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April 30, 2021

Docket No. APHIS-2017-0067-0091
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Wildlife Services

Submitted via Regulations.gov

RE: Docket No. APHIS-2017-0067-0091; Draft Supplement to the Environmental Assessment: Field Evaluation of HOGGONE Sodium Nitrite Toxicant Bait for Feral Swine

To Whom It May Concern:

The Animal Welfare Institute (AWI) submits these comments on behalf of our supporters in response to the USDA's posting on March 31, 2021 of the "Draft Supplement to the Environmental Assessment: Field Evaluation of HOGGONE Sodium Nitrite Toxicant Bait for Feral Swine" (Supplement).

Since 1951, AWI has been alleviating suffering inflicted on animals by humans. AWI pursues its mission through engagement with policymakers, scientists, industry, and the public. Our wildlife protection program seeks to reduce the detrimental impacts of human activities on wild animals and promotes humane, nonlethal solutions to conflicts with wildlife.

Legal Background

The National Environmental Policy Act (NEPA), 42 U.S.C. § 4321 et seq., is the nation's "basic charter for protection of the environment." In enacting NEPA, Congress declared a national policy of "creat[ing] and maintain[ing] conditions under which man and nature can exist in productive harmony."[1] NEPA was adopted to "promote efforts which will prevent or eliminate damage to the environment and biosphere" in order to "fulfill the responsibility of each generation as trustee of the environment for succeeding generations."[2] NEPA is intended to "ensure that [federal agencies] . . . will have detailed information concerning significant environmental impacts" and "guarantee[] that the relevant information will be made available to the larger [public] audience."[3]

[1] Or. Natural Desert Ass'n v. Bureau of Land Mgmt., 531 F.3d 1114, 1120 (9th Cir. 2008) (*quoting* 42 U.S.C. § 4331(a)).
[2] 42 U.S.C. §§ 4321, 4331(b)(1).
[3] Blue Mountains Biodiversity Project v. Blackwood, 161 F.3d 1208, 1212 (9th Cir. 1998).

Under NEPA, an environmental assessment (EA) is performed to determine whether a federal action has the potential to cause significant environmental effects.[4] While an EA is described as a "concise public document,"[5] it must "provide sufficient evidence and analysis for determining whether to prepare an [EIS] or [FONSI]."[6] Agencies are also obligated to "involve[] the public . . . to the extent practicable in preparing [EA]s."[7]

NEPA requires Wildlife Services to take a "hard look" at all the consequences of its proposed actions.[8] CEQ regulations state that agencies "shall ensure the professional integrity, including scientific integrity, of the discussions and analyses in environmental documents" and "make use of reliable existing data and resources."[9] Where scientific uncertainty is present, an agency must openly analyze the reputable opinions contrary to its proposed action.[10]

In order to satisfy the "hard look" mandate, NEPA requires agencies to "consider every significant aspect of the environmental impact of a proposed action" and "inform the public that [they have] indeed considered environmental concerns in [their] decision making process."[11] An agency's failure to use the most up-to-date information and tools available, or the inclusion of erroneous information, undermines the public's confidence in an environmental review document and renders it legally defective.[12] Accurate, up-to-date information is imperative for both the public and for the agency to assess the proposed action.

Though Wildlife Services prepared the 2017 EA under the 1978 regulations, in July 2020, the Council on Environmental Quality (CEQ) updated its NEPA regulations. This Supplement was prepared under the new CEQ guidance, which holds the agency to a new standard in preparation of the supplement to the 2017 EA.[13]

Take of Non-Target Species Has Not Been Sufficiently Addressed

The stated purpose of the Supplement is to examine the potential environmental impacts of conducting field trials incorporating new information that has become available from research findings and data gathering since the issuance of the Decision and FONSI in 2017.[14] Specifically, unexpected non-target mortalities during the 2018 field trial in Texas led to modifications to the bait station, the formulation of the bait, and the baiting strategy.

The Wildlife Services program subsequently conducted three small-scale trials in 2019 and 2020 to test the effectiveness of the changes made to the product and their effect on the human

[4] 40 C.F.R. § 1508.1(h).
[5] *Id.*
[6] *Id.* § 1501.5(c).
[7] *Id.* § 1501.5(e).
[8] Baltimore Gas and Elec. Co. v. Natural Res. Def. Council, 462 U.S. 87, 100 (1983).
[9] 40 C.F.R. § 1502.23.
[10] Sierra Club v. Bosworth, 199 F. Supp. 2d 971, 980 (N.D. Cal. 2002).
[11] Earth Island Institute v. U.S. Forest Serv., 442 F.3d 1147, 1153–54 (9th Cir. 2006) (abrogated on other grounds) (*citing* Kern v. U.S. Bureau of Land Mgmt., 284 F.3d 1062, 1066 (9th Cir. 2002)); *see also* Baltimore Gas and Elec., 462 U.S. at 100 (1983); 40 C.F.R. § 1506.6.
[12] Tribal Village of Akutan v. Hodel, 869 F.2d 1185, 1192 n. 1 (9th Cir. 1989).
[13] Supplement at 4.
[14] Supplement at 2.

environment.[15] Trials conducted in Australia and Alabama of the modified HOGGONE bait stations documented spillage of 0 to 130.2 g with an average of 55.4 g (or 6.51 g of active ingredient) that could end up on the ground.[16] The small-scale field trial conducted in Alabama in 2019 resulted in take of two non-target animals (both opossums).[17] In another small-scale trial in March 2020 in Texas, non-target take was "higher than expected" and resulted in the killing of 35 birds.[18] A third trial, which employed a bird deterrent device, was conducted in July 2020 at the same location in Texas and resulted in the taking of two rodents.[19]

The Supplement notes that, although the potential risk of non-target take has been reduced, "it would still be possible to take an unacceptable number of non-targets with the maximum amount of predicted spillage of 130.2g of bait (6.51g of active ingredient, or 0.23 oz.)."[20] A goal of 400 feral swine between the two designated geographic locations would require approximately 40 bait stations. According to the Supplement, based on a scenario of maximum bait spillage at the stations, "it would be possible to take up to 400 non-target blackbirds and 50 non-target raccoons/opossums."[21]

The 2021 study by Snow cited in the Supplement concludes "we expect we found a majority of the non-target species that were killed during toxic baiting, because [sodium nitrite] is fast-acting and most non-targets that consumed bait did not move far from the bait sites." [22] However, given data from new studies not included in the Supplement, we have concerns that non-target mortality caused by ingestion of vomited bait may be under-estimated. Ingestion of sodium nitrite at both lethal and sublethal doses is associated with vomiting, which occurs in roughly two-thirds of swine ingesting it, with exact incidence of vomiting varying depending on the study. The authors of the 2021 Snow study acknowledge that some pigs died or were presumed to have died outside of their transect grids.[23] While the Supplement described that approximately 90% of sodium nitrite residues in the stomachs of feral swine are lost within 3 hours after ingestion,[24] a study not included in the EA or Supplement (see below) notes that vomiting typically occurs between 27.2 and 47.9 minutes after ingestion of sodium nitrite, when significant degradation may not have occurred.[25] Since pigs may travel significant distances during the time frame in which vomiting can occur, they could spread the toxin throughout the environment by this route.

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[15] Supplement at 3.
[16] Supplement at 9–10.
[17] Supplement at 22.
[18] Supplement at 23.
[19] *Id.*
[20] Supplement at 24.
[21] *Id.*
[22] Snow, N. P., J.D. Wishart, J.A. Foster, L.D. Staples, and K.C. VerCauteren. 2021. Efficacy and risks from a modified sodium nitrite bait for wild pigs. *Pest Management Science.* 77:1623.
[23] EA at 25. Cowled, B.D., P. Elsworth, and S. J. Lapidge. 2008. Additional toxins for feral pig (*Sus scrofa*) control: identifying and testing Achilles' heels. *Wildlife Research.* 35:651–662; Carthage Veterinary Services. 2020. Pork Checkoff Research Report: Evaluation of sodium nitrite for mass euthanasia of commercial pigs (NPB #20-118).; Pipestone Veterinary Services. 2020. Pork Checkoff Research Report: Determine effective oral dosing of sodium nitrite for efficient euthanasia of adult swine using oral drench technique (NPB #20-122).
[24] Supplement at 26.
[25] Pipestone Veterinary Services, *supra* note 23.

Finally, we believe the Supplement has not sufficiently made the case that vultures are "at minimal to no risk" from sodium nitrite contained in pig carcasses. In a study cited in the Supplement, an LD_{50} of 663 mg/kg was determined for vultures.[26] The study reports no death or intoxication when vultures were fed on stomachs of feral pigs dosed with 600 mg/kg of sodium nitrite. However, as described below, there is accumulating evidence that pigs ingesting bait must consume significantly higher dosages of sodium nitrite (1,600 mg/kg) to achieve high mortality rates. Thus there is potential for vultures to be exposed to significantly higher amounts of sodium nitrite in field conditions compared to study conditions.

Review of Humaneness of the Proposed Action is Inadequate

"Humaneness & Ethics" is one of four issues supposedly evaluated in depth in the original EA.[27] According to the Supplement, the discussion of humaneness in the EA "focused on the pain or suffering that an animal may have from consuming a lethal dose of sodium nitrite."[28] However, the sole research reviewed was a very limited study conducted in Australia in 2009.[29] To our knowledge, the study was never peer-reviewed or published in any academic journal. Moreover, given the study's extremely small sample size of only five pigs, the statistical power of the analysis was too low to allow any inferences to be made regarding the reaction of pigs to the toxicant sodium nitrite.

In addition to a small sample size, the study's report contains an inadequate presentation and discussion of the findings. For example, the report notes an increase in serum cortisol—a hormone commonly used as an indicator of stress in animals—of eight- to ten-fold after consumption of the bait.[30] Yet, the report fails to explain how such a finding could be consistent with the report's conclusion that sodium nitrite intoxication is a humane method of killing. The report also mentions the results of a trial of the lethality of sodium nitrite conducted in 2006, where dyspnea (respiratory distress) was a common symptom and half of the feral pigs tested vomited one to three times prior to unconsciousness.[31] Dyspnea, caused by hypoxemia due to severe methemoglobinemia, is typically associated with anxiety and distress in a range of species. Yet, despite failing to even define the term "humane," the report concludes that, "[i]n the opinion of the authors, the symptoms leading to death and duration of display of these symptoms would suggest that sodium nitrite satisfies a general understanding of what a humane poison would be."[32]

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[26] Foster, J.A., J.C. Kinsey, J. Bustamante, N.P. Snow, L.D. Staples, and K.C. VerCauteren. 2018. Negligible risk of secondary mortality in turkey vultures (*Cathartes aura*) consuming wild pigs killed with HOGGONE®. International Wild Pig Conference, p.27, April 15-18, 2018. Oklahoma City, Oklahoma. Available at: https://www.wildpiginfo.msstate.edu/pdfs/2018agenda.pdf.

[27] Supplement at 5.

[28] Supplement at 27.

[29] Institute of Medical and Veterinary Science. 2010. Assessing the humaneness and efficacy of a new feral pig bait in domestic pigs. Report for the Australian Government Department of the Environment, Water, Heritage and the Arts. Canberra, Australia.

[30] *Id.* at 10.

[31] *Id.* at 8. The Supplement (at 25) notes that, in pen trials, 70% of feral swine who consumed a lethal dose vomited.

[32] *Id.* at 6.

A supplement to an EA is required when there are significant new circumstances or information relevant to the issues, such as humaneness, that have a bearing on the proposed action or its impacts.[33] While this Supplement reviews new information related to the risks to non-target animals posed by the reformulated HOGGONE bait and baiting strategy, it does not consider new information related to the effect of sodium nitrite on the target species.

In 2020, in response to meat supply chain disruptions caused by the COVID-19 pandemic, the pork industry in the United States funded two studies, through the Pork Checkoff, on the use of sodium nitrite to kill domestic pigs.[34] The Supplement fails to evaluate either of these relevant studies.[35]

The first study was conducted by Carthage Veterinary Services with a total of 110 pigs assigned to multiple treatment groups, as well as feed and water control groups.[36] The average time to death was 2 hours and 12 minutes, and nearly two-thirds of the pigs vomited during the feed treatment. Only 66% of the pigs across all treatment groups were killed by sodium nitrite consumption in this study.[37] Pipestone Veterinary Services conducted the second study with 40 market weight hogs and 10 sows divided into 5 treatment groups by dose.[38] According to the Pork Checkoff report of the research, "each dosage treatment group caused behavior signs indicative of stress in pigs," which included convulsions, gasping, vomiting, and vocalization. [39]

While both of these research studies use the term "euthanasia," the American Veterinary Medical Association's guidelines on euthanasia[40] do not recognize sodium nitrite as an acceptable method of euthanizing any species of animal, including domestic or feral swine. In fact, the authors of both studies reserve their support of the use of sodium nitrite for "constrained" circumstances.[41] AWI interprets "constrained circumstances" as those which involve imminent risk to animal and/or human health or safety. None of the three studies reviewed above offers justification for non-emergency killing feral swine with a toxicant, such as sodium nitrite, that results in serious animal distress.

New Research Raises Concerns Regarding Efficacy and Sublethal Effects

Information from the two 2020 Pork Checkoff studies also suggest the possibility that the new 5% formulation of the HOGGONE may result in increased sublethal poisonings and prolonged times until death.

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[33] 40 C.F.R. § 1502.9(d)(1)(ii).
[34] National Pork Board (NPB), *Pork Checkoff Research*, http://www.porkcheckoff.org/research.
[35] AWI acknowledges that the route of sodium nitrite administration differs in the NPB studies from the proposed action, and the NPB study subjects were domestic pigs as opposed to feral swine. However, these limitations apply as well to the Australia study, which was used by the EA to evaluate the humaneness of the proposed action.
[36] Carthage Veterinary Services, *supra* note 23.
[37] *Id.*
[38] Pipestone Veterinary Services, *supra* note 23.
[39] *Id.* at 7.
[40] American Veterinary Medical Association, AVMA Guidelines for the Euthanasia of Animals: 2020 Edition https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf.
[41] Pipestone Veterinary Services, *supra* note 18 at 3 ("The observed distress supports the reservation of sodium nitrite for constrained situations of depopulation.").

The Supplement refers to 400 mg/kg of body weight as the lethal dose of sodium nitrite in feral swine.[42] This number appears in numerous publications and appears to have been derived from a 2008 study by Cowled in which sodium nitrite was "concentrated with honey in the centre of a PIGOUT bait," and all feral pigs consuming more than 400 mg/kg died in under three hours.[43] This study states that the number of pigs used in the trial was insufficient to determine acute toxicity values and that its estimate of LD_{50} and LD_{95} values of 320 mg/kg and 475 mg/kg respectively "may have limited reference for appropriate doses for wild feral pigs."[44]

In contrast, the Carthage study obtained only a 66% mortality rate at an average dosage rate of 490 mg/kg.[45] Similarly, the Pipestone study suggested that at approximately 400 mg/kg (181 mg/lb), domestic hogs only exhibited 60% mortality.[46] In this study, a 90% mortality was reached only at doses of 543-600 mg/lb, or 1,197-1,323 mg/kg. These higher dosages are similar to what was ingested by feral pigs (1,600 mg/kg) of 10% HOGGONE in the 2017 study indicating 95% mortality over the course of two nights.[47] Together, these studies suggest that, when mixed in bait rather than honey, higher doses are required to achieve similar levels of mortality.

A major difference of the new formulation of HOGGONE is its concentration of 5% rather than 10%. While this change is intended to decrease risk to non-target species, it also potentially decreases the amount of sodium nitrite ingested by feral pigs; they would need to eat twice as much of the bait in order to ingest the same amount of sodium nitrite. The Pipestone study showed that time to death was quicker the higher the dosage, but "the frequency of behavior associated with animal discomfort (vocalization, vomiting, retching, head shaking, and convulsions) was not different" at different dosages.[48] This raises the concern that, with the 5% concentration of HOGGONE, many pigs will ingest lower doses that result in slower onset of death, sublethal poisoning, and more prolonged episodes of dyspnea, vomiting, and other forms of discomfort.

While a study performed with the 5% sodium nitrite bait reported an efficacy of removing wild pigs of 76.3-90.4%, this number was inferred via noting reductions in visitation to toxic baiting sites.[49] Other authors have noted that "observation of other group members" becoming ill after sodium nitrite ingestion may lead to learned aversion,[50] which could complicate interpretation of the decreased rate of visitation. Furthermore, the study with the 5% bait had no way of assessing time to death or duration of dyspnea, vomiting, or other symptoms of discomfort.

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[42] Supplement at 27.
[43] Cowled, B.D., P. Elsworth, and S.J. Lapidge. 2008. Additional toxins for feral pig (Sus scrofa) control: Identifying and testing Achilles' heels. *Wildlife Research*. 35:651–662.
[44] *Id.*
[45] Carthage Veterinary Services, *supra* note 23.
[46] Pipestone Veterinary Services, *supra* note 23.
[47] Snow, N.P.; Foster, J.A.; Kinsey, J.C.; Humphrys, S.T.; Staples, L.D.; Hewitt, D.G.; Vercauteren, K.C. 2017. Development of toxic bait to control invasive wild pigs and reduce damage. *Wildl. Soc. Bull*. 41, 256–263.
[48] Pipestone Veterinary Services, *supra* note 23.
[49] Snow, N. P., J.D. Wishart, J.A. Foster, L.D. Staples, and K.C. VerCauteren, *supra* note 22 at 1616–25.
[50] Foster, Justin A.; Martin, James C.; VerCauteren, Kurt . C.; Phillips, Greg E.; and Eisemann, John D. 2014. Optimization of Formulations for the Lethal Control of Feral Pigs. USDA National Wildlife Research Center - Staff Publications. 1772. https://digitalcommons.unl.edu/icwdm_usdanwrc/1772.

Conclusion

The Supplement fails to take the requisite "hard look" at 1) the "Effects on Non-Target and T&E Species," 2) "Humaneness/Ethics," and 3) the efficacy of the proposed use of HOGGONE sodium nitrite toxicant bait for feral swine. Scientific research not reviewed—or not adequately reviewed—by the Supplement raises serious questions about the impact of the proposed action on the human environment, as well as the ability of the proposed action to meet its stated objective. Wildlife Services must meet the standards of NEPA by providing its constituents with the necessary information to show the program has taken a hard look at the environmental impacts of its actions. The Supplement is deficient in this regard.

Thank you for consideration of our comments. Please do not hesitate to contact me by phone at (202) 446-2146 or by email at dena@awionline.org if you have any questions.

Respectfully submitted,

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RESEARCH REPORT

ANIMAL WELFARE

Title: Evaluation of Sodium Nitrite for mass euthanasia of commercial pigs – **NPB #20-118**

Investigator: Aaron Lower, DVM

Institution: Carthage Veterinary Service, Ltd.

Co-Investigators: Youngsoo Lee, Ph.D., Beau Peterson, Ph.D, Gustavo Silva, Ph.D
Joseph Connor, MS, DVM

Date Received: November 4, 2020

Industry Summary:
It is critical to develop feasible mass euthanasia technology that is humane, economical, safe, and less labor intensive. Microencapsulated sodium nitrite (meSN) feral swine bait has been developed and marketed by Animal Control Technologies Australia (ACTA) as a feed-based toxicant for feral swine control. This technology has not been applied to commercial swine in confinement barns, just feral swine. Sodium nitrite is a chemical commonly used in low concentrations as a preservative in processed meats. If consumed at high doses, sodium nitrite can convert hemoglobin to methemoglobin that is unable to transport oxygen in the blood. The reduced oxygen carrying capacity of the blood depletes the brain and tissues of oxygen, causing unconsciousness and death. Previous pilot trials showed that SN can effectively euthanize swine, however, has been difficult to achieve reliable ingestion when SN is added to commercial feed due to palatability issues.

110 early nursery weight pigs (6.2 kg average weight) were randomly allotted to one of the 11 treatments (one treatment per pen, 10 pigs per pen). Granular form SN (un-encapsulated) salt and microencapsulated SN were the two sources of SN used in the feeds. There were three different feed flavors or taste suppressor combinations. Dried molasses at 4.4% inclusion was the first feed flavor. Vanilla at 0.1% inclusion was utilized as the second flavor. A bitterness taste suppressor at 0.2% inclusion was utilized as the third treatment. The dosage of SN was targeted at 20 grams of SN/100kg of pig at 2 lbs offered per pig. The resulting concentration was 2.2% SN per kg of feed. The dosage of SN in the water was targeted for an intake of 20g of SN per pig with an intake estimation of 0.25 gallons per pig.

1	Feed
2	Feed + SN
3	Feed + SN + Molasses
4	Feed + SN + Taste suppressor
5	Feed + SN + Flavor
6	Feed + meSN
7	Feed + meSN + Molasses
8	Feed + meSN + Taste suppressor
9	Feed + meSN + Flavor
10	Water
11	Water + SN

Feed was removed 24 hours prior to offering treatments. The water treatment pens had water removed also 24 hours to trial start. Pigs were offered their respective treatments for 3 hours of ab libitum consumption.

Of the feed treatment groups, 53 of 80 pigs (66%) were euthanized by SN. Each feed treatment resulted in a range of 50-80% mortality regardless of treatment. 63% of the pigs vomited during the feed treatment. The average time to death was 2 hours and 12 minutes. The earliest pig died at 1 hour and 13 minutes and latest pig at 3 hours. The water treatment group (Group 11) failed to induce clinical signs or mortality. Average feed consumption was 0.14 kg/pig. Pigs on averaged consumed 0.49 g of SN per kg of body weight with a range of 0.20 to 1.09 g of SN per kg.

General timeline of clinical signs:

- 0-45 minutes: good feed intake
- 45-75 minutes: stop eating and huddle
- 75 minutes: start to observe vomiting. Progresses to ataxia and palor and then lateral recumbency.
- 90 minutes: earliest mortality. Most were 90-180 minutes.

All feed formulations performed similarly, with only a 30% mortality rate range between treatments. This method of euthanasia is promising for use in constrained circumstances for depopulation, however, it did not achieve 100% mortality in this trial. It is imperative that pigs receive a bolus of SN for a lethal amount. Increasing the inclusion rate may increase the mortality rate but at the risk of pigs being averse to consume the product. It would also be recommended that pigs have uncompetitive access to consume feed when SN feed is administered.

There were minor differences in intake and mortality between the following groups (meSN versus SN, molasses versus vanilla versus taste blocker groups, unflavored meSN and SN versus flavored and taste blocker). The flavorings and taste blockers did not improve intake and mortality in comparison to feed with meSN and SN by itself.

Pigs were averse to consuming water with SN solubilized. There was minimal intake and no clinical signs.

Sodium nitrite is a viable option for mass depopulation in constrained circumstances. It euthanized between 50-80% of the pigs when offered to commercial swine in this study. Further development is needed to increase the euthanasia rate of pigs, speed up time to death, and decrease the percentage of pigs that vomit.

For further questions or information on this study, please contact:
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Key Findings:

- Sodium nitrite euthanized 50-80% of the pigs per treatment regardless SN formulation used (microencapsulated SN or free form SN) or feed treatment (molasses flavor, vanilla flavor, bitterness taste blocker)
- Administration of sodium nitrite through the water did not achieve sufficient intake to induce mortality

Keywords: Euthanasia, depopulation, sodium nitrite, methemoglobinemia, feed

Scientific Abstract:
It is critical to develop feasible mass euthanasia technology that is humane, economical, safe, and less labor intensive. Microencapsulated sodium nitrite (meSN) feral swine bait has been developed and marketed by Animal Control Technologies Australia (ACTA) as a feed-based toxicant for feral swine control. This technology has not been applied to commercial swine in confinement barns, just feral swine.

110 early nursery weight pigs (6.2 kg average weight) were randomly allotted to one of the 11 treatments. Granular form SN (un-encapsulated) salt and microencapsulated SN were included at a dosage of SN targeted at 20 grams of SN/100kg of pig at 2 lbs offered per pig. The resulting concentration was 2.2% SN per kg of feed. Three different feed flavors or taste suppressor combinations were utilized including dried molasses at 4.4% inclusion, vanilla at 0.1% inclusion, and a bitterness taste suppressor at 0.2% inclusion. The dosage of SN in the water was targeted for an intake of 20g of SN per pig with an intake estimation of 0.25 gallons per pig.

Of the feed treatment groups, 53 of 80 pigs (66%) were euthanized by SN. Each feed treatment resulted in a range of 50-80% mortality regardless of treatment. 63% of the pigs vomited during the feed treatment. The average time to death was 2 hours and 12 minutes. The earliest pig died at 1 hour and 13 minutes and latest pig at 3 hours. Average feed consumption was 0.14 kg/pig. Pigs on averaged consumed 0.49 g of SN per kg of body weight with a range of 0.20 to 1.09 g of SN per kg.

All feed formulations performed similarly, with only a 30% mortality rate range between treatments. The flavorings and taste blockers did not improve intake and mortality in

comparison to feed with meSN and SN by itself. Pigs were averse to consuming water with SN solubilized.

Sodium nitrite is a viable option for mass depopulation in constrained circumstances. It euthanized between 50-80% of the pigs when offered to commercial swine in this study. Further development is needed to increase the euthanasia rate of pigs, speed up time to death, and decrease the percentage of pigs that vomit.

Introduction:

There is an urgent need for mass euthanasia technology in the US swine industry. In the spring of 2020, COVID19 disrupted pig production flows resulting in growing pig space shortages. Additionally, foreign animal diseases like African Swine Fever, if introduced domestically, will similarly disrupt market channels or require depopulation of infected premises. It is critical to develop feasible mass euthanasia technology that is humane, economical, safe, and less labor intensive. Microencapsulated sodium nitrite (meSN) feral swine bait has been developed and marketed by Animal Control Technologies Australia (ACTA) as a feed-based toxicant for feral swine control. This technology has not been applied to commercial swine in confinement barns, just feral swine. Sodium nitrite is a chemical commonly used in low concentrations as a preservative in processed meats. If consumed at high doses, sodium nitrite can convert hemoglobin to methemoglobin that is unable to transport oxygen in the blood. The reduced oxygen carrying capacity of the blood depletes the brain and tissues of oxygen, causing unconsciousness and death. Pigs are highly susceptible to methemoglobinemia as they have low levels of methemoglobin reductase (Smith et al., 1966), an enzyme that can reverse methemoglobin formation.

SN has several attractive features as a euthanasia method in depopulation strategies. The AVMA has previously approved SN as a mass euthanasia technique in constrained circumstances (AVMA Guidelines for the Depopulation of Animals: 2019 Edition). SN breaks down quickly, making it environmentally safe (USDA, 2017). There are low carcass residues limiting the risk to scavengers and allowing multiple carcass disposal options (Snow et al., 2018).

Little research has been conducted utilizing SN as a euthanasia method for domestic swine. The researchers involved in this study have completed in 2018, multiple pilot studies with commercial swine (unpublished). The results of those pilot trials showed that SN can effectively euthanize swine, however, has been difficult to achieve reliable ingestion when SN is added to commercial feed due to palatability issues. Flavoring or taste blocking agents may improve the ingestion by commercial swine when SN is added, resulting in predictable intake and death.

The aim is to identify the hog feed blend(s) including SN to encourage sufficient voluntary ingestion by commercial swine for a humane euthanasia technology.

Objectives:

1. Evaluate consumption and mortality of pigs consuming meSN (micro-encapsulated sodium nitrite) versus non-encapsulated SN without and with the addition of flavoring and bitterness taste blockers.

2. Evaluate the mortality of pigs consuming non-encapsulated SN through the water.

Materials & Methods

Animals:

A total of 110 early nursery weight pigs (6.2 kg average weight) were sourced and housed at the Carthage Innovative Swine Solutions, Veterinary Research Farm. Pigs were randomly allotted to one of the 11 treatments listed above in Tables 1 and 2 (one treatment per pen, 10 pigs per pen).

Materials:

A commercial feed composed of corn and soy was used as a base feed. Granular form SN (un-encapsulated) salt (Duda Energy, 99.9% pure food grade SN) and microencapsulated SN (Animal Control Technologies (Australia) Pty Ltd 46-50 Freight Drive, Somerton, VIC, 3062, Australia +61 3 9308 9688) were the two sources of SN used in the feeds.

There were three different feed flavors or taste suppressor combinations. Dried molasses at 4.4% inclusion was the first feed flavor. Vanilla at 0.1% inclusion (Lucta, Luctarom 32619Z) was utilized as the second flavor. A bitterness taste suppressor (Lucta, Luctarom Bitteroff "S" 5413Z) at 0.2% inclusion was utilized as the third treatment. The vanilla flavor and taste suppressor inclusion rate were added per the recommended by the manufacturer (Lucta). The molasses inclusion rate was based on recommendation from Dr. Youngsoo Lee, University of Illinois, Dept of Food Science and Human Nutrition, and targeted to be two times the inclusion rate of SN.

The dosage of SN was targeted at 20 grams of SN/100kg of pig at 2 lbs offered per pig. The resulting concentration as 2.2% SN per kg of feed. The sample preparation was completed at Integrated Bioprocessing Research Laboratory (IBRL, https://ibrl.aces.illinois.edu/) pilot plant at the University of Illinois, Urbana-Champaign.

Table 1:

In Feed — Weight in *grams* for 10 pigs

	Sample	Sample codes	Feed	SN	meSN	Molasses	Taste Supressor	Flavor	%SN
1	Feed	Con	9091						
2	Feed + SN	SN	8891	200					2.2%
3	Feed + SN + Molasses	SN+ML	8491	200		400			2.2%
4	Feed + SN + Taste suppressor	SN+TS	8891	200			18.2		2.2%
5	Feed + SN + Flavor	SN+FL	8891	200				9.1	2.2%
6	Feed + meSN	meSN	8871		220				2.2%
7	Feed + meSN + Molasses	meSN+ML	8471		220	400			2.2%
8	Feed + meSN + Taste suppressor	meSN+TS	8871		220		18.2		2.2%
9	Feed + meSN + Flavor	meSN+FL	8871		220			9.1	2.2%
		Total	79338	800	880	800	36	18	

Number of pigs		10
	lb/pig	g/pig
Feed intake	2	909.1
	lb	g
Base feed	20	9091
		g SN/pig
SN dose		20
		g meSN/pig
meSN dose		22
Molasses (82 Brix)	x2 of SN	
		% of feed
Taste supressor		0.2
		% of feed
Flavor		0.1

90% SN in meSN

*SN – free sodium nitrite; meSN – microencapsulated sodium nitrite; Taste suppressor – taste suppressor from a commercial partner; Flavor – vanilla flavored feed additive

The dosage of SN in the water was targeted for an intake of 20g of SN per pig with an intake estimation of 0.25 gallons per pig.

Table 2:

	In Drinking Water		For 10 pigs		
	Sample	Sample codes	Water (gal)	SN (g)	Water intake per pig estimation
10	Water	W Con	2.5		0.25 gal/pig
11	Water + SN	WSN	2.5	200	

This concentration is base on 20g SN/pig.
The test done by Swine Vet Center, P.A. used 1800g SN/ gal water with either 24 or 72 hours of offered water.
Soluble up to 84g SN/100g water (3000 g SN/1 gal water) at 25 C (77 F).

Administration:
Nursery feed and water were offered to all pens on a continuous basis for 48 hours to allow for acclimation. At 24 hours prior to offering treatment diets and water, feeders were emptied for all groups. The feed treatment groups were offered water, the water treatment pens had water removed. At the start of the trial, pigs were offered their respective treatments for 3 hours of ab libitum consumption. 20 lbs of feed were offered per treatment in a two hole feeder with 14'' wide holes. Water was offered through 1 cup per pen.

Measurements:
Pigs were individually numbered and weighed in each treatment. Treatments were monitored for feed intake at a group level, death, time to death, and vomiting

Results:
Control pigs (Group 1 – feed control and Group 10 – water control) did not show any clinical signs or mortality during the treatment.

Of the feed treatment groups, 53 of 80 pigs (66%) were euthanized by SN. Each feed treatment resulted in a range of 50-80% mortality regardless of treatment. 63% of the pigs vomited during the feed treatment. The average time to death was 2 hours and 12 minutes. The earliest pig died at 1 hour and 13 minutes and latest pig at 3 hours.

The water treatment group (Group 11) failed to induce clinical signs or mortality.

Table 3:

Treatment	Sample	Vomit	Mortality	Feed Consumed per pig (kg)	SN consumed per pig (g)	SN/kg	Average Time to Death	Range
1	Feed	0%	0%	Invalid Data				
2	Feed + SN	70%	60%	0.21	4.57	0.76	1:57	1:37 - 2:39
3	Feed + SN + Molasses	70%	60%	0.06	1.26	0.20	2:08	1:38 - 2:18
4	Feed + SN + Taste suppressor	70%	80%	0.31	6.77	1.09	2:01	1:13 - 2:49
5	Feed + SN + Flavor	60%	60%	Invalid Data			2:11	1:53 - 2:51
6	Feed + meSN	40%	60%	0.11	2.36	0.37	2:32	1:50 - 3:00
7	Feed + meSN + Molasses	70%	50%	0.06	1.26	0.21	1:59	1:40 - 2:25
8	Feed + meSN + Taste suppressor	60%	80%	0.11	2.36	0.36	2:19	1:40 - 3:00
9	Feed + meSN + Flavor	60%	80%	0.11	2.36	0.40	2:27	2:01 - 2:48
10	Water	0%	0%					
11	Water + SN	0%	0%					

*SN – free sodium nitrite; meSN – microencapsulated sodium nitrite; Taste suppressor – taste suppressor from a commercial partner; Flavor – vanilla flavored feed additive

Treatment 1 (Control) did not have feed offered during the trial period, so no consumption data is available. Treatment 5 (Feed + SN + Flavor) had invalid data from the feeder weights taken to determine pen consumption. Average feed consumption was 0.14 kg/pig. Pigs on averaged consumed 0.49 g of SN per kg of body weight with a range of 0.20 to 1.09 g of SN per kg.

Discussion:

The timeline of clinical signs is predictable with ab libitum feed intake of SN in commercial swine.

Timeline:

- 0-45 minutes: good feed intake
- 45-75 minutes: stop eating and huddle
- 75 minutes: start to observe vomiting. Progresses to ataxia and palor and then lateral recumbency.
- 90 minutes: earliest mortality. Most were 90-180 minutes.

In previous pilot projects, intake of meSN was low and inconsistent resulting in a mortality rate of less than 10%. The feed treatments of flavoring and taste blocking were to improve consumption of SN. Feed intake during this trial was much improved for all treatments, even those with just SN added to the feeds. All feed formulations performed similarly, with only a 30% mortality rate range between treatments. The rate of vomiting and retching is high (63%). An antiemetic, in combination with SN, should be evaluated.

This method of euthanasia is promising for use in constrained circumstances for depopulation, however, it did not achieve 100% mortality in this trial. It is imperative that pigs receive a bolus of SN for a lethal amount. When pigs begin to experience clinical signs, there will be no further consumption of SN feed. Dosing in this study was at 2.2% inclusion rate. In an effort to

overcome the intake concerns in previous work, we used a lower inclusion rate. The feral swine product has used formulations of 10% and moved to 5% inclusion rate. Increasing the inclusion rate may increase the mortality rate but at the risk of pigs being averse to consume the product. Work needs to be done to determine correct dose titration. Current information is based on group intake and weights to determine dosage. Additionally, increasing the feeder space will also have a positive impact on the bolus of SN consumed. In this trial, there were 5 pigs per feed space hole (14''). Effectively, there were 2 pigs eating out of the feeder space at a time. It would be recommended that pigs have uncompetitive access to consume feed when SN feed is administered.

There were minor differences in intake and mortality between the following groups:

- meSN versus SN
- Molasses versus vanilla versus taste blocker groups
- Unflavored meSN and SN versus flavored and taste blocker

It is interesting that there was not an appreciable difference in mortality rate between any of these groups. The flavorings and taste blockers did not improve intake and mortality in comparison to feed with meSN and SN by itself.

Pigs were averse to consuming water with SN solubilized. There was minimal intake and no clinical signs.

Sodium nitrite is a viable option for mass depopulation in constrained circumstances. It euthanized between 50-80% of the pigs when offered to commercial swine in this study. Further development is needed to increase the euthanasia rate of pigs, speed up time to death, and decrease the percentage of pigs that vomit.

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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**

Investigator: Brent Pepin

Institution: Pipestone Veterinary Services

Date Submitted 8/21/2020

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.

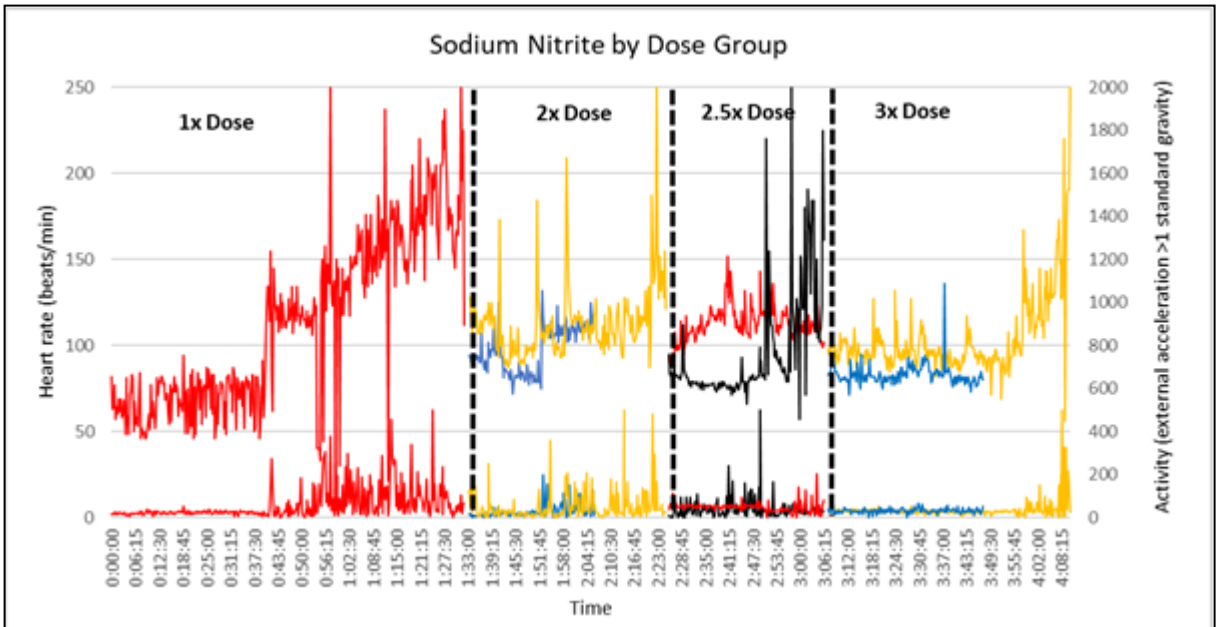

Figure 1: Heart rate (bpm) matched with general activity of the implanted pigs in each dose group over time. Measurements were taken every 15 seconds. One of the two implanted pigs in 1x group was euthanized by captive bolt and not included on the figure.

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.

For further questions or information on this study, please contact:
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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

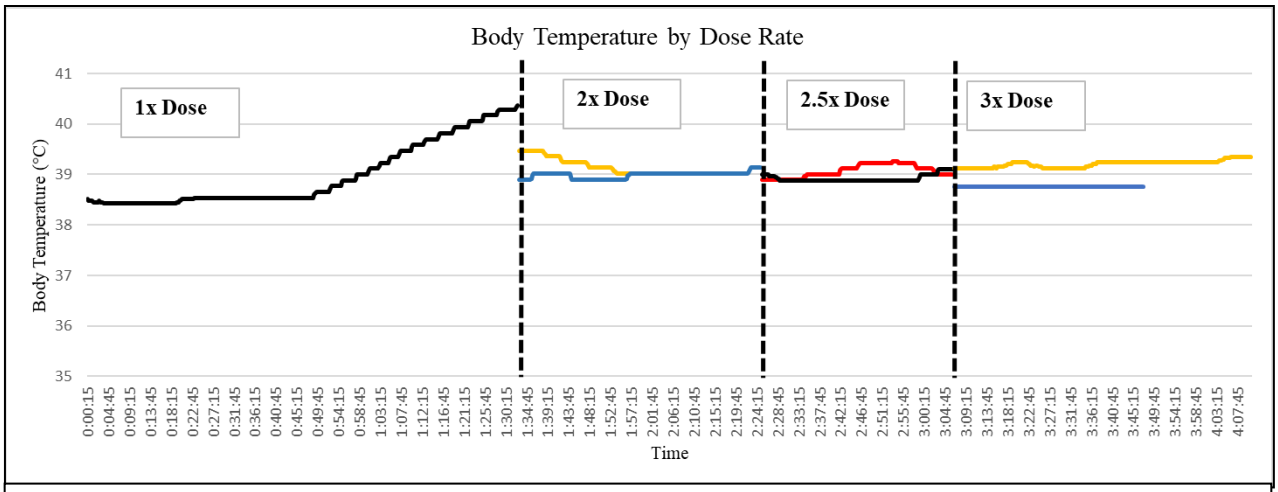

Figure 2: Body temperature (°C) of the implanted pigs in each dose group over time. Measurements were taken every 15 seconds. One of the two implanted pigs in 1x group was euthanized by captive bolt and not included on the figure.

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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