2022 SUPPLEMENT TO THE ENVIRONMENTAL ASSESSMENT:

Field Evaluation of HOGGONE® Sodium Nitrite Toxicant Bait for Feral Swine

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1.0 NEED FOR ACTION AND SCOPE OF ANALYSIS

1.1 Introduction

In 2017, the United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS), Wildlife Services (WS) prepared an Environmental Assessment (EA) titled Field Evaluation of HOGGONE® Sodium Nitrite Toxicant Bait for Feral Swine (USDA 2017). The EA analyzed the potential effects of conducting field trials of the sodium nitrite toxicant, HOGGONE® in Texas and Alabama on free-ranging feral swine. Based on the EA (USDA 2017), WS issued a Finding of No Significant Impact (FONSI) and Decision to conduct the study. The first phase of the trial was conducted in Texas from January-March 2018. WS deployed 14 bait stations in northcentral Texas. The Texas trial resulted in taking 109 feral swine and based on GPS transmitter information from 38 feral swine, the toxicant baiting resulted in approximately 66% overall lethality in 1-2 nights.

The baiting strategy was effective as a toxicant for free-ranging feral swine, however, during the trial, WS researchers discovered that feral swine were spilling or dropping more bait than observed in pen trials. This bait spillage caused the non-target take of several passerine birds, raccoons, turkeys and crows. Although this non-target take was adequately analyzed in the EA, it was considered a worst-case scenario and the non-target take was higher than WS was willing to accept. With remote cameras positioned at the bait stations, WS could observe and believe the primary reason for increased spilling and dropping of the bait was a palatability issue with feral swine. The trial was postponed until these issues could be resolved.

The palatability issue during the 2018 field trial in Texas led to modifications to the bait station, the formulation of the bait, and the baiting strategy. Specifically, WS revised the bait station to accept small, compacted trays of the SN toxic bait to limit the feral swine's ability to scoop the bait onto the ground. Secondly, the bait was reformulated to reduce the risks to non-target species. The original bait included 10% SN w/w that was microencapsulated and mixed into a matrix of peanut paste bait with crushed grains (Snow et al. 2016, Snow et al. 2017a). The reformulation, called HOGGONE® 2 included: 1) increasing the microencapsulation coating around the SN, 2) decreasing the SN concentration by 50% (i.e., to 4.965% SN w/w) to minimize the amount of SN deployed, and 3) using more finely milled grains to reduce the attractiveness to small granivorous birds.

WS also revised the baiting strategy to reduce the attractiveness of the bait sites to non-target animals. The amount of pre-baiting time with freely available whole-kernel corn (e.g. outside the bait stations) was decreased to 10 days from 14-16 days. Bait stations were also placed ~10–30 m away from the original pre-baiting sites where whole-kernel corn was used to draw feral swine to the area. Installing bait stations ~10-30 m away would keep granivorous birds attracted to remnant particles of whole kernel corn away from bait stations. Lastly, WS incorporated a deterrent device (Scare Dancer® Snake 6ft Cordless Inflatable Scarecrow: https://scare-dancer.com/collections/inflatable-scarecrows/products/snake-6-ft-cordless-inflatable-scarecrow) that is operated the morning following toxic bait deployment to scare non-targets away until an operator can arrive at the bait site and remove any spilled HOGGONE 2. These changes were implemented and analyzed in several small-scale trials in 2019 and 2020 to determine their effectiveness and environmental effects (USDA 2019a).

In July and August of 2021, a full-scale replication of the 2018 field trial was conducted in Texas and Alabama that incorporated all the changes and improvements made to the product and the baiting strategies evaluated in 2019 and 2020. This study replication along with any potential environmental effects, were analyzed in a Supplemental EA (USDA 2021).

Table 1. Timeline of NEPA documents and field trials.

Year	Month	Event	Description
2017	November	EA	Field Evaluation of HOGGONE® Sodium Nitrite Toxicant Bait for Feral Swine
2018	Jan/-March	Field Trial	Texas full-scale field trial (Winter)
2018	Dec	Field Trial	Queensland, Australia (Summer) small-scale field trial evaluating new bait formulation
2019	July	EA*	A Small Scale Field Evaluation of HOGGONE® 2 Sodium Nitrite Toxicant Bait for Feral Swine
2019	Aug	Field Trials	AL (Summer) Small-scale trial evaluating new bait formulation
2020	March	Field Trials	TX (Winter) Small-scale trial evaluating new bait formulation
2020	July	Field Trial	TX (Summer) Small-scale trial evaluating scare devices
2021	May	EA-Supplement	Supplement to 2017 EA to conduct 2021 field trials in TX/AL
2021	July	Field Trial	Texas full-scale field trial (Summer)
2021	August	Field Trial	Alabama full-scale field trial (Summer)
2022	TBD	EA-Supplement	Supplement to 2017 EA to conduct 2022 field trial in TX
2023	Jan/-March	Field Trial	Texas full-scale field trial (Winter)

* Independent EA to analyze the small-scale trials that were conducted in 2019 and 2020 to test the effectiveness of the changes made to the product and their effect on the human environment.

1.2 Need for Action

The purpose and the need for action in the EA will remain as addressed in Section 1.2 of the EA (USDA 2017). The 2018 trial took place in February/March. The most recent trial in Texas incorporated all changes and improvements developed in 2019 - 2020 and was conducted in July 2021 resulting in zero known non-target take. However, the potential risk for migratory birds was low during this timeframe. This trial will replicate the July 2021 trial but will take place in February/March.

The purpose and need for this Supplement is to analyze a trial replication in Texas to demonstrate the effectiveness of the deterrent devices for non-target animals during the same time period as the 2018 trial (February/March) when potential risk for exposure to migratory birds is highest and therefore more accurately replicating the 2018 trial. This Supplement will analyze and incorporate new information that has become available from research findings and data gathering since the issuance of the Decision and FONSI in 2017.

1.3 Objectives

The objectives of the study remain consistent and are described in Section 1.4 of the EA (USDA 2017). Additional objectives are focused on the effects of non-target birds and reducing their take after research findings and data gathering since the issuance of the Decision and FONSI in 2017. These additional findings are discussed and analyzed in this supplement.

1.4 Scope of Analysis / Site-Specificity

The EA analyzed a field study in two study sites in Texas and Alabama. The analysis evaluated the effects on humans and pets, terrestrial and aquatic environments, non-target and threatened and endangered species, and humaneness and ethics. This Supplement will only analyze a follow up trial in Texas, not Alabama. Due to fewer feral swine in the past study areas, it was necessary to expand the potential test sites to some additional adjoining counties in Texas while remaining within the same ecoregion discussed in (USDA 2017). The following counties have been added to the original (USDA 2017) list in Section 1.5.1 of site specific counties where the trial could occur: Childress, Dickens, Throckmorton, Haskell, King, Jones, and Stonewall. The site-specific criteria in (USDA 2017) Section 1.5.1 also remains valid for this supplement. The trial will likely only occur in one or two counties depending on the location and criteria discussed in Section 1.5.1. Unless otherwise discussed in this supplement, the scope of the analysis remains valid as addressed in the EA USDA (2017).

1.5 Relationships of Agencies During Preparation of this EA Supplement

Based on agency relationships, Memorandums of Understanding (MOU's), and legislative authorities, WS was the lead agency during the development of the EA and the supplement to the EA. WS was also responsible for the scope, content and decisions made. These relationships remain valid as addressed in Section 1.6 of the EA USDA (2017).

1.6 Documents Related to this EA Supplement

Documents identified and related to the EA in section 1.7 remain relevant for this supplement. In addition to the documents listed in the EA, new research and analysis has been conducted since the issuance of the Decision and FONSI in 2017 and is included in this supplement.

Field Evaluation of HOGGONE® Sodium Nitrite Toxicant Bait for Feral Swine: WS prepared an EA analyzing the potential effects of conducting field trials of the sodium nitrite toxicant, HOGGONE® in Texas and Alabama on free-ranging feral swine. A Decision and FONSI was signed November 20, 2017.

Supplement to the Environmental Assessment: Field Evaluation of HOGGONE Sodium Nitrite Toxicant Bait for Feral Swine: The WS program prepared a Supplement to USDA 2017 incorporating changes made to the product during small-scale trials that were conducted in 2019 and 2020. This Supplement was designed to be a full-scale replication of the 2018 trial and took place in Texas and Alabama in July/August 2021 (USDA 2021).

Study protocol – Product performance: field evaluation of HOGGONE® 2 for feral swine in winter/spring: WS-NWRC has prepared a detailed study protocol. This supplement has incorporated all relevant information from this protocol as it relates to any potential environmental effects on the human environment (USDA 2022).

1.7 Public Involvement

Public involvement is described in Section 1.8 of the EA and the FONSI (USDA 2017). This supplement will be made available for public review and comment through the publication of a legal notice announcing a minimum of a 30-day comment period. The legal notice will be published in the *The Austin Statesman* in Texas and sent to interested parties via the APHIS stakeholder registry, and posted on the APHIS website at http://www.aphis.usda.gov/wildlife_damage/nepa.shtml. This supplement and EA/FONSI/decision (USDA 2017) will be posted on http://www.regulations.gov Comments received on the supplement by the comment closing date will be fully considered for new substantive issues and alternatives before WS issues a decision. The public will be notified of WS decision in the same manner as the supplement to the EA.

1.8 Laws Related to this Discussion

Laws related to this discussion were identified and related to the 2017 EA in section 1.10 remain relevant for this Supplement. In addition to the laws and Executive Orders (EO) listed in the EA, new EOs and rule changes have taken effect since the issuance of the Decision and FONSI in 2017 and are included in this Supplement.

National Environmental Policy Act (NEPA) (42 U.S.C. 4321 et seq.). All federal actions are subject to NEPA (42 U.S.C. §§ 4321 et seq.). WS-NWRC follows CEQ regulations implementing NEPA (40 CFR 1500 et seq.) and USDA (7 CFR 1b) and APHIS implementing regulation (7 CFR 372) as part of the decision-making process. These laws and regulations generally outline five broad types of activities to be accomplished as part of any project: public involvement, analysis, documentation, implementation, and monitoring. NEPA also sets forth the requirement that all major federal actions be evaluated in terms of their potential to significantly affect the quality of the human environment for the purpose of avoiding or, where possible, mitigating and minimizing adverse impacts.

WS developed this EA (USDA 2017) under the 1978 NEPA regulations and existing APHIS NEPA implementing procedures. In July 2020, CEQ updated its NEPA regulations. These revised regulations went into effect on September 14, 2020. However, CEQ, in 2022 revised elements of these regulations ultimately restoring some provisions of the 1978 NEPA regulations during a Phase 1 Final Rule issued April 20, 2022. Phase 1, 40 CFR – 1508 revisions went into effect May 20, 2022. Therefore, this Supplement was prepared under provisions and elements of the 2020 CEQ rules while also incorporating the recent 2022 Phase 1 CEQ rule changes.

Executive Order on Advancing Racial Equity and Support for Underserved Communities Through the Federal Government. Executive Order (EO) 13985 promotes the fair treatment of people of all races, income levels, and cultures with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies. Advancing Racial Equity is the pursuit of equal justice and protection under the law for all environmental statutes and regulations without discrimination based on race, ethnicity, or socioeconomic status. Executive Order 13985 requires federal agencies to make Advancing Racial Equity part of their mission, and to identify and address disproportionately high and adverse human health and environmental effects of federal programs, policies, and activities on minorities and low-income persons or populations. WS evaluates all activities for their impact on the human environment and compliance with Executive Order 13985. WS would only use legal, effective, and environmentally safe methods, tools, and approaches for this trial. Work will be conducted exclusively on private land with landowner permission and monitored daily. WS does not anticipate that the proposed action would result in any adverse or disproportionate environmental impacts to underserved communities including minorities and persons or populations of low income.

Executive Order on Protecting Public Health and the Environment and Restoring Science to Tackle the Climate Crisis (EO 13990), and Executive Order on Tackling the Climate Crisis at Home and Abroad (EO) 14008: The WS program activities that may result from the alternatives would have a negligible effect on atmospheric conditions, including the global climate. The project is limited in scope and employs routine vehicle/ATV use and would meet requirements of applicable federal laws and regulations. No significant direct or indirect emissions of greenhouse gases would occur as a result of the proposed action.

2.0 ISSUES AND ALTERNATIVES

The issues analyzed in detail and the alternatives identified during the development of the EA are discussed in Chapter 2 of the EA (USDA 2017). The following issues were identified in the EA and remain relevant to this Supplement:

<u>Effects on Human Health and Pet Safety</u>

<u>Impacts on Terrestrial and Aquatic Environments</u>

<u>Effects on Non-Target and T&E Species</u>

<u>Humaneness / Ethics</u>

Other issues were identified in the EA but were not discussed in detail and the EA provided the rationale for doing so. Those issues are in Section 2.1.2 in the EA (USDA 2017) and remain relevant for this supplement.

2.1 Study Protocol and Product Description

The basic study protocol identified in Section 2.2 of the EA remains valid for this supplement, however, several changes were made to the product/protocol based on results from the original 2018 trial and from several small-scale trials in 2019 and 2020. These changes were implemented into the full scale replication study that took place in 2021 (USDA 2021). The changes and results from the 2021 trial are identified and discussed in chapter 2 and their potential effects are analyzed in chapter 3 of this Supplement to the EA.

The initial trial in north-central TX identified a palatability problem with the original HOGGONE feral swine bait. Approximately 1 kg of bait was dropped or spilled by swine at each bait station. WS predicted and analyzed the potential effects of spilling .01-1kg of bait with 1 kg being the worst-case scenario and unacceptable. To reduce exposure and attractiveness to non-targets (particularly granivorous birds), the manufacturer has reformulated the bait (HOGGONE 2) and some slight changes were made to the bait stations to reduce spillage. In cooperation with WS-NWRC, the manufacturer, tested the reformulated HOGGONE 2 and the new presentation method in Queensland, Australia and found spilled bait was significantly reduced (averaged ~55g outside of the bait stations). Results from this test also showed that granivorous birds did not appear to be attracted to the spilled HOGGONE 2, and none were found dead. Subsequent small-scale trials conducted in the U.S. also showed a substantial improvement in palatability and a reduction in spillage and hence, a reduction in non-target take because of these changes.

Several changes have been made to the original HOGGONE described in Section 2.2.1 of the EA (USDA 2017). The biggest adjustment is the overall concentration of the active ingredient, sodium nitrite (SN), the original formula consisted of a 10% concentration of SN and the revised formula is comprised of a 5% concentration. The original formula bait matrix consisted of black-colored peanut paste with milled flour and crushed grains. HOGGONE 2 is the same matrix but has removed the crushed grains to reduce the attractiveness to granivorous birds. Another change in the effort to increase palatability and decrease spillage was to increase the micro-encapsulation coating over the SN. Sodium nitrite has a strong salty taste and the inert food-grade polymer (Connovation Ltd., Manukau, NZ) micro-encapsulation coating helps conceal the taste. It is designed to dissolve in the high pH environment of the stomachs of feral swine. This coating was doubled from 5% to 10%.

Differences between HOGGONE 2 and the original HOGGONE:

Bait Formulation	HOGGONE 2	HOGGONE	Rational for Change
Percent sodium nitrite	5%	10%	To reduce the hazard presented to non-target species from spilled bait
Micro-encapsulation coating	10%	5%	To better protect the SN and make the bait more palatable to pigs, thereby reducing bait rejection and spilling
Bait matrix	Peanut paste with milled grain flour	Peanut paste with milled grain flour and crushed grains	To reduce the attractiveness of spilled bait to granivorous birds

Reformulated HOGGONE 2 in tray | Original HOGGONE in bucket

2.2 Description of Study / Data Analysis

The 2021 trial field tested HOGGONE 2 using the modified bait stations and baiting strategy described above in 1.1 and 2.1 during the summer months (June−August). The trial focused on the summer months to avoid high abundances of migrating birds that were deemed susceptible to spilled HOGGONE 2. Given the success of these summer 2021 trials at reducing non-target mortality, this trail will now assess the efficacy of HOGGONE 2 and non-target risks in winter/spring in Texas in 2023 when migrating birds should be abundant.

The study description and the data analysis discussed in Section 2.2.2 and 2.2.3 of the EA (USDA 2017) remain valid except for a few minor changes described here in this Supplement. These minor changes were also identified in the USDA 2021 Supplement but are repeated here for consistency, and to recognize these changes will also be implemented into this trial..

A winter/spring trial is not planned to occur in Alabama. This Supplement only analyzes a winter/ spring trial to be conducted in Texas. Additionally, the handling of captured feral swine has been modified from USDA 2017. Section 2.2.2 described using (3.3 mg/kg body weight of Telazol® plus 1.6 mg/kg body weight of xylazine). The immobilizing drug for this revised trial will be Medatomadine-Midazolam-Butorphenol (MMB) at a target dosage of 0.06 mg/kg Medetomadine, 0.30 mg/kg Midazolam, and 0.30 mg/kg Butorphanol, or a premixed combination of Butorphanol-Azapaperone-Medatomadine (BAM) at a target dosage of 0.026 ml/kg.

Section 2.2.2 describes capturing and marking raccoons at each location to test the effects of non-target species. In a small-scale trial in 2019, two non-target opossum were taken; therefore this revised trial will include marking opossum and raccoon in the same manner and quantity as described in 2.2.2.

Another minor change not incorporated into the original protocol is to move the bait station a minimum of 10 meters away from the initial pre-bait location. The bait station move is to help prevent birds and other non-target wildlife that may have become habituated specifically to that site. There may be small bits of grain remaining on the ground that may continue to attract these animals.

The bait station holding capacity and baiting protocols are also described in Section 2.2.2. Revisions to the bait stations and baiting protocols were made and described here. The redesigned bait boxes (Appendix B) hold 3.75 kg of bait on each side for a total of 7.5 kg for each bait box compared to the original box, which held a total of 20 kg of bait. The original protocol called for two consecutive nights of toxic baiting and this revised protocol calls for just one night of toxic baiting and post-baiting with non-toxic pre-bait (2 nights) until consistent visitation to bait stations resumes and a second night of toxic baiting could take place (maximum of three nights of toxic baiting per bait site). In addition to these changes, WS will remove or bury any spilled particles of toxic bait when bait stations are checked each morning.

Lastly, WS incorporated a deterrent device (Scare Dancer® Snake 6ft Cordless Inflatable Scarecrow: https://scare-dancer.com/collections/inflatable-scarecrows/products/snake-6-ft-cordless-inflatable-scarecrow) that will be activated the morning after a toxic baiting to scare non-targets away until an operator can arrive at the bait site and remove any spilled HOGGONE 2. The device will be set to operate from 1 hour before sunrise until WS arrives to the bait site.

Section 2.2.3 described systematic transects that will be walked by personnel following a toxic baiting to locate feral swine carcasses and non-target animals. Transect grids will remain 400m x 400m, 10m apart as described, but an additional grid of 50m x 50m that are 5m apart will be incorporated to enhance the surveillance of non-targets. In addition, WS may incorporate the use of drones to look for carcasses or VHF signals from transmitters.

2.3 Alternatives Considered in Detail

The following alternatives were developed to meet the objectives for a field trial in USDA (2017) and remain valid for this Supplement except for the product change (HOGGONE to HOGGONE 2) and other changes noted in the study description.

Alternative 1 – No Action – No Study

The no action alternative is the status quo. Under this alternative, a research study on the effectiveness of HOGGONE 2 sodium nitrite bait to control feral swine would not be conducted. The No Action alternative is required for comparative evaluation in an EA.

Alternative 2 – Proposed Action – Conduct the Study

This alternative consists of conducting a study to determine the effectiveness and environmental effects of HOGGONE 2 as a toxicant bait for feral swine as described in Sections 2.2.1, 2.2.2, 2.2.3 of (USDA 2017) and 2.1 and 2.2 of this Supplement.

2.4 Alternatives and Strategies Not Considered in Detail

Despite changes to the product and study protocols, the alternatives and strategies not considered in detail in (USDA 2017) remain valid for this Supplement.

3.0 ENVIRONMENTAL EFFECTS

This chapter discusses the beneficial and adverse environmental impacts of the Proposed Action and the No Action Alternative on environmental, human health and safety and threatened and endangered species. Each section includes information on existing conditions of the resource and the expected consequences or impacts of the alternatives.

Chapter 3 of this Supplement will vary in two ways from the EA (USDA 2017). First, due to the change in the product's active ingredient concentration (10% concentration SN to 5% concentration SN), the overall amount of SN to be used, and other procedural changes in the study, data from subsequent field trials in 2021, a new analysis is warranted. However, much of the original analysis will remain relevant in this analysis, depending on the resource. Any changes to the original analysis will be properly cited in this Supplement.

3.1 Issues Considered in Detail and Their Associated Impacts.

The issues identified in chapter 2 of the EA (USDA 2017) remain relevant for each alternative. The EA analyzed the environmental consequences of the No Action Alternative for the identified issues and compared the impacts with the projected environmental impacts of the Proposed Action. The environmental baseline, or status quo of the No Action Alternative for the EA provided the necessary benchmark to determine if the real or potential impacts of the Proposed Action are greater, lesser or the same for each issue. This Supplement will consider the direct, indirect and cumulative impacts on the resources.

3.1.1 Effects on Human Health and Pet Safety

The EA (USDA 2017) analyzed the No Action and the Proposed Acton alternatives and their potential effects on human health and pet safety in Section 3.2.1. The analysis addressed three basic issues or types of potential human exposure from conducting the proposed action: exposure to the bait from employees, exposure to hunters, and the possible exposure to feral swine meat markets. The analysis discusses these issues in detail and despite changes to the product and study protocols, the nature of these changes does not change the analysis in Section 3.2.1 in (USDA 2017). WS identified no indirect or cumulative impacts to human health and safety. The analysis remains consistent and relative to this Supplement.

3.1.2 Impacts on Terrestrial and Aquatic Environments

The EA (USDA 2017) analyzed the No Action and the Proposed Action alternatives and their potential impacts on terrestrial and aquatic environments in Section 3.2.2. The analysis focused on several issues: Environmental Fate of Sodium Nitrite, Aquatic Exposure Assessment, Risks to Aquatic Vertebrates, Risks to Aquatic Invertebrates, Risks to Aquatic Plants, Terrestrial Exposure Assessment, Risks to Terrestrial Vertebrates, Risks to Terrestrial Invertebrates, and Risks to Terrestrial Plants.

The primary means of analyzing these potential effects on the terrestrial and aquatic environment rely on the predicted amount of sodium nitrite that could enter the environment. The EA (USDA 2017) based the analysis on the original product with a 10% concentration of sodium nitrite and bait stations with a holding capacity of 20kg of product. The current product, HOGGONE 2, has a sodium nitrite concentration of 5% with a bait station holding capacity of 7.5kg. Therefore, the issues in Section 3.2.2 of (USDA 2017) are re-analyzed in this Supplement to reflect this change.

Environmental Fate of Sodium Nitrite

In order to assess whether or not the proposed study would have any effects on the terrestrial or aquatic environment, WS-NWRC will first analyze how the product HOGGONE 2 will specifically be used and what potential there is for the active ingredient sodium nitrite to enter the environment and to discuss the environmental fate of sodium nitrite.

The environmental fate describes the processes by which sodium nitrite moves and transforms in the environment. The environmental fate processes include: 1) mobility, persistence, and degradation in soil, 2) movement to air, 3) migration potential to groundwater and surface water, and 4) plant uptake.

The soil environment is composed of organic and inorganic material as well as air and water. Sodium nitrite does not adhere well to soil particles. Sodium nitrite remains a particulate in the air pockets in soil because it is not volatile. In the air (both above and within the soil) sodium nitrite gradually oxidizes to nitrate. However, in the presence of water, sodium nitrite immediately dissociates into sodium and nitrite ions. In water, the nitrite ions easily oxidize to nitrate, and nitrate is the more predominant compound of the two detected in groundwater (OECD 2005). Nitrate and nitrite are likely to remain in water until consumed by plants or other organisms (USEPA 2006). Biodegradation of nitrite in the environment occurs when bacteria (such as members of genus *Nitrobacter*) oxidize nitrites to nitrates. Then, anaerobic bacteria in soil and sediment reduce nitrates to nitrogen, which is absorbed into the nitrogen cycle. Bioconcentration or bioaccumulation of nitrite (cumulative effect) is not expected for residues that could occur in aquatic systems. Nitrite is highly soluble which is not typical for compounds that may accumulate in aquatic biota (OECD 2005). A low estimated bioconcentration factor (BCF) of 3.162 and the metabolism of nitrite by fish further supports the lack of potential for bioconcentration or bioaccumulation in aquatic habitats.

Aquatic Exposure Assessment

The anticipated use of the sodium nitrite product HOGGONE 2 will meaningfully reduce the possibility of exposure to aquatic environments. Using baits contained within a bait station will virtually eliminate the potential for off-site transport of sodium nitrite from drift and substantially reduce the potential for any runoff to aquatic systems. The possibility does, however, exist that some runoff could occur if baits are dropped or spilled on the ground during feeding. The amount of runoff from this type of scenario is expected to result in very low estimated residues. Most bait will be consumed in the bait station. Baits dropped on the ground by feeding swine will likely be consumed by feral swine or other animals, thereby, decreasing the probability baits would stay on the ground for a sufficient amount of time to allow for degradation and be susceptible to runoff.

To estimate what the risks would be in a typical baiting situation, WS-NWRC characterized potential residues in various-sized water bodies using several conservative assumptions. The total amount of bait in a bait station is 7.5 kg with 5 (4.965) percent of the material by weight containing sodium nitrite. This is the maximum amount of bait that would be in an individual bait station. Based on observed removal efficiencies of the bait noted in all past trials conducted in Australia, Alabama and Texas, the average amount of spillage ranged from 0 to 140.0 g averaging 36.1 g that could end up on the ground. At a 4.965% concentration, that amounts to 6.95 g of the active ingredient. The amount of material susceptible to runoff was set at 10 percent based on maximum runoff values for conventionally applied liquid pesticides. This value is conservative since it assumes that feral swine or non-target mammals and birds would remove none of the material on the ground, and all sodium nitrite would be susceptible to runoff instantaneously. Baits will degrade at different rates depending on the environmental conditions, and nitrite leaching and movement will occur slowly at varying rates instead of one large single runoff event indicative of broadcast liquid pesticide applications.

The above exercise calculated residues from three different aquatic habitats: a wetland, a small pond, and a drinking water reservoir. The dimensions of these water bodies are based on USEPA default assumptions for each habitat type. The water bodies are assumed to be static with no inflow or outflow, and residues are considered instantaneous with no degradation or partitioning. This is also a very conservative assumption since nitrite in runoff would be susceptible to a variety of transformation processes to less toxic forms of nitrogen or assimilation by terrestrial or aquatic plants or partitioning to soil/sediment (Bowden 1987). Residues were also assumed to be instantaneously distributed throughout the water column, as opposed to a chemical gradient with higher residues adjacent to the point source as observed under normal field conditions. Potential residues into flowing aquatic habitats were assumed to be captured using the three static water bodies that were used in this exposure assessment. Instantaneous surface water concentrations ranged from 0.0013 (mg/L) in a wetland habitat to 4.85×10^{-6} mg/L in a small drinking water reservoir. These are considered very conservative estimates of potential aquatic residues and are highly unlikely to occur but can be used for screening level purposes to compare to available aquatic effects data and determine the potential for risk to aquatic biota.

Risks to Aquatic Vertebrates

The below section summaries available nitrite toxicity data for aquatic vertebrates. Nitrite toxicity varies considerably between different fish species, ranging from highly toxic to practically non-toxic. Cold tolerant freshwater species such as salmonids appear to be the most sensitive fish species with median lethality concentrations (LC_{50}) in the low part per billion (µg/L) range while marine fish and cyprinids appear to be more tolerant to sodium nitrite exposure with median lethality values greater than 100 parts per million (mg/L).

Sublethal acute and chronic effects have also been noted in fish species at concentrations below median lethality values (Jensen 2003, Kroupova et al. 2005, Jensen 2007, Russo 2006). Nitrite at sublethal concentrations can result in methemoglobinemia as well as affect the gill, brain and liver, where it can accumulate (Margiocco et al. 1983). Effects on swimming performance, food consumption and growth, ability to survive hypoxic conditions and increased pathogen susceptibility have been reported in acute sublethal dosing studies (Eddy and Williams 1987, Carballo and Munoz 1991, Carballo et al. 1995, Russo et al. 1981). These effects result in decreased fitness, reducing reproduction potential and predator avoidance and increased susceptibility to other natural and anthropogenic stressors. Acute sublethal responses that have been observed in fish exposed to sodium nitrite have also been observed in chronic studies. Hilmy et al. (1987) noted effects on erythrocyte (red blood cell) count, hemoglobin content and hematocrit (percentage of red blood cells) counts during a six month exposure to nitrite at 1/10 (2.8 mg/L), the median lethal concentration for the African mudfish, (*Clarias lazera)*. In another long-term exposure study, steelhead trout (*Onchorhynchus mykiss*) were exposed to sodium nitrite concentrations ranging from 0.015 to 0.060 mg/L for six months. Methemoglobinemia was slightly elevated compared to controls observed at each test concentration; however, no effects on growth or other hematological abnormalities were noted at concentrations ranging up to 0.030 mg/L. The highest test concentration (0.060 mg/L) resulted in hypertrophy, hyperplasia, and lamellar separation in the gill epithelium (Wedemeyer and Yasutake 1978).

Amphibian sensitivity to nitrite is comparable to the range of sensitivities reported for acute lethal exposures to fish. Marco et al. (1999) reported 96-hr median lethality values ranging from 0.59 mg/L for the northwestern salamander (*Ambystoma gracile*) to greater than 5.0 mg/L for the Oregon spotted frog (*Rana pretiosa*), the northern red-legged frog (*R. aurora*), and the Western toad (*Bufo boreas*). In another study using small-mouthed larval salamanders (*A. texanum*), the 96-hr LC_{50} value was reported as 1.09 mg/L suggesting that larval salamander species may be more sensitive than tadpole species (Huey and Beitinger 1980a). Shinn et al. (2008) reported five and six-day median lethality values of 127.6 and 116.4 mg/L for larval Perez's frog (*Pelophylax perezi*) and Mediterranean tree frog (*Hyla meridionalis*). Sensitivity was shown to increase with longer exposure time, which is typical for most chemicals.

Smith (2007) reported no lethal or sublethal effects of nitrite concentrations ranging up to 20 mg/L for the wood frog (*R. sylvatica*). A similar lack of lethal or sublethal effects has also been noted in the bullfrog (*R. catesbiana*) at concentrations up to 10 mg/L (Huey and Beitinger 1980b; Smith et al. 2004). However, sublethal effects have been noted in other amphibian species at lower test concentrations. Marco and Blaustein (1999) documented developmental and behavioral effects at a concentration of 3.5 mg/L for the Cascades frog (*R. cascadae*) resulting in increased susceptibility to predation. Griffis-Kyle (2005, 2007) reported sublethal effects on growth and development in 30-day exposures using embryos and larvae of wood frogs and eastern tiger salamanders (*A. tigrinum tigrinum*) at concentrations ranging from 0.3 to 6.0 mg/L. This variability, even within the *Rana* genus, is due to the type of endpoint measured, water chemistry during the test exposures, and potential differences in physiological adaptation related to lower ion uptake or a more effective methemoglobin (metHb) reductase enzyme system for those species and life stages that are less sensitive.

<u>Risks to Aquatic Invertebrates</u>

Aquatic invertebrate acute toxicity to sodium nitrite ranges from highly toxic to nearly non-toxic with median lethality values ranging from approximately 1.0 mg/L to greater than 500 mg/L.

Chronic toxicity of nitrite has also been evaluated in different aquatic invertebrate species. Water chemistry, in particular chloride levels, can also influence the effect on toxicity of nitrite to aquatic invertebrates with increasing chloride concentrations reducing toxicity (Lin and Chen 2003, Russo 2006, Alonso and Camargo 2007). Chen and Chen (1992) reported Maximum Allowable Toxicant Concentrations (MATC) of 4, 2 and less than 2 mg/L in 10, 30 and 60-day exposures for the giant tiger prawn (*Penaeus monodon*) and reported EC_{50} values at 60 days for length and weight were 26.20 and 22.45 mg/L. Armstrong et al. (1976) found larval giant Malaysian prawn (Macrobrachium rosenbergii) to have LC_{50} values of 6-12 mg/L. Kelso et al. (1999) in a reproductive study using the freshwater cladoceran, (*Daphnia magna*), reported a significant linear negative impact on the length and weight of adult cladocerans as well as reproduction at concentrations ranging from 2.5 to 40 mg/L. Dave and Nilsson (2005) reported nitrite-related reproductive and adult effects in a chronic study using another freshwater cladoceran (*Ceriodaphnia dubia*) at the lowest test concentration 0.0157 mM (converted to 1.08 mg/L by multiplying the molecular weight of sodium nitrite 68.9953 g/mole). Chen et al. (2011) demonstrated impacts on growth and reproduction in a twelve-day exposure for the freshwater rotifer (*Brachionus calyciflorus*) at 10 mg/L nitrite but not at 3 and 6 mg/L suggesting a No Observable Effect Concentration (NOEC) of 6 mg/L.

Mollusks have been shown to have much higher tolerances to nitrates and nitrites than crustaceans and aquatic insects (Soucek and Dickinson 2012). Soucek and Dickinson 2012 also conducted a review of the literature that found five species of mollusks to have LC_{50} for nitrite that ranged from 15.6 mg/L to 535 mg/L. Epifanio and Srna (1975) found tolerance levels of 330-736 mg/L in juveniles and adults in the clam (*Mercenaria mercenaria)* and the oyster (*Crassostrea virginica)*. Widman and Meseck (2008) found bay scallops (*Argopectien irradians irradians)* to have LC_{50} levels at 345.13 mg/L. Furthermore, considering that most nitrite would likely oxidize to nitrate in water, increasing those tolerances substantially anywhere from 2 to 10 times higher depending on the species (Soucek and Dickinson 2012).

Risks to Aquatic plants

Available toxicity data for aquatic plants is limited primarily to algal species. Algal sensitivity to sodium nitrite is low with a reported 72-hr EC_{50} value for the green algae (*Scenedesmus subspicatus*) of greater than 100 mg/L. No sublethal effects were noted at the highest test concentration used in the study resulting in a NOEC of 100 mg/L. Comparative experiments using several species of green and blue-green algae suggest that blue-green algae are more sensitive based on photosynthesis inhibition when exposed to 1.0 mM (68.9 mg/L) nitrite (Wodzinski et al. 1978). Risk to aquatic plants from nitrite would also be negligible based on the available toxicity data endpoint of a NOEC of 100 mg/L. Toxicity to aquatic plants is several orders of magnitude above any of the residues expected in various water bodies.

Summary of Aquatic Risks

The risk of nitrite exposure from HOGGONE 2 applications was evaluated by comparing the estimated residues in a typical wetland and pond to the range of acute and chronic toxicity data for aquatic vertebrates and invertebrates and is summarized in Figure 1.

All acute and chronic toxicity endpoints were several orders of magnitude above the range of estimated acute aquatic concentrations suggesting a lack of risk to aquatic fauna. As previously stated, the estimated aquatic values for nitrite are conservative since they would decrease rapidly due to degradation and uptake from other biota. The assessment's estimate of aquatic residues also assumed that baiting stations would be established adjacent to aquatic habitats. A setback buffer of 25 feet from aquatic habitats is required under the current HOGGONE 2 label and will further reduce the potential for acute or chronic nitrite exposure to aquatic organisms.

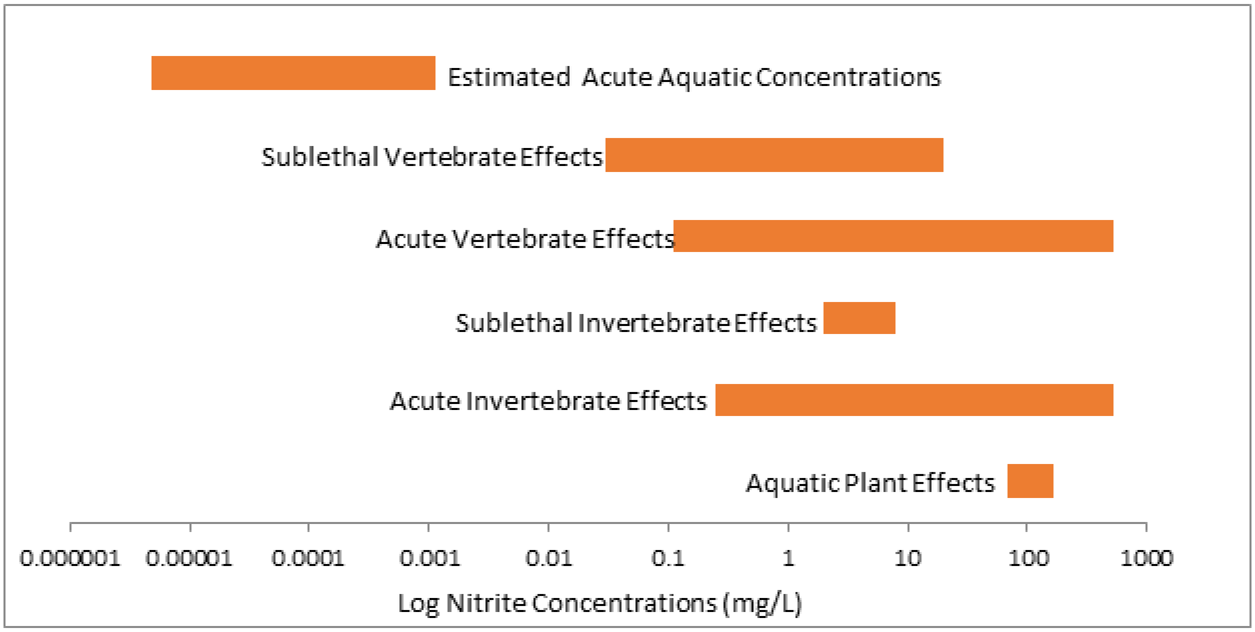

Figure 1. Aquatic vertebrate and invertebrate risk characterization for nitrite.

It is anticipated that toxic baiting in most situations would be for one day. It may be necessary to reapply a toxic bait for an additional day if it is found that some feral swine did not receive a toxic dose. Therefore, WS-NWRC does not expect there to be any chronic or cumulative nitrite exposure due to a short application period. Furthermore, referring to figure 3, there are still wide safety margins between the estimated acute residues and the chronic toxicity data. The available data for aquatic vertebrates, invertebrates and plants demonstrate that the estimated residues of HOGGONE 2 in aquatic habitats present risks to these organisms that are insignificant and discountable. This includes direct risk from nitrite exposure and any indirect risk to available food items and habitat.

Terrestrial Exposure Assessment

The primary exposure pathway to terrestrial wildlife will be via the dietary route. Exposure may occur for those animals that can access bait from the bait station itself or from bait that falls on the ground during feeding events. As mentioned above in the aquatic assessment, based on the recent trials, bait spillage ranged from 0 to 140.0g of bait on the ground with 36.1g being the average. WS will use the worst-case scenario of 140.0g of spillage possible at each bait site for this assessment. A detailed analysis of the concentrations' potential effects on non-target and threatened and endangered terrestrial wildlife is addressed below in Section 3.1.3.

Risk to Terrestrial Vertebrates

Sensitivity of different mammalian species to sodium nitrite is correlated to levels of MtHb reductase which converts methemoglobin to hemoglobin. Lapidge and Eason (2010) demonstrated the relationship between MtHb reductase and lethality for several mammal species in Figure 2. A statistically significant positive correlation was observed between MtHb reductase levels and lethality suggesting that reductase levels can be used to estimate lethality for other mammal species where toxicity data is unknown for sodium nitrite. The correlation between lethal doses and data regarding MtHb reductase levels demonstrates that domestic animals such as dogs and some livestock are particularly sensitive to the effects of sodium nitrite toxicity.

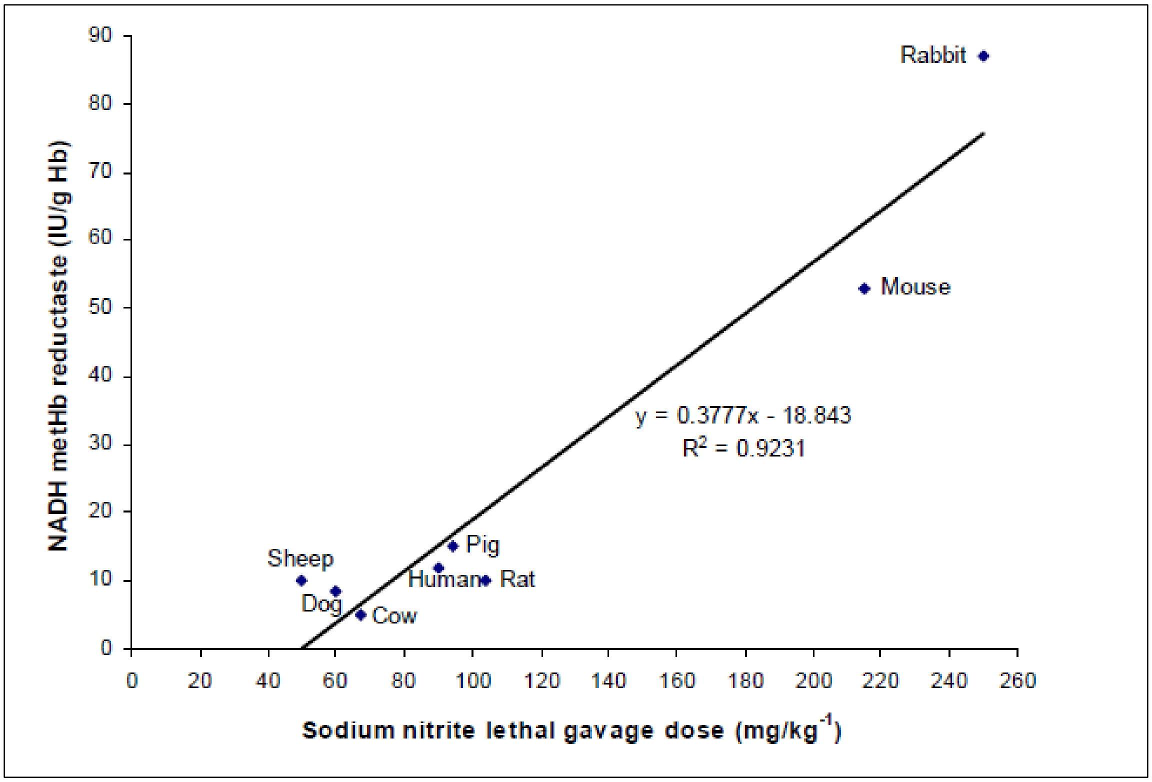

Figure 2. Regression between sodium nitrite lethal doses and NADH (Nicotinamide Adenine Dinucleotide (NAD^+) reduced by oxidization) MtHb reductase levels in various mammal species (from Lapidge and Eason 2010)

Lethality in mammals can occur when methemoglobin levels exceed 70 percent. However, many sublethal responses may occur at lower levels and may be ecologically relevant. Clinical signs of nitrite exposure can appear in some species of mammals when methemoglobin levels reach 20 percent (Bruning-Fan and Kaneene 1993). Ataxia (lack of coordination), dyspnea (shortness of breath), and general weakness are typical signs of nitrite toxicosis and could impact non-target mammals' ability to avoid predation and impact other behavioral and physiological responses. However, any potential sub-lethal effects are expected to be short-lived based on the rapid metabolism of sodium nitrite observed in various mammals. Lapidge and Eason (2010) summarized data from previous studies in the rat, sheep, dog and horse and observed the elimination half-life ($T_{1/2}$) of sodium nitrite in plasma to range from 29 to 62.5 minutes based on a range of doses.

Chun-Lap Lo and Agar (1986) compared MtHb reductase levels in erythrocytes (red blood cells) from eleven newborn and adult mammal species. They found that except for the rabbit and humans, levels were higher in newborns when compared to adults of the same species. These results are consistent with previous work except for cattle and pigs which demonstrated that newborns were shown to have less MtHb reductase levels compared to adults (Agar and Harley 1972).

Dietary exposure may also occur from consumption of potentially contaminated drinking water. Strnad and Persin (1983) reported average methemoglobin levels of 16.5 percent in fourteen day-old ring-necked pheasant (*Phasianus colchicus*) chicks exposed to 15 mg/L of sodium nitrite in drinking water; liver and kidney dysfunction were also reported at this exposure. Other studies exposing birds to a range of nitrite concentrations in drinking water have demonstrated similar impacts to those observed from feeding studies in various test species at concentrations of 200 mg/L (Bruning-Fan and Kaneene 1993). However, this exposure pathway is anticipated to be insignificant or discountable since conservative estimated aquatic residues presented above in the aquatic assessment are well below concentrations that would be expected to result in adverse effects.

No nitrite toxicity data appears to be available for reptiles and the terrestrial phase for amphibians. USEPA Office of Pesticide Programs (OPP) assumes that bird sensitivity to pesticides is representative of the potential effects to reptiles, however, some uncertainty is presumed with this assumption. Differences in metabolism and other physiological adaptations and life history traits are unique to reptiles and not shared with birds. Uncertainty regarding nitrite sensitivity of reptiles compared to birds and other non-target vertebrates can be addressed by assessing available information regarding MtHb reductase levels. Reductase levels are equal to or greater in reptiles than in birds suggesting sensitivity to the effects of nitrite poisoning would be similar, or less, for reptiles (Board et al. 1977). A similar trend was also observed when comparing MtHb reductase levels in nucleated erythrocytes (red blood cells) between birds and the adult bullfrog suggesting similar sensitivity between terrestrial phase amphibians and birds (Ito et al. 1984).

Risk to Terrestrial Invertebrates

Acute exposure data using the earthworm demonstrates moderate toxicity with a 48-hr LC_{50} ranging from 100 to 1000 µg/cm^3 (Roberts and Dorough 1984). Elevated soil nitrite concentrations impact soil microorganisms responsible for methanogenesis and other degradation processes (Banihani et al. 2009, O'Reilly and Colleran 2005). Other studies have shown some nitrite-related impacts to soil-borne terrestrial invertebrates. However, these studies are typically conducted with sewage sludge and contain other pollutants that could be responsible for adverse impacts; thus, these studies would have limited ecological relevance in evaluating the impacts of the use of sodium nitrite as a feral swine toxicant. Some nitrite toxicity information is available for non-soil-borne terrestrial invertebrates. Sarikaya and Cakir (2005) conducted feeding studies using the larval fruit fly, (*Drosophila melanogaster*), and found no effects on survival when exposed to 25 mM sodium nitrite until pupation. Ionescu et al. (1990) reported 100 percent mortality to honeybees (*Apis mellifera*) when exposed to a 1 percent solution of sodium nitrite with a maximum allowable concentration of 1 mg/L. More recently, Leonard (2016) evaluated the effects of SN on honeybees and found a NOED of 100µg (0.1 mg/L) and a LOED of 400µg (0.4 mg/L).

Most recently, Shapiro et al. (2017) evaluated the primary and secondary poisoning risks to several surrogate species in New Zealand when exposed to a new SN toxicant registered in New Zealand for brushtail possums (*Trichosurus vulpecula)* and feral swine that has a very similar formulation as HOGGONE. Shapiro evaluated the risks to the cave weta (Family: Rhaphidophoridae) a common native New Zealand invertebrate similar to a grasshopper or cricket. These invertebrates were commonly found sheltering in bait stations. They could potentially access and consume baits that could cause direct mortality or consume sub-lethal amounts of bait and then be eaten by other non-targets such as birds.

Shapiro et al. (2017) collected sixteen cave weta and allowed them direct access to bait for a two-week period. All cave weta were alive after the trial suggesting there was no primary poisoning. Following the direct exposure trial, cave weta were euthanized and assayed to determine if any trace of SN could be detected. One cave weta was found to have SN residue of 10 µg suggesting the potential for bioaccumulation and secondary poisoning is extremely low. Furthermore, this concentration was just above the minimum detection level and the author suggests it could have been the result of some bait material contaminating the weta when collected at the conclusion of the trial. The authors suggest that based on the dietary 50% lethal dose (LD_{50}) calculated for chickens, a 1 kg chicken would need to consume over 25,000 weta (each with a residue of 10µg) in quick succession to receive an LD_{50} dose.

Risk to Terrestrial Plants

Available toxicity data for terrestrial plants suggested effects can occur when nitrite soil or soil water concentrations exceed 1.0 mg/L. Effects on root and shoot growth, dry matter yield and chlorosis have been observed in crops such as lettuce, tomato and tobacco (Phipps and Cornforth 1970, Hamilton and Lowe 1981, Hoque et al. 2007). Wheat seedlings exhibited nitrite-related effects to root growth in exposure to 1 mM (68.9 mg/L) sodium nitrite (Tari and Csiszar 2003).

As discussed above, the amount of sodium nitrite that could inadvertently end up on the ground as a result of spillage from a bait station would be minimal. Predicted values would still be far below the 1.0 mg/L concentration has been shown to affect plants negatively. It should also be noted that sodium nitrite would also be susceptible to degradation to other forms such as nitrogen that are less toxic and can be assimilated by plants. Similar to soil invertebrates the risk to terrestrial plants is low and would only occur in areas where bait contacts the ground and decomposes. However, due to degradation of the bait and sodium nitrite, extremely low concentrations and low bioavailability, potential effects would be transient and specific to soil under, and immediately adjacent to, any spilled bait. Removing spilled bait as required by the label would further reduce the availability of sodium nitrite to terrestrial plants.

Summary of Terrestrial Risks

Overall, risks to terrestrial vertebrates, invertebrates and plants are expected to be minimal based on the proposed use and available effects data. Some terrestrial vertebrates and invertebrates may be attracted to spilled bait as a food source. Still, any potential risk would be limited to individuals actively feeding on the bait and would not result in population-level impacts or any cumulative effects. A more detailed analysis of non-target mammals and birds is presented below in Section 3.1.3. The lack of toxicity at relevant doses to pollinators such as the honeybee and the low potential for exposure to pollinators suggests they would not be at risk from the proposed use of sodium nitrite. The risk to soil-borne invertebrates would be possible if bait was left on the ground and allowed to degrade in place adding sodium nitrite levels to the soil resulting in exposure. However, current protocols and the proposed product label would prevent that much bait from ending up on the ground and therefore WS-NWRC does not believe this to be an exposure risk. Any dietary exposure to vertebrates from contaminated water is shown to be insignificant based on the estimated aquatic residues presented above. These levels are also shown to be insignificant for terrestrial plants as well.

Other indirect and cumulative effects of this study that may affect the terrestrial environment such as trails accessing bait sites or other human activities were also considered. Bait sites will be visited daily most likely via a 4x4 vehicle or an ATV. Access on private land would be from established trails or roads and access off those trails would either be by an ATV or by foot. Any foot traffic or ATV traffic off established trails would be minimal. It would not only be desirable to leave a “minimal footprint” to prevent any environmental damage such as trampling of vegetation, erosion from new trails etc., but it would also likely be beneficial to the effectiveness of the control program and the study so that feral swine are not disturbed or frightened from the area. Therefore, all efforts will be made to keep disturbances in the area to a minimum.

3.1.3 Effects on Non-target and T&E Species

The EA (USDA 2017) analyzed the No Action and the Proposed Action alternatives and their potential effects on non-target and T&E species in Section 3.2.3. Much like the analysis above (Section 3.1.2) on the impacts to the aquatic and terrestrial environment, a key factor in the analysis relies specifically on the predicted amount of sodium nitrite that could enter the environment and potentially affect non-target or T&E species or their habitat. The EA (USDA 2017) based the analysis on the original product with a 10% concentration of sodium nitrite and bait stations with a holding capacity of 20kg of product. The current product, HOGGONE 2, has a sodium nitrite concentration of 5% with a bait station holding capacity of 7.5kg. Although much of the original analysis in Section 3.2.3 of (USDA 2017) remains valid, this Supplement will re-analyze the potential effects to non-target and T&E species to reflect the changes made to the product, other changes to the study protocol and incorporate data from small-scale trials conducted in 2019-2020 and data from the 2021 full-scale replication of the 2018 trial.

Effects on Threatened and Endangered Species

WS-NWRC reviewed the status, critical habitats designations, and current known locations of all species listed as threatened, endangered, or candidates within the counties where the study site in Texas could be located and species effects determinations were made. The species list, designated critical habitat, and effect determinations made for those species in Texas in Section 3.2.3 of the EA (USDA 2017) is no longer valid due to several species status changes and changes to the counties in which the study could take place. WS-NWRC originally considered nine counties (Archer, Baylor, Cottle, Foard, Hall, Knox, Motley, Wichita, and Wilbarger) for the study site in 2017. However, due to multiple trials in 2019, 2020, and 2021, it was necessary to expand the site to some additional adjoining counties. Therefore, this trial will also consider the T&E species in these additional counties: Childress, Dickens, Throckmorton, Haskell, King, Jones, and Stonewall. Below is an updated table showing the current species, their status and WS-NWRC's updated determinations.

Species listed as threatened, endangered or candidate in sixteen Texas counties.

Common Name	Scientific Name	Status†	Determination‡
ANIMALS			
Clams			
Texas fawnsfoot	*(Truncilla macrodon)*	PT,PCH	NE
Birds			
Whooping crane	*(Grus americana)*	E	NE
Red knot	*(Calidris canutus rufa)*	T	NE
Piping Plover	*(Charadrius melodus)*	T	NE
Insects			
Monarch Butterfly	*(Danaus plexippus)*	C	NE
Fish			
Smalleye Shiner	*(Notropis buccula)*	E, CH	NE
Sharpnose Shiner	*(Notropis oxyrhynchus)*	E, CH	NE

†T=Threatened; E=Endangered; C=Candidate; PT=Proposed Threatened; CH=Critical Habitat; PCH=Proposed Critical Habitat
‡NE=No effect;

WS-NWRC made No Effect determinations for all the species listed in Texas in the original EA (USDA 2017) and provided a letter to the USFWS Texas Ecological Services Field Office in Arlington, TX on June 5, 2017. With improvements to the bait formulation and study protocol, WS believes any risks to T&E species are further reduced and therefore species effect determinations remain the same.

Rationale for Species Effect Determinations

Three aquatic species (1 clam and 2 fish) are listed in the proposed Texas counties. Aquatic species and their critical habitat were closely evaluated because excess nitrite in aquatic systems can be very detrimental to aquatic species. The direct, indirect and cumulative effects were analyzed. This detailed analysis of aquatic impacts is discussed above in Section 3.1.2. The risk of nitrite exposure from HOGGONE 2 applications was evaluated by comparing the estimated residues in a typical wetland and pond to the range of acute and chronic toxicity data for aquatic invertebrates and vertebrates. In all estimates, sodium nitrite concentrations were orders of magnitude below the range of estimated acute aquatic concentrations for aquatic species suggesting a lack of risk to aquatic fauna. This includes direct risk from nitrite exposure and any indirect risk to available food items and habitat.

WS believes any other operational activities, including trapping, handling of animals, euthanasia, transporting equipment, and carcass disposal for this study, will have no effect on aquatic species that may be found in or near the potential study sites because the activities would not modify habitats, occur in waterways, or affect individual animals. Therefore, WS has determined the proposed action will have no effect on the Texas fawnsfoot, smalleye shiner, and the sharpnose shiner or their critical habitat.

The monarch butterfly is listed as a candidate species in the proposed Texas counties. The monarch butterfly feeds exclusively on plants during its entire life cycle so there is no risk of direct exposure to sodium nitrite. As discussed above in Section 3.1.2, the amount of sodium nitrite that could inadvertently end up on the ground as a result of spillage from a bait station would be minimal. Predicted values would still be far below the 1.0 mg/L concentration that has been shown to have negative effects on plants. So WS believes there would be no indirect or cumulative effects via plant consumption. Furthermore, the proposed trial would take place during the fall/winter months when monarchs would not be present in the area. Therefore, WS has determined the proposed action will have no effect on the monarch butterfly.

There are three T&E bird species listed in the proposed Texas counties. Whooping cranes breed in Canada during the summer months and spend the winters on the Texas coast near Rockport, TX. Whooping cranes only migrate through the proposed study site counties in Texas and will not be migrating during the proposed study timeframe. Furthermore, study site locations would not be located in whooping crane habitat and WS-NWRC does not anticipate any whooping cranes to be present during the study timeframe. Rationale for the red knot and the piping plover are similar in that WS does not anticipate the species to be present during the time of the proposed study. Bait stations would also not be located in preferred habitat of these species and therefore WS has made no effect determinations for these species.

Direct Impacts on Non-target Mammals and Birds

Direct exposure refers to the ability of a non-target animal to directly access and consume bait either directly from the bait station or from spilled bait on the ground next to the bait station. Non-targets represent any animal other than the target species (feral swine). The primary exposure pathway to terrestrial non-target wildlife will be via the dietary route. Evaluation of potential bait formulations in the United States has demonstrated that a variety of non-target organisms may be attracted to bait stations (Campbell and Long 2007, Campbell and Long 2009, Campbell et al. 2011, Long et al. 2010). This includes wild species and domestic animals; similar observations were also noted outside of the United States (Massei et al. 2010).

Snow et al. (2016) identified white-tailed deer and raccoons as the most frequently observed non-target species that visited placebo HOGGONE bait sites without using bait stations in Texas. Foster (2011) also found that raccoons and deer were very susceptible to SN, and therefore, would be a serious non-target concern. Previous studies also identified raccoons as the primary non-target species accessing predecessor prototypes of bait stations for feral swine (Long et al. 2010, Campbell et al. 2011, Campbell et al. 2013). Given these findings, the original study conducted in January 2018 in Texas focused on raccoons as the primary non-target species at potential risk from direct exposure to toxic HOGGONE in bait stations. From the results of the 2018 trials and subsequent trials in 2019, 2020 and 2021, non-target birds became a primary focus of concern (Snow 2020, Snow et al. 2021,2022). Although raccoons remained a primary focus, the majority of the modifications made to the baiting strategy were directed toward minimizing risks to non-target birds and are described in more detail below.

The bait station (Appendix B) lids will have 13.6 kg (30 lbs) of magnetic pressure holding the lids shut ensuring that only larger animals such as feral swine would have direct access when the sodium nitrite bait is deployed. This bait station delivery device will significantly decrease the potential for exposure to most non-target organisms. Snow et al. (2017c) found that 13.6 kg of magnetic pressure excluded raccoons but allowed 75% of feral swine access to the bait station. Therefore, it was assumed in the original HOGGONE trial in 2018, all non-target birds and mammals smaller than a raccoon would be completely excluded from the bait station. Subsequent trials have proven the effectiveness of the magnetic lids.

The challenge with non-target birds has not been a direct access problem. It has resulted from feral swine dropping and spilling bait outside the bait stations while feeding, which allows birds to scratch and pick up small particles of spilled bait after feral swine have left the bait station. Therefore, another modification that would be implemented to reduce the potential for taking non-target birds is moving the bait stations a minimum of 10 m away from the pre-bait locations. This technique would allow birds to scratch the ground and feed on spilled non-toxic pre-bait at the “old” bait site while the “new” bait site (10 m away) would contain toxic bait. The change is not substantial enough to affect feral swine feeding but could significantly reduce bird feeding at the new toxic site. A recent unpublished pen trial of 40 red-winged blackbirds was tested using this scenario and allowed to feed freely next to a toxic bait station and resulted in the taking of 1 blackbird.

To establish risks to various species, WS reviewed several studies. All species have varying degrees of sensitivity to SN. Therefore, acute oral dosing studies have been conducted with several test species with values demonstrating moderate to high toxicity from sodium nitrite exposure (Table 2).

Table 2. Acute oral median lethality values for various mammals to sodium nitrite.

Test Species	Test	Toxicity Values (mg/kg)	Reference
Brushtail possum (*Trichosurus vulpecula)*	Acute Dietary Toxicity	122	Shapiro 2016
Raccoon (*Procyon lotor*)	Acute Oral Toxicity	58	Foster 2011
New Zealand rabbit (*Oryctolagus cuniculus*)	Acute Oral Toxicity	124	Dollahite and Rowe 1974
White-tailed deer (*Odocoileus virginianus*)	Acute Oral Toxicity	154	Foster 2011
Feral swine (*Sus scrofa*)	Acute Oral Toxicity	133	Foster 2011

Available acute oral dosing studies show moderate toxicity of sodium nitrite to birds. Acute dietary testing has shown some mixed results. Stafford (2011a,b,c), using the northern bobwhite and mallard demonstrates that sodium nitrite is practically non-toxic to surrogate bird species representing upland game birds and waterfowl. No toxicity or sublethal effects were noted at the highest test concentration (Table 3). However, more recently, Shapiro (2017) dosed domestic chickens and mallards with SN paste bait registered for brushtail possums in New Zealand and found dietary LD_{50} values to be approximately 254.6 mg/kg for both species. Soniat (2012) and Stafford (2011a) showed sodium nitrite LD_{50} toxicity values for red-winged blackbirds to be 119.8 mg/kg and the Northern bobwhite to be 619 mg/kg. Ley (1986) reported an LD_{50} value of 588 mg/kg for the domestic turkey testing a nitrite-based fertilizer. Sublethal effects and measured methemoglobin levels were consistent with nitrite being the causal agent for mortality.

Table 3. Standardized acute avian toxicity values for sodium nitrite.

Test Species	Test	Toxicity Values	Reference
Red-winged blackbird (*Agelaius phoeniceus*)	Acute Oral Toxicity	LD_{50}:< 119.8mg/kg LOEL: 119.8 mg/kg NOEL: 71.1 mg/kg	Soniat (2012)
Turkey Vulture (*Cathartes aura*)	Acute Oral Toxicity	LC_{50}: 663 mg/kg NOEL: 75 mg/kg	Foster (2018)
Northern Bobwhite (*Colinus virginianus*)	Acute Oral Toxicity	LD_{50}: 619 mg/kg LOEL: 418 mg/kg NOEL: 251 mg/kg	Stafford (2011a)
Domestic chicken *(Gallus gallus domesticus)*	Acute Dietary Toxicity Acute Oral Toxicity	LC_{50}: 254.6 mg/kg LC_{50}: 68.5 mg/kg	Shapiro (2017)
Domestic Mallard *(Anas platyhynchos domesticus)*	Acute Dietary Toxicity Acute Oral Toxicity	LC_{50}: 254.6 mg/kg LC_{50}: 68.5 mg/kg	Shapiro (2017)
Mallard (*Anas platyhynchos*)	Acute Dietary Toxicity	LC_{50}: > 5000 ppm LOEL: undetermined NOEL: > 5000 ppm	Stafford (2011b,c)
Northern Bobwhite (*Colinus virginianus*)	Acute Dietary Toxicity	LC_{50}: >5000 ppm $LOEL_{(BW)}$: 5000 ppm $NOEL_{(BW)}$: 2995 ppm	Stafford (2011a)

Available sublethal feeding studies using domestic and wild bird species show multiple physiological endpoints that may be impacted by sodium nitrite. Atef et al. (1991) dosed cockerels (immature male chickens) for four weeks at a dose of 1.7 g sodium nitrite/kg feed. Significant negative effects were seen on weight gain, erythrocyte (red blood cell) counts and glutamic pyruvic transaminase. Creatinine and urea levels suggested immune, liver and kidney impacts. No NOEC was determined since only one dose was tested. Average (±SD) percent methemoglobin levels were 25.6 percent (±4.0) in sodium nitrite exposed birds which was statistically significant from control birds at 1.1percent (±0.5).

During the original 2018 study (USDA 2017), to ensure there was no direct exposure to any non-target animals such as black bears, large dogs or any other large non-target animal that may be capable of opening a 30 lb. lid, each bait station was monitored with remote cameras. Observation from these remote cameras confirmed that no non-target animals could access the bait stations; therefore, bait station lid tension will remain unchanged for this study. However, the other possible direct exposure route for non-target wildlife analyzed in the original 2018 study (USDA 2017) was the exposure to non-targets via spilled bait around the bait stations. Initial controlled pen studies revealed that spillage should be very low but when the trial was conducted with free ranging feral swine in 2018, they reacted differently than captive feral swine by dropping and spilling about 3.7% (Snow et al. 2021) of the bait which resulted in what was analyzed as a worst-case scenario in the original EA (USDA 2017).

During the 2018 study, 179 non-target animals were taken (Table 4) from 14 bait stations as a result of birds and raccoons eating spilled bait from the ground. This resulted in an average take of 12.8 non-targets per bait station.

Table 4. Non-Target Species taken in 2018 Texas field trial of HOGGONE.

Non-target Species found near bait site in 2018 study	Number found	Mean Distance from bait site (meters)
White-crowned sparrow	121	21.7
Red-winged blackbird	26	109.6
Dark-eyed junco	11	32.9
Northern cardinal	3	29.3
Meadowlark spp.	2	36.6
Brown-headed cowbird	1	40.9
American Crow	3	124.3
Wild turkey	4	104.7
Northern raccoon	8	86.8
Total	179	42.1

Following these results (Table 4), in an effort to make improvements, WS-NWRC collaborated with the HOGGONE manufacturer in Australia to make modifications to the product and revise the baiting strategies which are detailed in Sections 1.2, 2.1, and 2.2. of this Supplement. Also discussed in detail in Section 3.1.2, the Environmental Fate section, the 2018 (USDA 2017) study estimated the possibility of spilling approximately 20 g of active ingredient. The revised product (assuming maximum amount of spillage observed in all trials) with a reduced concentration and a decrease in the total amount of bait in the bait stations has reduced the estimated spillage to approximately 6.95 g of active ingredient. Therefore, the HOGGONE 2 improvements have reduced the potential of active ingredient spillage by about two-thirds.

The manufacturer in collaboration with WS-NWRC conducted the first trial to test these improvements. A small-scale trial of similar size (14 bait stations) incorporating these modifications was conducted in Queensland, Australia, in December 2018. The Australia trial results showed a substantial decrease in spilled bait (Mean of 1.22kg in the 2018 Texas trial, down to 0.055kg in the 2018 Australia trial) while maintaining an effective lethality rate for feral swine. There were 3 non-targets (Australian ravens; *Corvus coronoides*) which resulted in 0.2 non-targets per bait station (Snow 2021). Although not completely comparable to the U.S. because non-target species and conditions are different in Australia, these results were still very encouraging and provided evidence and rationale for pursuing additional trials in the U.S.

WS-NWRC conducted a second Environmental Assessment (USDA 2019) to analyze the potential effects of conducting a second trial incorporating most of the modifications described in this Supplement. This second small-scale trial was conducted in the same locations described in the first EA (USDA 2017). The first of these trials took place in August 2019 in Alabama. Bait spillage from five bait sites was difficult to measure due to muddy conditions but was estimated to be less than 0.01kg based on visual inspections. The trial resulted in two non-target opossums (Table 5) for 0.4 non-targets per bait station which was consistent with the earlier trial in Australia.

Table 5. Non-Target Species taken in Alabama 2019 from small-scale trials of HOGGONE 2.

Non-target Species found near bait site in Alabama 2019	Number found	Mean Distance from bait site (meters)
Virginia opossum	2	94.6
Total	2	94.6

The next small-scale trial took place in March 2020 in Texas in the same locations described in the EA (USDA 2017). Estimated spillage was again difficult to detect due to the conditions but sites were visually inspected, and spillage was estimated at 0.08kg. Non-target take was higher than expected and resulted in the take of 35 birds for an average of 7 non-targets per bait station (Table 6).

Table 6. Non-Target Species taken in Texas 2020 from small-scale trials of HOGGONE 2.

Non-target Species found near bait site in Texas 2020	Number found	Mean Distance from bait site (meters)
Dark-eyed Junco	28	18.4
White-crowned sparrow	5	18.4
Chipping sparrow	2	18.4
Total	35	18.4

These results (Table 6) prompted WS-NWRC to explore more ways to exclude or deter birds, (particularly small passerine birds), from feeding in and around bait stations to reduce the chance of non-target take further. WS-NWRC evaluated four bird deterrent devices and identified the Scare Dancer® (a 6-foot cordless inflatable scarecrow shaped like a snake) as the most effective and easy-to-operate device (Snow 2020). To evaluate the effectiveness of the Scare Dancer, WS-NWRC developed another trial that occurred in July 2020 in Texas in the same locations described in the EA (USDA 2017). This trial consisted of 10 bait stations, 5 sites to be operated with the use of the Scare Dancer and 5 sites without the Scare Dancer. The Scare Dancer was deployed and programed to begin operation 1 hour before sunrise. The concept was to scare/harass birds or other non-targets from the bait sites immediately following a toxic baiting. Most feral swine visited and fed at the bait stations before the Scare Dancer was set to operate and therefore did not result in decreased feral swine take. Average bait spillage remained consistent with the past small-scale trials at 0.023kg. Of the 5 bait stations without the Scare Dancer, WS-NWRC identified 2 non-target rodents and 0 birds for an average of 0.4 non-targets per bait station. Of the 5 bait stations that deployed the Scare Dancer, no non-targets were found (Table 7).

Table 7. Non-Target Species taken in Texas 2020 with/without Scare Dancer®.

Non-target Species found without the use of the Scare Dancer	Number found	Non-target Species found with the use of the Scare Dancer	Number found
Plains pocket mouse	1	0	0
North American deer mouse	1	0	0
Total	2	Total	0

The results in Table 7. identified two important findings. First, the timing of the trial (July vs March) may have reduced the risks to non-targets (particularly passerine birds) because there would have been fewer birds present in the area due to seasonal migration. Secondly, the Scare Dancer appeared to be an effective deterrent to birds following the deployment of HOGGONE 2 (Snow 2020).

To confirm these positive results, WS-NWRC conducted a full-scale replication of the 2018 trial during July/August of 2021 in Texas and Alabama (Snow et al. 2021, 2022), incorporating all elements from the above trials. WS-NWRC conducted the first of these trials in Texas. This full-scale replication of the 2018 trial was conducted in the same locations described in the first EA (USDA 2017). Bait spillage from 8 bait sites averaged 0.14 kg. Toxic baiting occurred for two non-consecutive nights which yielded a total of 64 feral swine taken with zero non-targets found (Table 8).

In Alabama, the 2021 trial replication resulted in an average spillage of 0.06 kg from 11 bait sites. Toxic baiting was also offered for two non-consecutive nights and yielded a total of 70 feral swine and one confirmed non-target taken. Four other non-targets were found during transect surveys (2 raccoons, 1 opossum, 1 snake) but after further investigating, two were ruled deaths from other causes (1 opossum, 1 snake) and two (2 raccoons) were ruled unlikely and not confirmed to be killed by HOGGONE 2 (Snow et al. 2022).

Table 8. Non-Target Species taken in Texas/Alabama 2021 Full Scale Trial Replication.

Non-target Species found near bait sites in Texas 2021	Number found	Non-target Species found near bait sites in Alabama 2021	Number found
NA	0	Opossum	1
Total	0	Total	1

Summarizing the above data and tables 4-8 indicates a reduction in non-target take from the modifications made to the product and the overall application strategy from 2018 to present. The 2018 trial took 179 non-targets with 14 bait stations for an average of 12.8 non-targets per bait station. This was a worst-case scenario and the trial was immediately discontinued. With the modifications made to the product, and promising results from a 2018 trial in Australia, subsequent small-scale trials have shown substantial improvements. The 2019 Alabama trial resulted in 0.4 non-targets per bait station, a 97% improvement. The March 2020 Texas trial resulted in 7 non-targets per bait station, a 45% improvement. The July 2020 Texas trial resulted in 0.4 non-targets without the deployment of the Scare Dancer, a 97% improvement and with the deployment of the Scare Dancer, zero non-targets were taken resulting in a 100% improvement. The 2021 trial (Texas and Alabama combined) resulted in one confirmed non-target take at 0.05 non-targets per bait station, a 99% improvement over the 2018 trial.

However, based strictly on the active ingredient, it would still be possible to take an unacceptable number of non-targets with the maximum average amount of predicted spillage of 140.0 g of bait (as observed in all trials since 2018, 6.95 g of active ingredient, or 0.25 oz.). For example, the LD_{50} of sodium nitrite for raccoons is 58 mg/kg. A group of raccoons weighing 4 kg each would need to eat 232 mg (58mg/kg x 4 kg) of active ingredient for half of them to be killed. Thus, it would appear that 6.95 g of SN would take several raccoons but in reality, raccoons eat 5% of their body weight per day, approximately 200 g (4 kg = 4000 g x .05% = 200). Therefore one raccoon could consume all of the spilled bait resulting in the death of one or maybe two raccoons per bait station. However, many of the spilled particles of bait may be too small for raccoons to pick up and consume, but large enough for smaller animals to detect and potentially consume lethal doses.

In the case of birds, passerine birds appear to be relatively sensitive to SN compared to other birds (e.g. ducks, chickens). Of the passerine species tested, zebra finches have an LD_{50} of 107 mg/kg and red-winged blackbirds have an LD_{50} of 120 mg/kg). An average red-winged blackbird weighs about 56 g, therefore they would need to eat approximately 6.7mg (120mg/kg x .056kg) of SN or about 134mg of 5% concentrated bait to $LD_{50.}$ They likely consume about 4 grams of food per day. This indicates that 35 blackbirds could reach LD_{50} of which half could be killed (140.0 g bait divided by 4 grams per bird). This could be possible if a flock of birds was to feed on the precise amount of spilled bait. However, this is unrealistic, because bait would not be widely and evenly scattered, and birds are not evenly distributed. A more realistic estimate may be 5-10 non-target blackbirds or less could be taken as a result of intensively feeding on spilled bait at each bait station, but this would still be a worst-case scenario. Passerine birds with smaller body sizes than blackbirds could be taken at higher rates.

The study protocol calls for baiting 3-9 feral swine sounders in Texas. As a high estimate, 9 sounders with an average of 22 feral swine per sounder would be approximately 200 feral swine. This would require approximately 20 bait stations to administer lethal doses to approximately 200 feral swine. Based on the scenarios above, it would be possible to take up to 200 non-target blackbirds and 25 non-target raccoons/opossums. Again, this would be a scenario where there is an average maximum amount of bait spilled (140.0 g) at every bait station. This level of spillage is not realistic or expected. Based on the best available data from a trial in Australia and the five small-scale trials conducted in the U.S. (Snow et al. 2020, 2021,2022), non-target animals averaged 1.2 per bait station. With the deployment of 20 bait stations, a likely estimate of non-target take would be 24 non-target animals. If observed in the same ratios, 10% would be mammals (approximately 2.4) and 90% birds (approximately 21.6) for the entire study. However, with the modifications to the baiting strategies described in this supplement, WS believes non-target take will be substantially less than described here.

<u>Summary of Direct Impacts to Non-target Species</u>

Although there is a chance to expose some non-target birds and mammals directly to spilled bait, WS-NWRC believes the risk is minor and short-term and would not result in any chronic or cumulative effects. Overall, some non-targets may be attracted to the bait as a food source, but any potential risk would be limited to individuals actively feeding on the bait and would not result in any population-level impacts.

<u>Indirect and Cumulative Impacts to Non-Target Mammals and Birds</u>

Indirect effects to mammals or birds would primarily refer to secondary poisoning concerns. WS-NWRC analyzed the possibility and the effects of an animal consuming another animal, insects or plants that may have been exposed to sodium nitrite and other potential secondary or unintended effects. WS-NWRC identified four possible routes of secondary exposure to SN. First, and probably the least likely concern is exposure via consuming contaminated drinking water. As noted in 3.1.2., the estimated aquatic residues are orders of magnitude below any effects data for all aquatic species and are also far below safe drinking water standards. There is virtually no risk of secondary exposure to SN via drinking water to non-target wildlife. Secondly, the consumption of vertebrates, invertebrates or plants that may have been exposed to SN is considered. The impacts of SN exposure to plants and invertebrates are also discussed in Section 3.1.2. The effects to aquatic plants and invertebrates, and terrestrial plants and invertebrates were shown to be minuscule and insignificant. Furthermore, due to the biological process in which SN converts hemoglobin to methemoglobin (MtHb) and the protective enzyme MtHb reductase quickly reversing the de-oxidizing effects of nitrite, no bioaccumulation of nitrite occurs. Lapidge and Eason (2010) summarized data from previous studies in the rat, sheep, dog, and horse and observed the elimination half-life ($T_{1/2}$) of sodium nitrite in plasma to range from 29 to 62.5 minutes based on a range of doses.

Also noted in Section 3.1.2, Shapiro et al. (2017) evaluated the risks to the cave weta (Family: Rhaphidophoridae), a common native New Zealand invertebrate similar to a grasshopper or cricket. One out of 16 wetas collected and analyzed was found to have SN residue of 10 µg, suggesting the potential for bioaccumulation and secondary poisoning is extremely low. Shapiro et al. (2017) go on to suggest that based on the dietary LD_{50} calculated for chickens, a 1 kg chicken would need to consume over 25,000 weta (each with a residue of 10µg) in quick succession to receive an LD_{50} dose. Therefore it is highly unlikely that there would be any secondary exposure (indirect or cumulative) effects to an animal that may consume a plant or animal that could have received a sub-lethal dose of SN.

Another potential route of secondary exposure identified was the possible exposure to feral swine vomitus (vomited material) to non-target wildlife. Pen studies have shown that 70% of feral swine vomited after consuming a lethal dose of HOGGONE. Snow et al. (2017b) evaluated the potential risks of vomitus (vomit) to non-targets and found that residual SN in vomitus degraded quickly in a hot, humid environment. Residual SN was found to have decreased by 50% in less than 4 days and had nearly completely degraded in 25 days. The authors also noted that vomitus was difficult to accurately weigh and collect because it primarily had a liquid consistency and that undigested bait was usually found in scarce amounts. Vomitus would also likely be randomly distributed, making it difficult for non-target animals to find. In addition, the residual SN in vomitus is exposed to the digestive tract, and therefore, the micro-encapsulation would have been dissolved, giving the vomitus a strong salty taste that is likely aversive to scavengers or non-target wildlife. Given these parameters, WS-NWRC believes it would be highly unlikely that non-target wildlife would find and consume enough vomitus to receive a lethal dose and therefore minimizing any indirect and cumulative effects.

Lastly, and likely the most probable concern, would be for non-target or scavenger species that may consume carcasses of feral swine that have consumed a lethal dose of SN. This concern is discussed in detail below.

Indirect Impacts on Scavenging Species

The potential for scavenging species such as predators, free-ranging dogs, vultures, raptors, and any other non-target animals that may consume carcasses has been analyzed. Sodium nitrite is metabolized quickly by feral swine that consume the product with negligible residues reported in muscle tissue. Lapidge et al. (2012) and (Snow et al. 2017b) found residual SN in muscle, liver, and eye tissue to be very low (average 3.2 mg/kg). The U.S. Food and Drug Administration regulates that no more than 200 mg/kg of sodium nitrite can be used as a preservative in meat products.

Risk estimates for non-target animals e.g. bald eagle (*Haliaeetus leucocephalus*) show they would need to consume more than 300 times their daily food consumption rate to exceed an acute oral dose of sodium nitrite based on residues that could occur in muscle tissue. Similar estimates of low risk have also been shown for other scavengers (Snow et al 2017b). Snow et al. (2019) found no observable effects to coyotes when allowed to feed freely on SN-dosed feral swine carcasses for 24 hours. Sixteen coyotes were given feral swine carcasses, 8 coyotes were given SN-dosed feral swine carcasses and 8 coyotes were given placebo-dosed carcasses. There were no mortalities and no difference in the consumption rates for each group. Another study by Texas Parks and Wildlife Department (TPWD) assessing the secondary effects of SN on turkey vultures (*Cathartes aura*), has shown SN from carcasses is minimal to no risk to vultures (Foster 2018). TPWD dosed 4 feral pigs at 600 mg/kg of SN (one and a half times the lethal dose) and presented them to 4 groups of 5 vultures (3 treatment and 1 control group). Vultures fed freely on the carcasses for one week. The entire carcasses were consumed (with the exception of the hair and bones) by vultures, and no effects were observed.

Despite the extremely low residues found in muscle tissue and the apparent lack of risk, the digestive tract (stomach, stomach contents, and the small intestines) showed elevated levels of SN and hence a greater risk of exposure to scavengers. However, Snow et al. (2017b) found that approximately 90% of sodium nitrite residues in the stomach of feral swine are lost within three hours due to metabolism and degradation. These residues in the stomach contents are susceptible to environmental degradation, reducing the time for exposure to scavenging non-target animals. Estimates assuming that scavengers only feed on undigested stomach contents show potential acute risk (Snow et al. 2017b). However, these estimates are conservative since they don't assume any degradation of sodium nitrite and that scavengers would preferentially feed only on undigested stomach contents. Shapiro et al. (2016) cited that SN has an aversive taste, so it must be encapsulated to mask the taste. Once the SN has been consumed and the encapsulation removed by the acidic stomach, it again, becomes very unpalatable to potential scavengers. Wade and Brown (1982) also suggest that many predators/scavengers will choose to consume rumen or stomachs last and that bald eagles typically do not eat the stomachs.

Muscle tissue that makes up a larger percentage of biomass from a feral swine would also be present for scavenging and with negligible sodium nitrite concentrations. In most cases scavengers would preferentially consume muscle tissue over stomach contents, reducing sodium nitrite exposure to non-target wildlife. Snow et al. (2019) saw evidence of this when only 2 of the 16 coyotes consumed stomachs in a pen study. Those 2 were also in the placebo group, meaning there was no SN in the stomach. Of the 8 coyotes that were feed SN dosed carcasses, none consumed stomachs. Scavengers would also have to consume stomach contents quickly to receive a lethal dose since consuming it over a longer period would allow sodium nitrite metabolism, reducing the potential for acute risk (Snow et al 2017b).

In another study, TPWD fed HOGGONE *ad libitum* to four pigs, and the stomach and upper intestines were harvested. Only the stomachs and upper intestine (1 pair/cage) were presented to 3 groups of 5 turkey vultures and a control group of 2 vultures. Birds were monitored visually and with a remote camera for 24 hrs. Stomachs and tissues were nearly completely consumed, only a portion of the food bolus from stomachs were not consumed. The remaining contents were removed after 24 hours to avoid forced consumption of the food bolus. No mortalities or effects have been observed. Although the residual concentrations of SN in the stomach and intestines are high enough to be lethal to vultures, it is hypothesized that the vultures are not able to consume them fast enough before effects are quickly reversed by the protective enzyme MtHb reductase to produce any observable effects (Foster 2018).

Summary of Indirect Impacts to Non-target Species

WS-NWRC identified four possible routes of secondary exposure to SN:

1) Potential exposure via consuming contaminated drinking water. The estimated aquatic residues are significantly below any effects data for all aquatic species and are also far below safe drinking water standards, therefore there is almost no risk of secondary exposure to SN via drinking water to non-target wildlife.

2) Consumption of vertebrates, invertebrates or plants that may have been exposed to SN. Due to the biological process in which SN effects are quickly reversed by the protective enzyme MtHb reductase in animals, no bioaccumulation of nitrite occurs. Therefore, it is highly unlikely that there would be any secondary exposure (indirect or cumulative) effects to an animal that may consume a plant or animal that could have received a sub-lethal dose of SN.

3) Possible exposure to feral swine vomitus (vomited material) to non-target wildlife. WS-NWRC found that vomitus degraded quickly in the environment and that it would be randomly distributed, making it difficult for non-target animals to find to consume. In addition, any exposed SN in vomitus would have a strong salty taste that is likely aversive to scavengers or non-target wildlife. Therefore, it is unlikely that non-target wildlife would find and consume enough vomitus to receive a lethal dose, minimizing any indirect and cumulative effects.

4) Non-target or scavenger species that may consume carcasses of feral swine that have consumed a lethal dose of SN. Sodium nitrite is metabolized quickly by feral swine that consume the product with negligible residues reported in muscle tissue. Although low residues of SN are found in muscle tissue, the digestive tract (stomach, stomach contents, and the small intestines) have showed elevated levels of SN presenting a greater risk to scavengers. However, approximately 90% of sodium nitrite residues in the stomach of feral swine are lost within a few hours due to metabolism and degradation. Furthermore, once the SN has been consumed and the encapsulation removed by the acidic stomach, it becomes very unpalatable to potential scavengers.

In summary, there are some minimal risks to scavenging species but given the biological properties of SN and the available data, these risks are not considered cumulative, but very minor and short term.

3.1.4 Humaneness / Ethics

The EA (USDA 2017) analyzed the No Action and the Proposed Action alternatives and their potential effects on humanness and ethics. The discussion in the EA focused on the pain or suffering that an animal may have from consuming a lethal dose of sodium nitrite. Therefore, the humaneness discussion in Section 3.2.4 of EA remains valid and relevant to this supplement. The American Veterinary Medical Association (AVMA) also updated its guidelines for the euthanasia of animals in 2020 (AVMA 2020) and new information was reviewed for this Supplement. In addition, changes made to the concentration of the product (i.e.,10% reduced to 5%) are not expected to change the product's humanness but does warrant some additional discussion and analysis.

It is plausible the reduction in SN concentration from 10% to 5% may cause pigs to ingest a lower dose resulting in a slower onset of death and therefore potentially causing a less humane death. A recent study confirmed this theory while evaluating the humaneness of sodium nitrite's use for mass euthanasia (Pepin 2020). During an emergency depopulation of commercial pig facilities caused by COVID-19 supply chain disruptions, the Research Report (Pepin 2020) found increased concentrations of SN given by an oral drench resulted in a quicker onset to death than lower concentrations. Although not directly comparable because of the differences in how the SN was administered, the findings do indicate that higher doses of SN resulted in quicker death.

However, an important distinction between Pepin (2020) and field testing of HOGGONE 2 is the criteria used by the US Food and Drug Administration to evaluate the humaneness of drugs or other substances used for euthanasia and depopulation of domestic animals differs considerably from free ranging wildlife or feral animals. Based on euthanasia guidelines from AVMA (AVMA 2020), section S7.6.1 Free Ranging Wildlife states *"Because of the variety of situations that may be encountered, it is difficult to strictly classify methods for termination of free-ranging wildlife as acceptable, acceptable with conditions, or unacceptable. Furthermore, classification of a given method as a means of euthanasia or humane killing may vary by circumstances. These acknowledgments are not intended to condone a lower standard for the humane termination of wildlife. The best methods possible under the circumstances must be applied, and new technology and methods demonstrated to be superior to previously used methods must be embraced…….acknowledging that the quickest and most humane means of terminating the life of freeranging wildlife in a given situation may not always meet all criteria established for euthanasia"*

The earlier version of HOGGONE (10% concentration) was developed with the idea that higher concentrations would result in a quicker onset of death and would potentially be more effective and humane. The decision to reduce the concentration from 10% to 5% assumed that decreasing the concentration would increase palatability for feral swine, therefore resulting in more bait consumed and less bait spilled on the ground, resulting in fewer impacts to non-targets and the environment. Snow (2021) showed that pigs consumed more bait with a 5% concentration compared to the amounts consumed when fed the 10% concentration. By consuming more bait, even at a lower concentration, NWRC believes intoxication rates were similar or perhaps better than the higher concentrated HOGGONE resulting in similar times of death.

Current WS-NWRC research also indicated distances feral swine moved after consuming a lethal dose were similar between the 10% bait and 5% bait, providing little evidence of differences of effects between the baits (Snow et al. 2021). Additionally, field efficacy was estimated at 66% mortality with a 10% bait and was estimated at 76–90% with a 5% bait (Snow et al. 2020). WS-NWRC acknowledges that a higher concentration of SN could shorten the time of death but is also dependent on how palatable the bait is and subsequently how much bait is consumed. Additionally, WS-NWRC has an obligation to strike a reasonable balance between administering an efficient, humane, safe, toxicant to wild free ranging feral swine, while taking every reasonable precaution to avoid negative impacts to non-target animals and the environment. Based on the most recent research, the reduction in concentration from 10% to 5% SN, within the constraints of the circumstances, has accomplished this goal.

3.2 Summary of Impacts

This study will likely only result in the removal of approximately 200 or less feral swine from the study site in Texas. An improvement in habitat conditions in the immediate area where swine are removed could be expected. This improvement would be a result of less habitat destruction from feral swine. Although the removal of approximately 200 feral swine at the study site would likely be beneficial, it would still be considered minor and insignificant due to the small scale of the study.

The analysis suggests that based on the methodology of the study, the amounts or concentrations of sodium nitrite used and its potential exposure to the terrestrial and aquatic environment, any direct or indirect effects of conducting the study would be insignificant or discountable. Due to the short-term time frame of this study, any cumulative effects are expected to be insignificant.

WS-NWRC hopes to use information from a successfully completed field trial to apply for EPA registration of the product HOGGONE 2. However, even if the field trial is successful and WS-NWRC pursues registration, EPA registration is not certain. This study is designed with EPA guidelines to provide the required efficacy data for EPA registration. The registration of a product necessitates several data requirements that include but are not limited to product chemistry, toxicology (human), ecological effects, environmental fate, residue chemistry (for food use), and product performance (lab and field studies, i.e., Proposed Action).

A considerable amount of these data requirements have not been completed. Therefore, due to the complexity and the potential timeframe of the registration process (2-3 years), the other data requirements for EPA registration, and the unknown results of this proposed study, WS-NWRC believes any cumulative effects analysis of the potential registration of the product would be premature and inappropriate at this time due to the lack of completed data that could be used in a meaningful analysis.

Based on the analysis in this Supplement to the EA (USDA 2017), WS-NWRC has determined that the Proposed Action is not expected to have significant or adverse direct, indirect or cumulative impacts on to human health and pet safety, the terrestrial and aquatic environment, non-target and T&E wildlife or the humaneness of research activities. Based on experience, the methods and strategies considered in this document are limited in nature, and will not result in significant environmental impacts. The EA (USDA 2017) provided two tables in section 3.4.1 summarizing the environmental effects on the issues addressed in the EA. These summary tables remain relevant and are applicable to this Supplemental summary of impacts.

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APPENDIX A. Literature Cited

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APPENDIX B. Bait Station Specifications

APPENDIX C: Responses to public Comments

There was a total of 4 comment letters submitted during the public comment period. Some commenters expressed more than one topic in their comment. These comments have been summarized into 4 topics and are addressed below.

1. **All the commenters recognized the damages that feral swine cause and expressed their support for the Proposed Action to continue the field evaluation of Hoggone® 2.**

Response: Wildlife Services (WS) agrees that feral swine are detrimental to many resources. These comments are consistent with peer reviewed research referenced in the EA and Supplement and relate to the purpose and need of the EA. The Need for Action is addressed in 1.2 of the EA, and 2.2 of the EA describe that it is the mission of WS and its research arm, NWRC, to evaluate damage situations and develop methods and tools to reduce or eliminate damage and resolve land use conflicts. WS' thanks the commenters for the letters of support for the field evaluation of Hoggone® 2.

2. **Several commenters expressed their support for the development and potential future registration and use of the product.**

Response: WS appreciates those comments in support of this important research.

3. **Several commenters expressed pleasure with the diligence NWRC has taken to research and improve the product while continuing to reduce the effects of sodium nitrite on non-target wildlife.**

Response: WS appreciates those comments and agrees that the effects on T&E species and non-target wildlife is adequately addressed in section 3.2.3 of the EA and 3.1.3 in the EA Supplement. Section 3.1.3 in the Supplement also addresses the possible indirect and cumulative effects of the proposed action on non-target wildlife and scavengers in detail.

4. **Several commenters expressed their support for the research but were concerned with how long the research and potential future registration process is taking.**

Response: WS understands that this research has taken several years to complete. However, WS-NWRC takes the research and product development very seriously. It is imperative that the product is effective at controlling feral swine while also having no significant effect on the environment.