and ease of equipment maintenance. Eleven participants noted many vocalizations "*for several minutes*" during CO_2 and thus expressed concern over a longer time for animals to lose consciousness. However, CO_2 was noted for having "*quiet equipment*" compared to N_2F, but concerns were higher regarding "*challenges in* CO_2*-supply and availability*", "*difficult/complicated setup*", along with "*worker safety [being] a concern*". Water-based foam received positive comments regarding the "*availability of materials*", "*ease and scalability of the setup*", and "a*ll equipment is readily available and easy to operate... This has minimum expense and is easily trainable*". However, a couple of participants noted that it was "*unpleasant to view the escape attempts*" at the initiation of the foam build up and that there might be a risk of public debate regarding "*airway occlusion vs physiologic hypoxia*". On the contrary, one participant had noted that observing foam-based methods is less disturbing due foam grouping/blanketing animals, whereas CO_2 carcasses were uncovered and thus easily discernable.