RESEARCH REPORT

ANIMAL WELFARE

Title: Determine effective oral dosing of sodium nitrite for efficient euthanasia of adult swine using oral drench technique – **NPB #20-122**

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Industry Summary:
The objective of this study was to determine the oral dose for efficient and effective euthanasia of domestic swine by sodium nitrite. Sodium nitrite is currently "Permitted in Constrained Circumstances" by the American Veterinary Medical Association for swine depopulation events. However, the use of sodium nitrite in domestic pigs is poorly understood. Swine have a strong taste aversion to sodium nitrite, making it difficult to provide adequate dosages through the feed and water for depopulation. This study used an individual oral drench approach to dosing domestic market weight and adult pigs at four different dosages. A standard oral dose of 181 mg/lb. of body weight, taken from a previous study in feral hogs, was used (1x), twice the standard dose (2x), 2.5 times the standard dose (2.5x), and 3 times the standard dose (3x). Pigs were dosed by oral drench individually using an air-compressor powered hook drench gun designed for administering oral medication to cattle. The behavior response of each animal following sodium nitrite administration was observed by the behaviors defined in Table 1. Monitoring devices that measure and record individual animal body temperature, heart rate, and activity were surgically placed under the skin of eight animals. Two implanted animals were placed in each treatment group.

Table 1: Ethogram of definitions of the behaviors and recorded measurements used to monitor physiological response to sodium nitrite administration

Behavior	Definition	Variables Recorded
Convulsions	Involuntary contraction of skeletal muscles[1] (tonic, clonic, or both) and paddling	Latency to onset and frequency
Gasping	Low frequency, very deep breathing through the wide-open mouth with large abdominal movements and stretching of the neck	Latency to onset
Head shaking	Vigorous, rapid, and purposeful movements of the head from side to side (at least two consecutive movements)	Frequency
Loss of coordination	Loss of balance, stumbling, or diminished muscle control	Latency to onset
Loss of posture	Animal collapses into recumbent position with no evidence of posture control and does not regain posture or show further evidence of awareness	Latency to onset
Respiratory arrest	Permanent cessation of respiratory movements (minimum of 60 seconds without a breath)	Latency to onset
Retching	Making the sounds and movements of vomiting but not producing the ejection of contents from mouth[3]	Frequency
Vocalization	Pig emits an audible bout of a squeal or grunt[2]	Frequency of bouts
Vomiting	Ejection of gastrointestinal contents through the mouth	Latency to onset and frequency

[1]Tonic defined as prolonged generalized contraction. Clonic defined as alternating contraction/relaxation in quick succession. Paddling defined as involuntary walking/running/galloping motion of the limbs
[2]A bout is defined as a single discreet event or a period of a continuous event with a < 1-second pause. A pause >1-second is the end of the bout
[3]Definition for our study was derived from the description of vomiting behavior

At the 1x dose, sodium nitrite successfully euthanized 6 of 10 (60%) pig's after 2 hours of observation with the average time of death due to sodium nitrite of 1 hour 23 minutes. At 2x, 10 of 10 (100%) pigs died due to sodium nitrite with an average time to death of 47 minutes. At 2.5x dose, 9 of 10 (90%) pigs died due to sodium nitrite with an average time to death of 42 minutes after 2 hours of observation. At 3x, 9 of 10 (90%) pigs died due to sodium nitrite with an average time of 34 minutes after 2 hours of observation. The time to death was found to be statistically different ($p<0.05$) between the dosages by a linear relationship along with the time to onset of convulsions, gasping, loss of coordination, loss of posture, and vocalization. This linear relationship means the time to death, or adverse behavior response was significantly quicker as the dosage increased. The time of onset of vomiting was not found to be significantly different between the treatment groups. The frequency of convulsions, head shaking, retching, vocalization, and vomiting were not significantly different between the treatment groups.

The data from this study suggests that the 3x rate (543-600mg/lb.) is the best dosage for oral drench in market weight pigs of those evaluated. The 3x rate provided the shortest time to death while still providing enough time to allow pigs to walk out of the barn before dying. The frequency of the behaviors associated with animal discomfort (vocalization, vomiting, retching, head shaking, and convulsions) was not different among the treatment groups.

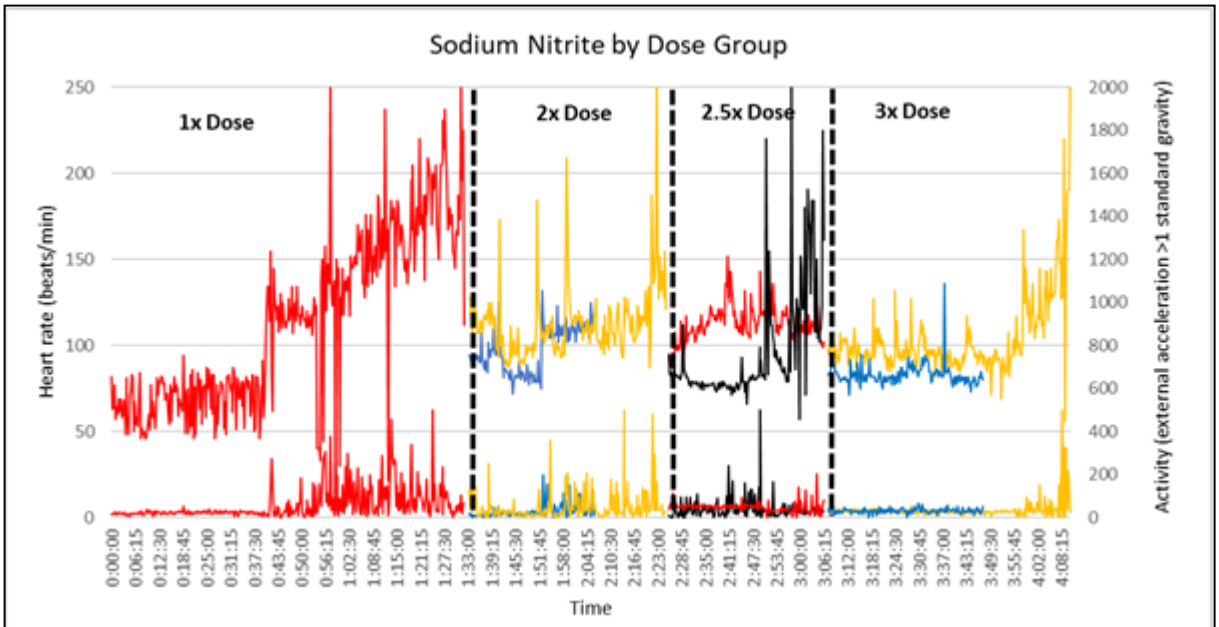

Figure 1: Heart rate (bpm) matched with general activity of the implanted pigs in each dose group over time. Measurements were taken every 15 seconds. One of the two implanted pigs in 1x group was euthanized by captive bolt and not included on the figure.

The implant data (Figure 1) reflected fewer spikes in pig activity levels as the dose of sodium nitrite increased. Heart rate appeared to increase the longer the pigs remained alive after the sodium nitrite dose was administered. The 3x dose seemed to provide the least amount of spikes in pig activity after administration compared to the other dosing groups. The body temperature between the treatment groups remained relatively consistent from the time of administration to the time of death, except for the pig in the 1x group, where body temperature continued to rise over time.

After determining the best dosing rate in the market weight animals, that rate was applied to10 sows, and the same behavior responses were monitored. With the high success rate and a short time to death of the 3x dose market weight group, the 3x dose rate was used in the 10 sows. An oral drench of sodium nitrite successfully euthanized 9 of 10 sows (90%) with an average time to death of 31 minutes.

The average time to dose a group of ten pigs with a team of 2-3 people was 14 minutes, ranging from 12 to 17 minutes (average of 1minute and 24 seconds/pig). The team consisted of 1 to 2 people corralling an individual animal with a hinged sort board and one person administering the sodium nitrite via the hooked oral drench gun. Occasionally, individual animals would need to be snared during the administration process if they could not be adequately restrained with a sorting board.

The oral drench administration of sodium nitrite is a viable approach to euthanizing market weight and adult swine in depopulation events under constrained circumstances. The pigs do experience behaviors indicative of distress after the dosing of sodium nitrite. The observed distress supports the reservation of sodium nitrite for constrained situations of depopulation. When possible, more humane euthanasia methods (e.g., CO2, captive bolt, gunshot, etc.) should be used unless circumstances prevent it. For a large scale depopulation event, euthanizing a high number of animals with the oral drench of sodium nitrite may be too timely and labor restrictive.

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Key Findings:

- A linear relationship between sodium nitrite dosage in time to death was observed with shorter time of death as dosage rate increased
- 3x the regular documented oral dosage provided the shortest time to death compared to the lower dosage rates evaluated

- Sodium nitrite does invoke behavioral signs suggestive of distress before death in commercial swine
- Sodium nitrite should be reserved for only constrained situations for swine depopulation when other euthanasia methods are unsuitable or not available for the given circumstances

Keywords (5): Sodium nitrite, depopulation, euthanasia, behavior, oral drench

Scientific Abstract:
Sodium nitrite has been used in various countries for the control of feral swine by creating toxemia, causing lethal methemoglobinemia. Little current literature is available on the use of sodium nitrite for the euthanasia of commercial swine. Potential advantages for the use of sodium nitrite in constrained situations as in a foreign animal disease depopulation event include the ability to walk animals out of the building before they die. This study aimed to determine an effective oral drench dosing rate of sodium nitrite, develop a standard procedure for the use of oral drench for swine depopulation, and to measure the physiological response of pigs exposed with toxic levels of sodium nitrite. Forty (40) market weight hogs (pigs averaging 290lbs) and 10 adult sows (commercial female pigs that have had at least 1 litter) were used in this study. The market weight animals were divided into 4 treatment groups. Treatment groups were based on the dose of sodium nitrite administered by oral drench at the oral lethal dose rate of 181mg/lb. of body weight and then 2x, 2.5x, and 3x oral lethal dose rate. Two pigs in each treatment group were implanted with a monitor, 48 hours in advance, to measure heart rate, activity, and body temperature. After the sodium nitrite administration, each pig was monitored for signs of distress by a previously defined ethogram for swine behavior. The most effective and humane dosing rate of the four evaluated was then applied to 10 adult sows to ensure the process worked in heavy adults. The 3x dosing rate provided the shortest time interval from oral drench to death in the market weight animals. Implant data revealed less frequent spikes in both heart rate and activity, the higher the dosage rate. Time to the start of convulsions, gasping, loss of coordination, loss of posture, and vocalization were all statistically significant by a linear relation with a shorter time interval associated with the higher dosing. The 3x dosing rate applied to sows was also successful. Based on the results of the study, sodium nitrite by oral drench is a viable option for the depopulation of swine. However, the labor required to complete the dosing processes may outweigh the benefits. The behavior and physiological signs of distress of the animals support the American Veterinary Medical Association's current recommendation of sodium nitrite as "Permitted in Constrained Circumstances."

Introduction:

Sodium nitrite ($NaNO_2$) has been used in other countries for the control of feral swine and other wild animals considered to be pests (Snow et al., 2016; Shapiro et al., 2016). The knowledge for the potential use of sodium nitrite in domestic swine for depopulation events (e.g., foreign animal disease or major market disruption) is limited. Sodium nitrite ingestion causes a lethal rise in methemoglobin in the animal leading to lethargy, ataxia, dyspnea, and death (Cowled et al., 2008). Methemoglobin is the stable oxidation of hemoglobin's ferric iron, which prevents the release of oxygen to the tissues (Bradberry, 2011). A potential advantage of sodium nitrite use is that animals may have time to walk outside of the barn before death occurs. Walking the animals outside eliminates the immensely laborious task of removing carcasses from the barn. When encapsulated, sodium nitrite requires up to 3 hours to cause death when consumed at the lethal dose for swine (USDA, 2018). The lethal dose of sodium nitrite in feral pigs is >400mg/kg (~181 mg/lb) for freely consumed bait (Cowled et al., 2008). However, getting domestic animals to consume the product is problematic due to taste aversion likely due to the salty and bitter properties of the product (Lewis, 1999; Sharpio et al., 2016). Unfortunately, it appears the withholding of water before administration in the water is necessary to convince the animals to consume it. Sodium nitrite has a water solubility of 70-80g/100ml with 20°C (68°F) water. This solubility limit means reaching a minimum lethal dose of 55g for a ~300pound pig requires 78.6ml of water, assuming the animal swallows the whole dose. Sodium nitrite is documented to be unstable in solution, requiring it to be prepared fresh or kept on ice before use (Misko et al., 1993). An air-compressor powered oral-bolus device commonly used in cattle wormer dosing can provide a quick and efficient method for administering the high volume of liquid required. The hook shape of the drench device holds its place in the pig's mouth. The administration process is similar to altrenogest dosing performed commonly in mature gilts for estrus synchronization.

Withholding water to convince animals to drink sodium nitrite freely creates a welfare concern. The requirement to withhold water is also not efficient for time-sensitive events, like a foreign animal disease depopulation. Determining the effectiveness of oral drenching may provide a method for depopulation where animals can be walked outside of the barn and not have to withhold from water. Utilizing the results from this trial will establish an efficient protocol of dosage for

sodium nitrite for depopulation via oral drench administration. This study will also evaluate the physiological and behavioral response of commercial swine to sodium nitrite toxicity, which has not been previously documented.

Objectives: From your research proposal.

1. Determine the ideal oral drenching dosage of sodium nitrite for swine depopulation (providing enough time for animals to walk outside of barn)
2. Develop a standard operating procedure for swine euthanasia via oral drench
3. Determine the physiologic response of the pig exposed to sodium nitrite

Materials & Methods:

Animals:
A total of 40 market weight (average weight 290lbs) and 10 sows (average weight 383lbs) were used in this study. These numbers are similar to the number of animals used in previous swine euthanasia and behavior studies (Sadler et al., 2014; Sutherland et al., 2017 and Kells et al., 2018).

Sodium nitrite solution:
99% sodium nitrite granular powder was used for solution preparation. Solutions were prepared by pre-weighing milligrams of sodium nitrite to be combined with a gallon of water using the assumed solubility of 70g of sodium nitrite into 100ml of solution. For every gallon of a solution prepared for dosing, 2649.5g of sodium nitrite powder was added, providing a final concentration of 0.7g sodium nitrite/ml of solution. The solution was made fresh, immediately before each dosing procedure began.

Sodium nitrite dosing:
To provide a 181mg/lb. of body weight dose of sodium nitrite solution to an average market weight pig (~290lbs), a dosing range of 181-200mg/lb. of body weight was targeted. This extended range is to account for the volume of water that may not be swallowed by the pig during the dosing. The treatment groups of 1x (181-200mg/lb), 2x (362-400mg/lb), 2.5x (452.5-500mg/lb), and 3x (543-600mg/lb) were used in this study. The 1x group received 80ml of sodium nitrite solution by oral drench, 2x group received 150ml, 2.5x group received 200ml, and the 3x group received 250ml of sodium nitrite solution.

The dosing level used on the sows was based on the ideal dosing determined by observations on the market weight animals. The dosing level used was the rate that provided market animals the shortest time to death while still allowing enough time to walk out of a building. The same solution and dosing rate method was used for administration to both sows and market weight animals.

An air-compressor powered hooked drench gun (typically used for liquid wormer administration to cattle) was used (Valbazen, Zoetis) was used to administer the oral drench. Pigs were restrained individually in the corner of a pen using a hinged sort panel. Each animal had the hook of the drench gun placed in its mouth and was administered their calculated dose. Immediately after administration, pigs were numbered and walked into a corralled area for monitoring.

Behavior measures:
The behavior of each animal was scored and recorded according to the ethogram defined in Table 1 (table definitions derived from Sutherland et al., 2017; Kells et al., 2018; and Sadler et al., 2014). The time each animal received the oral drench was recorded. All pigs in each group were numbered 1-10 on their backs for easy identification for monitoring. At least 3 people were evaluating the animals according to the definitions in Table 1. Animal's number, time of behavior event, and type of event were recorded during the observations until the time of death was confirmed. Death was confirmed by the observation of respiratory arrest and confirming the absence of a corneal reflex.

Vitals and activity monitoring:
Before sodium nitrite administration, two pigs in each dosing category (8 pigs total) were sedated for the implantation of an internal monitor device. The monitor measured the animal's heart rate via ECG measurements, activity levels by changes in external acceleration by values >1 standard gravity, and body temperature (°C). Animals were implanted 48 hours before the start of sodium nitrite administration to obtain appropriate base-line information from the implanted animals for appropriate readings. Implant readings were taken once every 30 minutes until the day of sodium nitrite administration when readings were taken every 15 seconds.

Statistical analysis:
For parameters with a normal distribution, the linear ANOVA model was performed using a significant p-value ($p \leq 0.05$).

Animal Use:
Animals were used under the guidelines and approval of the Pipestone Research Institutional Animal Care and Use Committee (IACUC) protocol ID# 2020-006.

Results: Report your research results by objective.

1. Determine the ideal oral drench dosage of sodium nitrite for swine depopulation

A statistically significant linear relationship ($p<0.05$) was found between the treatment groups, showing the higher the dosage, the shorter time to death after sodium nitrite administration. The dosage of at least 2x the lethal oral dose was needed for the highest success rate, as seen in Table 2.

Table 2: Rate of euthanasia success rate and mean time to death by dose treatment group

Dose[1]	Death rate	Mean of time to death[2]
1x (181-200mg/lb.)	6/10 (60%)	83 minutes
2x (362-400mg/lb.)	10/10 (100%)	47 minutes
2.5x (452.5-500mg/lb.)	9/10 (90%)	42 minutes
3x (543-600mg/lb.)	9/10 (90%)	34 minutes
3x – Sows[3]	9/10 (90%)	31 minutes

[1]Dosage used in each group of 10 market weight (290lb. average) animals
[2]Only accounts for the mean of animals that died from the sodium nitrite
[3]Same dosage rate as the 3x market group used on 10 adult sows (543-600mg/lb.)

2. Develop a standard operating procedure for swine euthanasia via oral drench

By use of the air-compressor powered drenching device, a team of 2-3 people could administer the sodium nitrite solution to ten pigs in 14 minutes on average (ranging from 12-17 minutes). This rate provides an estimate of 1 minute 24 seconds/pig for dosing with 1 team of people. A hinged sort board worked best for restraining animals for placing the hooked drench device into their mouth for the oral drench to the back of the animal's throat. Occasionally, an individual animal would need to be snared for restraint for solution administration.

3. Determine the physiological response of the pig exposed to sodium nitrite

Table 3: Frequency of behavior parameters by dose treatment group of market weight pigs and a group of sows

Behavior Parameter	Treatment Group (n=10 per group)				
	1x	2x	2.5x	3x	Sows (3x):
Convulsions	8	10	9	9	19
Head Shaking	2	0	0	0	1
Retching	1	1	0	2	5
Vocalization	9	5	5	6	5
Vomiting	1	2	4	4	17

Table 4: Time to onset of behavior parameters and linear statistical significance in market weight animals

Parameter for time to onset	1x	2x	2.5x	3x	p-value
Time to convulsions, min	80.2	44.6	38.03	29.88	<0.05
Time to gasping, min	89.5	34.38	39.38	34.5	<0.05
Time to head shaking, min	Not enough frequency to calculate				
Time to loss of coordination, min	75.17	40.67	35.83	26	<0.05
Time to loss of posture, min	83.96	34.75	46.5	23	<0.05
Time to respiratory arrest (death), min	83.12	47.3	41.08	35.78	<0.05
Time to retching, min	Not enough frequency to calculate				
Time to vocalization, min	76.48	49	43.5	31.67	<0.05
Time to vomiting, min	33.9	47.9	34.04	27.2	0.38

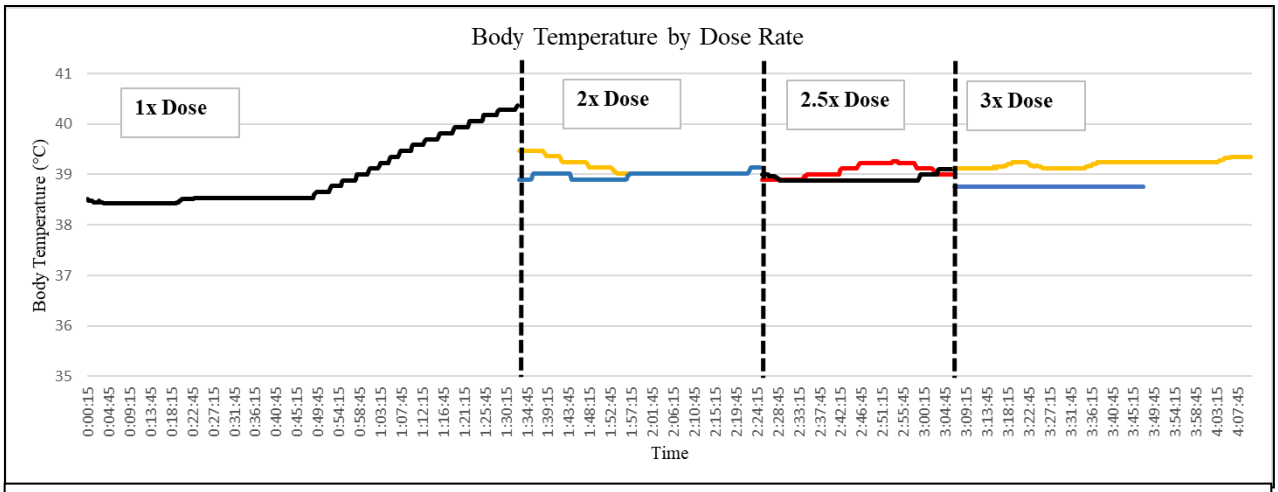

Figure 2: Body temperature (°C) of the implanted pigs in each dose group over time. Measurements were taken every 15 seconds. One of the two implanted pigs in 1x group was euthanized by captive bolt and not included on the figure.

As seen in Table 3, each dosage treatment group caused behavior signs indicative of distress in pigs. The frequency of the behaviors was not statically different between groups within the market weight pigs. Sows numerically had a higher frequency of convulsions and vomiting than all the treatment groups in the market weight animals. Time to the start of convulsions, gasping, loss of coordination, loss of posture, and vocalization was a significant linear relationship ($p<0.05$) of the higher the dosage, the shorter time to onset (Table 4). The frequency of head shaking and retching was not high enough to be analyzed between treatment groups. Figure 1 shows the heart rate and activity of each dosage treatment group over time, with the least spikes of activity seen in the 3x group. Figure 2 shows the body temperature of the implanted pigs in each dosage treatment group.

Discussion:

Sodium nitrite has been evaluated for use in controlling feral swine populations. However, little information is available on the use in commercial, domestic pigs. The oral dosage used in bait stations for feral hogs of 181.4mg/lb. of body weight produced the lowest successful death rate (Table 2). The results of this study support that oral drench of sodium nitrite needs to be at least twice the oral dose rate (>362mg/lb.) for the method to be consistently and repeatedly successful. The lethal dose to kill 50% of the test population (LD50) for sodium nitrite has been previously documented at 60.3mg/lb. in pigs with a minimal lethal dose reported from 40.8-51.3mg/lb. by oral drench (Cowled, B.D. et al., 2008; Foster, 2011). The current study would suggest an LD50 closer to the recommended oral feed dose of 181.4mg/lb. that has been previously documented for feral hog bait stations (Cowled, et al., 2008; Sharpiro et al., 2015).

The linear relationship found shows that as the dose increases, the time of death is shorter. Even at the 3x dose (543mg/lb.), time to death averaged >30 minutes in both market weight and adult sows. This time may provide ample opportunity for

animals to be walked out of the building after dosing before death. Whichever dosing rate was used, animals still experienced behaviors indicative of distress with no difference in frequency among the market weight animals (Table 3). However, at the higher dosage, the animals experienced distress and attained death significantly sooner. These quicker time intervals suggest the higher rate may be more humane as it shortens the animal's time of discomfort. This increase in welfare may be supported by the activity data recorded by the implanted monitors (Figure 1) in which the animals at the 3x rate provided fewer spikes in activity compared to the other treatment groups. As seen in Figure 2, the body temperature of the animal in the 1x treatment group continued to elevate until death, which may also suggest increase discomfort in that animal. The increasing spike in body temperature was not observed in the other treatment groups.

With the average dosing rate in this study of 1 pig every 1 minute 24 seconds per team of 2-3 people, it would take at least 28 hours to dose 1200 pigs (assuming no breaks taken). Even with 2 teams of people at the same rate, 14 hours would be needed for the administration process. Although doable with enough people, the oral drench approach may not be the most appropriate for a large number of animals. Due to the taste aversion to sodium nitrite's bitter, salty qualities, getting the animals to drink the solution freely is difficult (Lewis, 1999; Sharpio et al., 2016). The advantages of sodium nitrite for depopulation in a foreign animal disease situation include the ability to walk animals outside before they die and the reduction in bloodshed. The reduction of blood-loss is important as diseases like African Swine Fever can spread very readily by blood contact (Chenais et al., 2019).

Based on the results of the current study, sodium nitrite by oral drench is a viable option for the depopulation of swine. However, the labor required to complete the dosing processes may outweigh the benefits. The signs of distress experienced by sodium nitrite support the American Veterinary Medical Association's current classification of "Permitted in Constrained Circumstances" for depopulation events (Leary et al., 2019).

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