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SUPPLEMENT TO THE ENVIRONMENTAL ASSESSMENT:

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

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APPENDICES

1.0 NEED FOR ACTION AND SCOPE OF ANALYSIS

1.1 Introduction

An Environmental Assessment (EA) was prepared by the United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS), Wildlife Services (WS) to analyze the potential effects on the human environment to conduct field trials of HOGGONE® in Texas and Alabama on free ranging feral swine in 2017. 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.

The baiting strategy was relatively effective as a toxicant for free-ranging feral swine, however, during the trial, it was discovered that feral swine were spilling or dropping more bait than what was observed in pen trials. This bait spillage caused the non-target take of several passerine birds, some non-target 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 was able to 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.

In March 2018, the Australian HOGGONE® product developer, in collaboration with WS's National Wildlife Research Center (NWRC) began work to resolve the palatability issues with the bait and have made a number of important changes to further reduce any non-target risks with the product. These changes were implemented and analyzed into several small-scale trials in 2019 and 2020 to determine their effectiveness USDA (2019).

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). This supplement to the EA examines the potential environmental impacts of conducting field trials that would incorporate new information that has become available from research findings and data gathering since the issuance of the Decision and FONSI in 2017.

The unexpected non-target mortalities during the 2018 field trial in Texas led to modifications to the bait station, the formulation of the bait, and the baiting strategy. Specifically, WS revised the bait station to accept small, compacted trays of the SN toxic bait to limit the ability of feral swine from scooping 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. Finally, WS revised the baiting strategy to reduce the attractiveness of the bait sites to non-target animals. The amount of pre-baiting time was also decreased with freely available whole-kernel corn by 4–6 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 to avoid any remnant particles of whole-kernel corn that might attract granivorous birds. Finally, 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 and an analysis of their potential effects are addressed in this supplement.

1.3 Objectives

The objectives of the study remain consistent and are described in section 1.4 of the EA (USDA 2017). Additional objectives have focused on the effects of non-target birds 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. Unless otherwise discussed in this supplement, the scope of the analysis and the site-specificity 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.

Environmental Assessment – A Small Scale Field Evaluation of HOGGONE 2 ® Sodium Nitrite Toxicant Bait for Feral Swine: The WS program prepared a separate 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 USDA (2019).

Study protocol – Field Evaluation of HOGGONE 2 ® for Feral Swine: 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. However, the study protocol incorporates specific details regarding methodology that is not covered or applicable to this supplement and therefore is not included in this document.

1.7 Public Involvement

Issues related to the proposed action were initially developed by WS-NWRC. The document was noticed to the public through http://www.regulations.gov and on the APHIS website at: http://www.aphis.usda.gov/wildlife_damage/nepa.shtml. An invitation for public comment on the pre-decisional EA was sent to over 4000 individuals and organizations interested in feral swine projects in Alabama and Texas. Notices of the proposed action were also posted in the *Montgomery Advisor* in Alabama and *The Austin Statesman* in Texas, each with statewide circulation. There was a 30-day comment period for the public to provide input on the pre-decisional EA. There were 88 comments

received from the public after review of the pre-decisional EA. All comments were analyzed to identify substantial new issues or alternatives. A decision and FONSI was signed November 20, 2017. No comments were received.

This supplement, along with the EA (USDA 2017), and the associated Decisions and FONSI 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 at a minimum in the *Montgomery Advisor* in Alabama and *The Austin Statesman* in Texas, sent to interested parties via the APHIS stakeholder registry, and posted on the APHIS website. Comments received during the public involvement process will be fully considered for new substantive issues and alternatives.

1.8 Laws Related to this Discussion

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.

Pursuant to NEPA and CEQ regulations, the EA (USDA 2017) documented the analysis for potential impacts of a proposed federal action, informed decision-makers and the public of reasonable alternatives capable of avoiding or minimizing adverse impacts, and served as a decision-aiding mechanism to ensure that the policies and goals of NEPA were infused into the federal agency action. The EA was prepared by integrating as many of the natural and social sciences as warranted, based on the potential effects of the proposed action. The direct, indirect, and cumulative impacts of the proposed action were analyzed.

The EA also identified several other laws relevant to the proposed action in section 1.10 USDA (2017). The EA recognized the Endangered Species Act, the National Historic Preservation Act, Executive Order on Environmental Justice, Executive Order on Protection of Children from Environmental Health and Safety Risks, Invasive Species - Executive Order 13112, The Native American Graves and Repatriation Act of 1990, and the Federal Insecticide, Fungicide, and Rodenticide Act. These laws remain relevant and valid for this supplement.

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. The updated regulations include elements of the One Federal Decision policy, codify case law and CEQ guidance, revised regulations to reflect current technologies and agency practices, eliminated obsolete provisions, and improved the format and readability of the regulations. These revised regulations went into effect on September 14, 2020. This Supplement was prepared under this new CEQ guidance.

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 during the scoping of the EA and remain relevant to this Supplement:

Effects on Human Health and Pet Safety

Impacts on Terrestrial and Aquatic Environments

Effects on Non-Target and T&E Species

Humaneness / Ethics

Other issues were identified in the EA but were not discussed in detail and 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

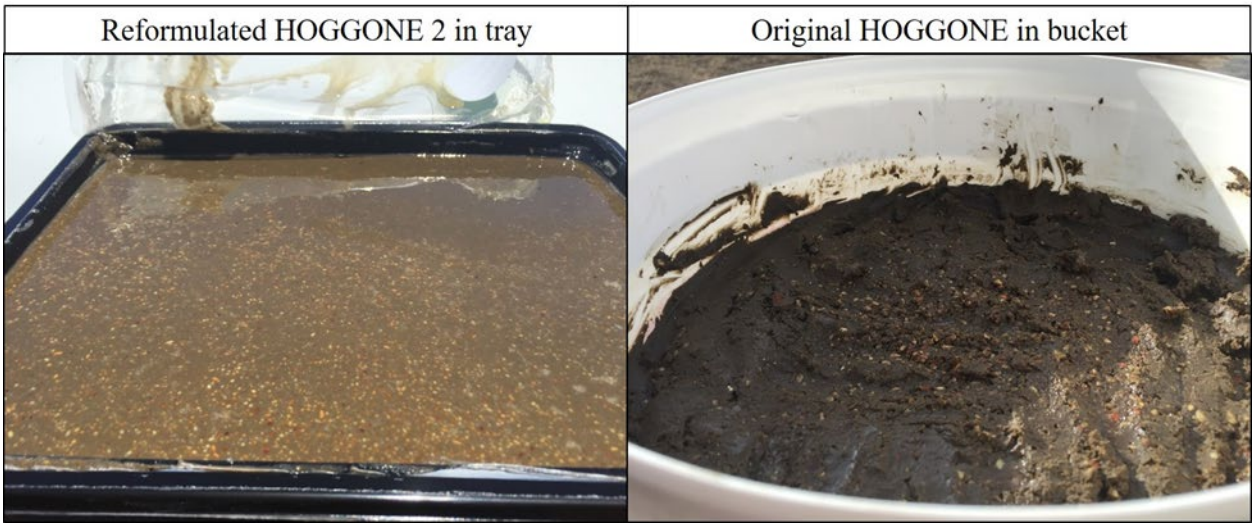
The basic study protocol identified in section 2.2 of the EA remains valid for this supplement, however, several changes were made to the product based on results from the original 2018 trial and from several small-scale trials in 2019 and 2020. These changes are identified and discussed in chapter 2 and their potential effects are analyzed in chapter 3 of this Supplement to the EA (USDA 2017).

The original study protocol called for targeting 3-9 sounders (average of 6-16 pigs per sounder) of feral swine per geographic location. 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 manufacture has reformulated the bait (HOGGONE 2 ®) and some slight changes were made to the bait stations to reduce spillage. The manufacture, in cooperation with WS-NWRC tested the reformulated HOGGONE 2 and the new presentation method in Queensland, Australia and found that 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 palpability and a reduction in spillage and hence, a reduction in non-target take as a result 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 the crushed grains removed 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 and 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 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 attractiveness of spilled bait to granivorous birds



2.2 Description of Study / Data Analysis

The description of the study and the data analysis discussed in section 2.2.2 and 2.2.3 of the EA (USDA 2017) remains valid except for a few minor changes described here in this Supplement.

Handling of captured feral swine will be conducted with the aid of chemical immobilization drugs. 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 and therefore this revised trial will include marking opossum as well as 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. This is to help prevent birds and other non-target wildlife that may have become habituated specifically to that site and 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 that held a total of 20 kg of bait. The original protocol called for two nights of toxic baiting and this revised protocol calls for just one night of toxic baiting at a time. 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-targets. 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 there being 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, 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. Secondly, the effects analysis in the EA (USDA 2017) was structured under the CEQ's 1978 implementing regulations which were revised and put into effect September 14, 2020.

This new analysis is structured and follows the new CEQ's definitions and will analyze changes to the human environment from the proposed action or alternatives that are reasonably foreseeable and have a close causal relationship to the proposed action or alternatives. This includes those effects that occur at the same time and place as the proposed action and may include those effects that are later in time or farther removed in distance from the proposed action or alternatives.

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 of the issues. This Supplement will consider the impacts of the changes to the study, their close causal relationship to the proposed action, the effects that may occur at the same time as the proposed action, and those that may occur later in time or father removed from the proposed action.

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). 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 to the terrestrial and aquatic environment rely specifically 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 rather 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 as a particulate in the air pockets in soil because it is not volatile. In the air (both above the soil 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 present in soil and sediment reduce nitrates to nitrogen, which is then absorbed into the nitrogen cycle. Bioconcentration or bioaccumulation of nitrite 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 any exposure to aquatic environments. The use of baits that are 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 percent of the material by weight (5% concentration) 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 recent trials conducted in Australia and small-scale studies in Alabama and

Texas (Snow et. al 2021), the amount of spillage ranged from 0 to 130.2 g with an average of 55.4 g that could end up on the ground. At 5% concentration, that amounts to 6.51g of 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 that all of the 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.

Residues from the above exercise were calculated for three different aquatic habitats: a wetland, small pond and 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 0.00198 parts per billion (µg/L) or .00000198 (mg/L) in a wetland habitat to 5.5 X 10^{-8} mg/L in a small pond. These are considered very conservative estimates of potential aquatic residues and 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 provides a summary of available nitrite toxicity data for aquatic vertebrates. Nitrite toxicity varies considerably between different fish species, ranging from highly toxic to practically non-toxic (Figure 1). Cold tolerant freshwater species such as salmonids appear to be the most sensitive fish species with median lethality concentrations (LC50) 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 types of effects result in decreased fitness, reducing reproduction potential and predator avoidance as well as 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).

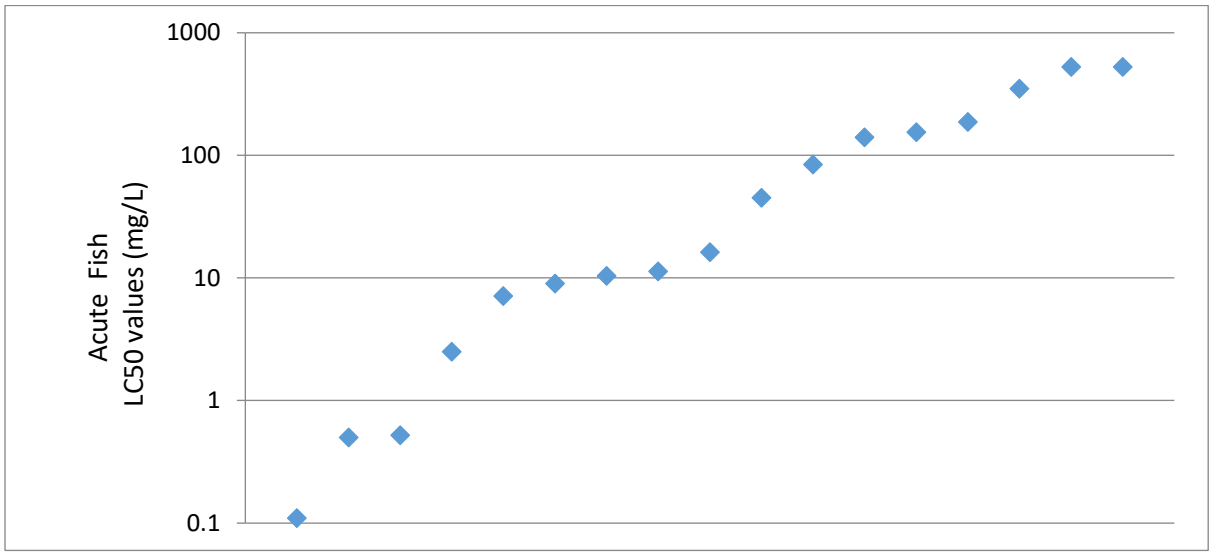


Figure 1. Distribution of acute nitrite sensitivity to freshwater and marine fish species.

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.

Risks to Aquatic Invertebrates

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 (Figure 2).

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 marine shrimp (*Penaeus monodon*) and reported EC50 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 LC50 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 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).

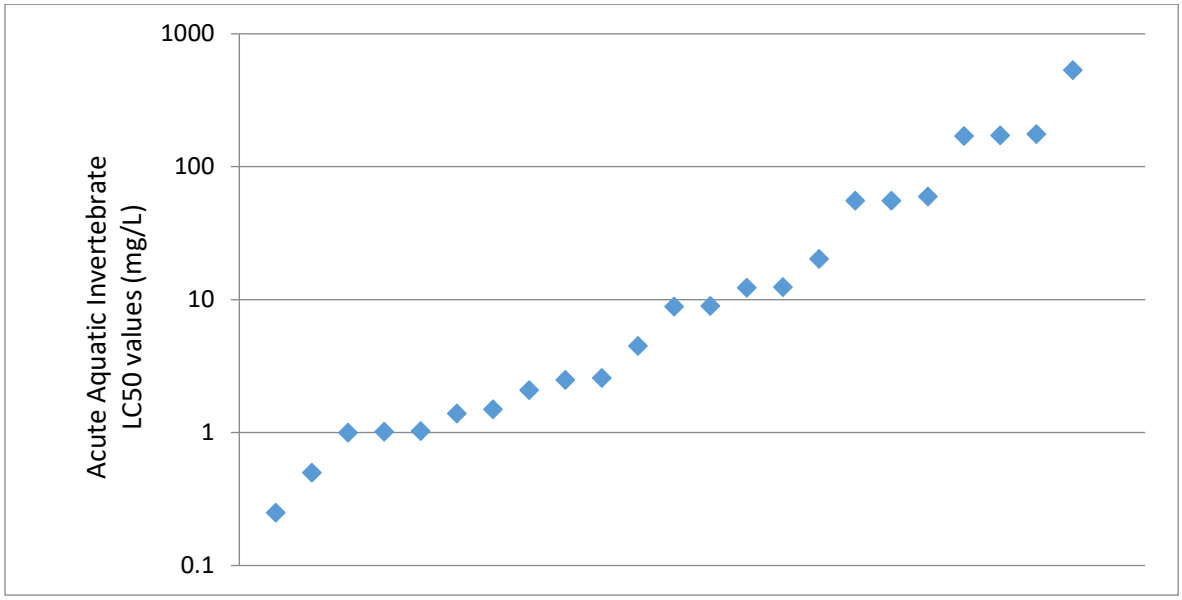


Figure 2. Distribution of acute median lethality concentrations (LC_{50}) for nitrite toxicity to freshwater and marine aquatic invertebrates.

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 that would be 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 3.

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 estimate of aquatic residues in this assessment 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.

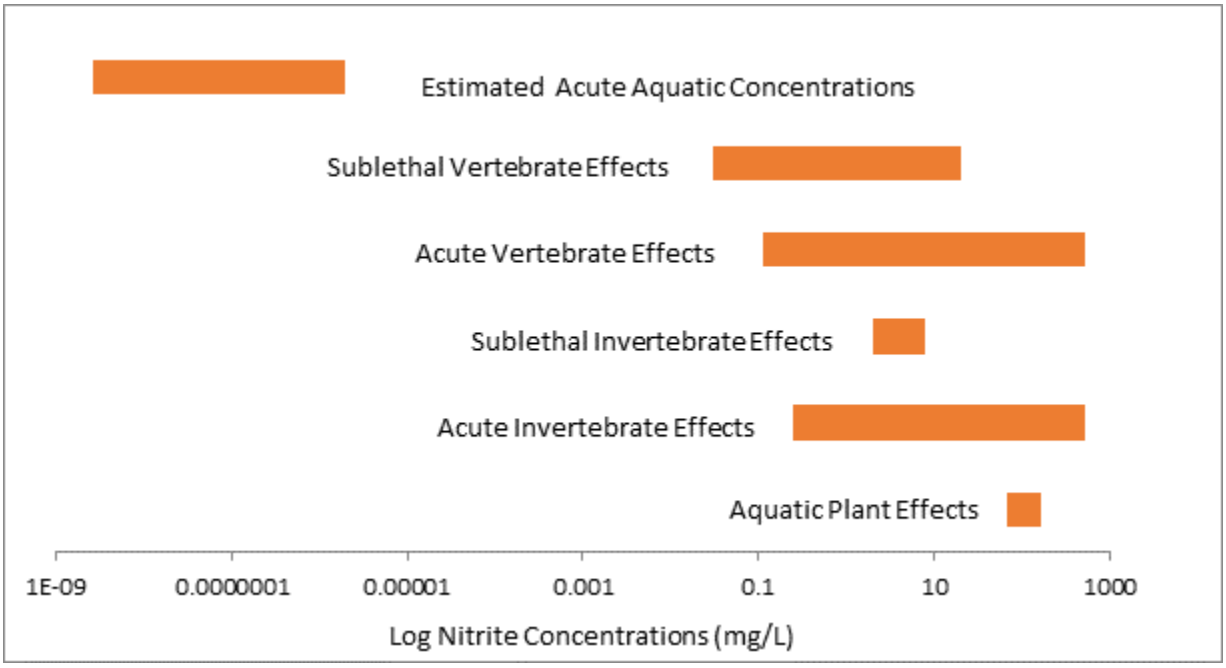


Figure 3. Aquatic vertebrate and invertebrate risk characterization for nitrite.

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 nitrite exposure due to a short application period. Furthermore, referring to figure 3, there are still wide margins of safety 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 presents risks to these

organisms that are insignificant and discountable. This includes risks that may occur at the same time and those that may occur later in time or father removed from these bait sites.

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 130.2g of bait on the ground with 55g being the average. For this assessment, we will use the worst-case scenario of 130.2g of spillage possible at each bait site. A detailed analysis of the potential effects these concentrations may have on non-target and threatened and endangered terrestrial wildlife is addressed below in 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 4. 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.

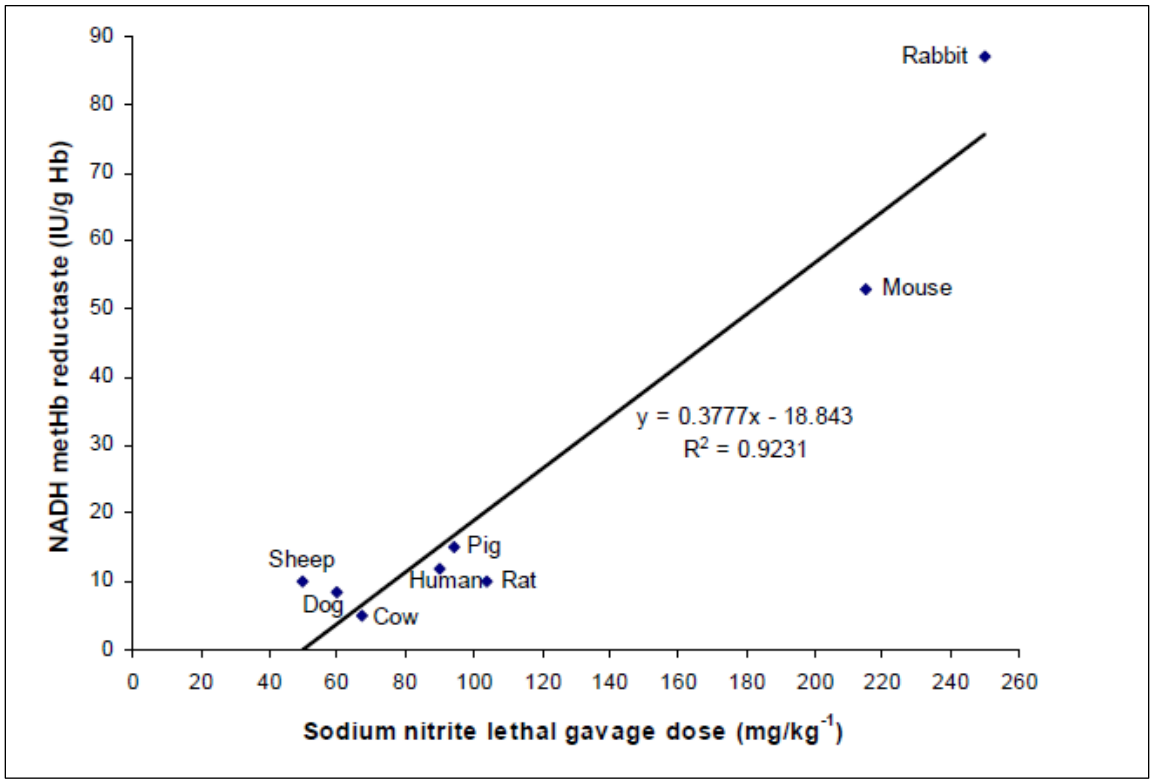


Figure 4. Regression between sodium nitrite lethal doses and NADH (Nicotinamide Adenine Dinucleotide (NAD^+) reduced by oxidization) MtHb reductase levels in various mammal species (from Lapidge and Eason 2010)

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 some of the typical signs of nitrite toxicosis and could impact the ability of non-target mammals to avoid predation as well as 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 and found that with the exception of 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 to 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 $\mu g/cm^3$ (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, but 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 and 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 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.

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 exposures 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 that have been shown to have negative effects on plants. 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. The removal of 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, but any potential risk would be limited to individuals actively feeding on the bait and would not result in population level impacts. A more detailed analysis of non-target mammals and birds is presented below in 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 SOPs 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 effects that may be further removed from the 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., 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, so, 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 effect 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-analyzed 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.

<u>Effects on Threatened and Endangered Species</u>

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 two study sites in Alabama and Texas would be selected. Species effects determinations were made for each study location and where applicable, were submitted to the USFWS for concurrence pursuant to Section 7 of the Endangered Species Act.

In Alabama, WS-NWRC considered three counties (Bullock, Mason and Montgomery) for the study site and these species listed in those counties. The species list, designated critical habitat and effect determinations made for those species in Alabama in Section 3.2.3 of the EA (USDA 2017) remain unchanged and is still valid. WS-NWRC' provided a review of the proposed study and these effect determinations in the form of a Biological Assessment (BA) on March 16, 2017 to the USFWS Alabama Ecological Services Field Office located in Daphne, Alabama. The USFWS provided a letter of concurrence on April 27, 2017.

In 2019, WS-NWRC planned to conduct a small-scale trial in Alabama with the revised HOGGONE® 2 formula (5% concentration) and due to the changes in the bait formulation described in the 2017 BA, WS-

NWRC decided to re-initiate the informal consultation. WS described the changes in the bait formulation and the study protocol (which reflect the current formulation and protocols) in a letter to the USFWS on May 7, 2019. WS received a letter of concurrence from the USFWS regarding the bait and protocol changes on June 25th, 2019. With improvements made to the formulation and protocol, WS believed any effects on non-targets and T&E species would be reduced, however, erroring on the conservative side, WS chose to keep the species effect determinations the same as in the original March 16, 2017 BA.

In Texas, WS-NWRC considered nine counties (Archer, Baylor, Cottle, Foard, Hall, Knox, Motley, Wichita and Wilbarger) for the study site and the T&E species in those counties. 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) remains valid with one exception, the least tern was delisted from the federal threatened and endangered list effect February 12, 2021. WS-NWRC made No Effect determinations for the species listed in Texas and provided a letter to the USFWS Texas Ecological Services Field Office located in Arlington, TX on June 5, 2017. With improvements made 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.

Section 3.2.3 in the EA (USDA 2017) sub-heading *Dissimilarities of Threatened and Endangered Species between Study Sites* describes differences between the study sites in Alabama and Texas and provides rational for the different species effects determinations made for each location. The sub-heading *Alabama Species* and *Texas Species* provide additional rational for the species determinations made in each location. These sections and rational remain valid and are still relevant to this Supplement.

<u>Effects on Non-target Mammals and Birds that May Occur at the Same Time</u>

An effect that may occur at the same time 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 have 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 that white-tailed deer and raccoons were the most frequently observed non-target species that visited placebo HOGGONE® 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.

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 access to 75% of feral swine to the bait station. Therefore, it was assumed in original the HOGGONE® trial in 2018 that all non-target birds and mammals smaller than a raccoon will be completely excluded from the bait station.

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 from the pre-bait locations just prior to toxic baiting. 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 effect feral swine feeding but could significantly reduce bird feeding at the new toxic site. A recent unpublished pen trial of 40 red-winged blackbirds were tested using this scenario and allowed to feed freely next to a toxic bait station and resulted in the taking of 1 blackbird.

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 1).

Table 1. 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 have 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 2). 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 blackbird 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 2. 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 were able to access the bait stations and therefore bait station lid tension will remain unchanged for this study. However, the other possible direct exposure route or an effect that may occur at the same time and place for non-target wildlife that was 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, a total of 179 non-target animals were taken (Table 3) 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 3. 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 3), 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 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 with a reduced concentration and a decrease in the total amount of bait in the bait stations has reduced the estimated spillage to approximately 6.5 g of

active ingredient. Therefore, the HOGGONE® 2 improvements have reduced the potential of active ingredient spillage by about two thirds.

The first trial to test these improvements was conducted by the manufacturer in collaboration with WS-NWRC. 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 2018 Texas trial, down to 0.055kg in 2018 Australia trial) while maintaining an effective lethality rate for feral swine. There were 3 non-targets (Australian ravens) which resulted in 0.2 non-targets per bait station (Snow 2021). These results, although not completely comparable to the U.S. because non-target species and conditions are different in Australia, were still very encouraging and provided evidence and rational to pursue 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 4) for 0.4 non-targets per bait station which was consistent with the earlier trial in Australia.

Table 4. 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 5).

Table 5. 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 5) prompted WS-NWRC to explore more ways to exclude or deter birds (particularly small passerine birds) from feeding in and around bait stations to further reduce the chance of non-target take. 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 first light (i.e. 05:17 am). 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 6).

Table 6. 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

These results in Table 6. 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).

This data indicates a reduction in non-target take from the modifications made to the product and the overall application strategy. 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 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.

However, based strictly on the active ingredient, it would still be possible to take an unacceptable number of non-targets with the maximum amount of predicted spillage of 130.2g of bait (6.51g of active ingredient, or 0.23 oz.). For example, a group of 4 kg raccoons would each need to eat 232 mg (58mg/kg x 4 kg) of active ingredient for half of them to be killed. This would appear that 6.51 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) and 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 (of those 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 it would be possible to take 33 (130.2 g bait divided by 4 grams per bird) average sized red-winged black birds or similar species if they were to feed on the maximum amount of spilled bait observed in Snow et al. (2021). This could be possible if a flock of birds were to feed on the precise amount of spilled bait. However, this is not realistic, 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 sounders at each geographic location (Alabama/Texas). As a high estimate, 9 sounders with an average of 22 feral swine per sounder would be approximately 200 feral swine at each geographic location. This would require approximately 40 bait stations total for both locations to administer lethal doses to approximately 400 feral swine. Based on the scenarios above, it would be possible to take up to 400 non-target blackbirds and 50 non-target raccoons/opossums. Again, this would be a scenario where there is a maximum amount of bait spilled (130.2g) at every bait station. This is not realistic or expected. Based on the most available data from a trial in Australia and the three

small-scale trials conducted in the U.S. (Snow et al. 2020, 2021), non-targets averaged 1.2 per bait station. With the deployment of 40 bait stations, a likely estimate of non-target take would be 48 non-targets. If observed in the same ratios, 10% would be mammals (approximately 5) and 90% birds (approximately 43) for the entire study encompassing both geographical locations. However, with the additional modifications to the baiting strategies described in this supplement, WS believes non-target take will be sustainably less than described here.

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 exposure 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.

Effects to Non-Target Mammals and Birds that may occur later in time from the Proposed Action

Effects that may occur at a later time 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 the consumption of contaminated drinking water. As noted above 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 is also discussed above in 3.1.2. The effects to aquatic plants and invertebrates, 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 reverses 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 above in 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) goes 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 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.

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.

Effects on Scavenging Species that May Occur at a Later Time

The potential for scavenging species such as predators, free-ranging dogs, vultures, raptors and any other non-target animal 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 such as the bald eagle show they would need to consume greater 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. In another study by 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 treatments 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 scavengers would preferentially feed only on undigested stomach contents. Shapiro et al. (2016) cited that SN has an aversive taste and therefore 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 predator/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 the exposure of sodium nitrite 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 of time would allow metabolism of sodium nitrite, reducing the potential for acute risk (Snow et al 2017b).

In another study, TPWD fed HOGGONE® *ad libitum* to four pigs, 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 with 2 vultures. Birds were monitored visually and with 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 at 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 to produce any observable effects (Foster 2018). In summary, there are some minimal risks to scavenging species but given the biological properties of SN and the available data, these risks are 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. Changes made to the concentration of the product (10% reduced to 5%) is not expected to change the humanness of the product. Evidence of this was confirmed during the small-scale trials where Snow et al. (2021) found the distance swine traveled after consuming a lethal dose of SN did not vary substantially from each bait concentration. Therefore, the humaneness discussion in section 3.2.4 of EA remains valid and relevant to this supplement.

3.2 Summary of Impacts

This study will likely only result in the removal of approximately 200 feral swine from each study site in Texas and Alabama. 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 each 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 effects that may occur at the same time or at a later time from conducting the study would be insignificant or discountable. Due to the short-term time frame of this study (approximately 6 months in each location), it is not reasonably foreseeable that any effects would occur farther removed in distance or later in time from the proposed action.

WS-NWRC recognizes that registration of the product HOGGONE® 2 could be considered an effect that could occur later in time. However, registration is not certain but could be a reasonably foreseeable future action providing the study results were positive. 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 effects that may occur later in time such as 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 impacts 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 any reasonably foreseeable future actions from this Proposed Action 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 B. Bait Station Specifications

APPENDIX C: Responses to public Comments