Photo courtesy of Marvin Moriarty/USFWS

ENVIRONMENTAL ASSESSMENT

FIELD STUDIES TO ASSESS THE SAFETY AND EFFECTIVENESS OF WHITE-NOSE SYNDROME VACCINE CANDIDATES IN BATS

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ACRONYMS

ACUC	Animal Care and Use Committee
BSL	Biosafety level
cal	Calnexin
DNA	Deoxyribonucleic acid
EA	Environmental assessment
EIS	Environmental impact statement
LD50	Lethal dose 50%
NWHC	National Wildlife Health Center
Pd	*Pseudogymnoascus destructans*
RCN	Raccoon poxvirus
SCID	Severe combined immune deficient
sp	Serine protease
tk	Thymidine kinase
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
WNS	White-nose syndrome

ENVIRONMENTAL ASSESSMENT OF FIELD STUDIES TO ASSESS THE SAFETY AND EFFECTIVENESS OF WHITE-NOSE SYNDROME VACCINE CANDIDATES IN BATS

1. INTRODUCTION

The U.S. Geological Survey (USGS) National Wildlife Health Center (NWHC) in Madison, WI, is proposing to conduct field studies to assess the safety and effectiveness of vaccine candidates that have been developed for oral use in bats and are designed to control white-nose syndrome (WNS) in free-ranging bats. The experimental vaccine candidates use raccoon poxvirus to carry genes of *Pseudogymnoascus destructans*, the causative agent of WNS. The field studies will assess the ability of the vaccine candidates to confer protective immunity against WNS to bats that ingest the vaccines. Prevention of WNS in bats is a vital concern for ongoing conservation efforts for bats.

Pilot field studies will be performed in restricted sites in Minnesota (See Figure 1), Texas (See Figure 2), and Wisconsin (See Figure 3). in areas where little brown bats (*Myotis lucifugus*) and tricolored bats (*Perimyotis subflavus*) are known to inhabit to assess the safety and effectiveness of the vaccine candidates. The specific sites will be selected just prior to field work based on the presence of bats and the total population.

Maps for Minnesota, Texas, and Wisconsin sites

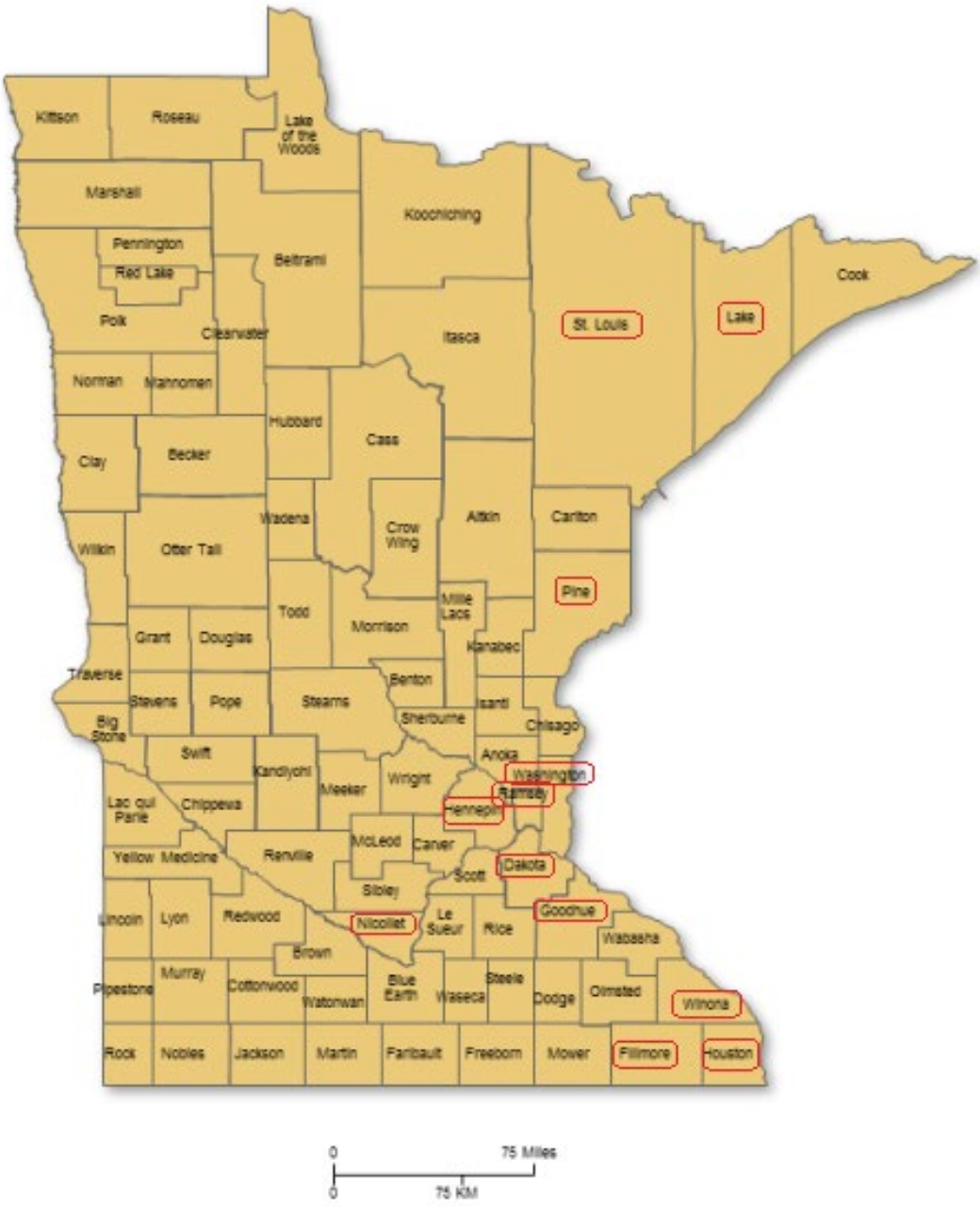

Figure 1: Selected Minnesota counties for field studies – Dakota, Fillmore, Goodhue, Hennepin, Houston, Lake, Nicollet, Pine, Ramsey, St. Louis, Washington, and Winona

Figure 2: Selected Texas counties for field studies – Freestone and Leon

Figure 3: Selected Wisconsin county for field studies – Pierce

2. HISTORY

White-nose syndrome, caused by the fungus *Pseudogymnoascus destructans* (Lorch et al., 2011), is an emerging disease of hibernating bats in North America (Blehert et al., 2009). The fungus thrives in the dark, cold, and moist environments of caves used by bats for hibernation and is well adapted for growth on the skin of hibernating bats (Verant et al., 2012). The disease is named for the fuzzy white fungal growth that appears on the muzzles, ears, and/or wings of affected bats. The fungus invades the wing membranes of bats, altering their ability to regulate body temperature, hydration status, and other important physiological processes (Cryan et al., 2012; Reeder et al., 2012; Verant et al., 2014). Infected bats often exhibit abnormal behavior during hibernation, including more frequent arousals, daytime flying, and movement toward cave mouths. This increase in activity uses up stored fat reserves which can eventually lead to severe emaciation and death (Reeder et al., 2012). First recognized in little brown bats (*Myotis lucifugus*) in New York State in the winter of 2006-2007, WNS has steadily spread west and south and has been confirmed as far west as the state of Washington.

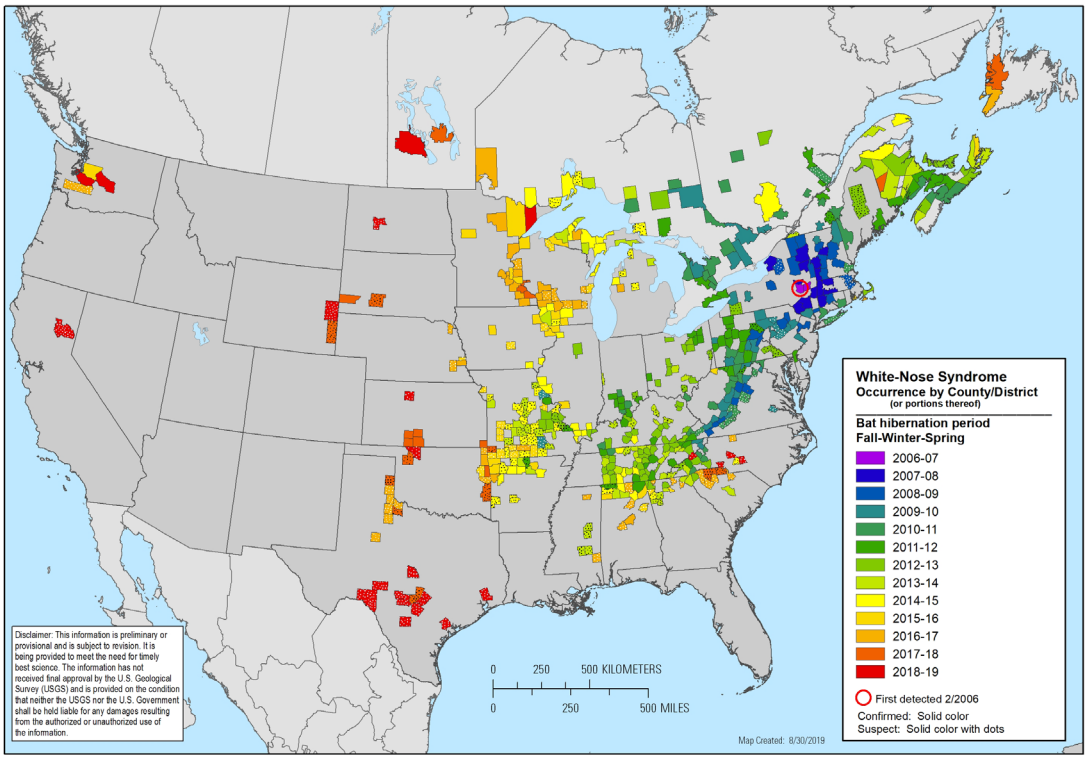

Figure 4: Citation: White-nose syndrome occurrence map – by year (2019). Data Last Updated August 30, 2019. Available at: https://www.whitenosesyndrome.org/

3. PURPOSE AND NEED FOR THE ACTION

The purpose of the proposed action is to assess the safety and effectiveness of WNS vaccine candidates in wild bats after application in the field. The need for these actions is explained below.

Since being recognized as an emerging disease of hibernating bats in North America, WNS has caused an estimated 80% decline in bat populations in the northeastern United States (USFWS, https://www.fws.gov/cno/newsroom/highlights/2017/bat_week_2017, accessed March 2018.) and has been identified in bats from 31 states and five Canadian provinces (USFWS, https://www.whitenosesyndrome.org/about/where-is-it-now, accessed March 2018). Nine species of cave-hibernating bats have been confirmed with WNS (USFWS, https://www.whitenosesyndrome.org/about/bats-affected-wns, accessed March 2018). Among these are the endangered gray bat (*Myotis grisescens*) and the endangered Indiana bat (*Myotis sodalis*). In 2015, the Northern long-eared bat (*Myotis septentrionalis*) was listed by the USFWS as threatened because of WNS, which has caused a population decline of up to 99% at many hibernation sites in the northeastern US (USFWS, https://ecos.fws.gov/ecp0/profile/speciesProfile?sId=9045, accessed July 2017). WNS has the potential to negatively impact populations of more than half the bat species in the US and has been described as "resulting in the most precipitous wildlife collapse of the past century" (Bat Conservation International, http://www.batcon.org/our-work/regions/contact-bci/usa-canada/white-nose-syndrome, accessed July 2017).

The National Plan for Assisting States, Federal Agencies, and Tribes in Managing White-Nose Syndrome in Bats (USFWS, 2011) acts to coordinate surveillance of and responses to WNS by federal, state, and tribal agencies and non-governmental partners. These collaborations have led to greater understanding of WNS including management strategies designed to limit the spread and disease effects of *Pseudogymnoascus destructans*. Many of these approaches include environmental treatment to inhibit fungal growth on bats or in hibernacula. Another method under investigation is vaccination of bats to prevent WNS.

Oral vaccine candidates developed and tested jointly by the USGS NWHC and University of Wisconsin (Madison, WI), are intended as a preventative method for controlling WNS in bats. The vaccine candidates are genetically modified viral vaccines, using attenuated raccoon poxvirus (RCN) as a vector for orally delivering critical *Pseudogymnoascus destructans* antigens to bats. The first is calnexin (cal), a highly conserved ascomycete fungal antigen that has been shown to protect mice against a variety of fungal pathogens (Wüthrich et al., 2015). Indeed, mouse T-cells specific for calnexin (Wüthrich et al., 2011) proliferated in the presence of *Pseudogymnoascus destructans* in initial studies (Wüthrich et al., 2015). The other is a subtilisin-like or serine protease (sp) that was identified in two separate studies as a major proteolytic component of *Pseudogymnoascus destructans* (O'Donoghue et al., 2015; Pannkuk et al., 2015) and likely facilitates tissue invasion by the organism. As major components of the secretome of *Pseudogymnoascus destructans,* these antigens may be attractive targets of the host's specific immune response and disrupting their activity may limit the ability of *Pseudogymnoascus destructans* to cause disease. Initial laboratory trials have demonstrated that intranasal and intramuscular injections of RCN-cal or RCN-cal/RCN-sp vaccines at least

partially protect little brown bats from developing WNS after challenge with *Pseudogymnoascus destructans* (Rocke, unpublished data).

Raccoon poxvirus has been shown to be highly safe in numerous animals (Esposito et al., 1988, 1989, 1992; Fekadu et al., 1991; DeMartini et al., 1993; Osorio et al., 2003a; Mencher et al., 2004; Rocke et al., 2004a, 2006, 2008ab, 2010ab, unpublished; Tripp et al., 2015), including black-footed ferrets, prairie dogs, dogs, cats, sheep, mice, etc.

Glycerin jelly, a semi-solid paste that is easily applied to surfaces, readily ingested by bats, and stable for the vaccine, has been selected for field delivery of the vaccine candidates. Glycerin jelly is composed of 46% glycerin, 46% water, 7% gelatin, and 1% phenol. Glycerin, also known as glycerol, naturally occurs in foods and animals as a component of triglycerides. It is a common food additive recognized as generally safe by the Food and Drug Administration with no known carcinogenic, mutagenic, or teratogenic effects. Previous work using glycerin jelly as an oral vaccine vehicle showed no adverse effects in Brazilian free-tailed bats (*Tadarida brasiliensis*) (Stading et al., 2016) or vampire bats (*Desmodus rotundus*) (Rocke, unpublished data).

Rhodamine B (<0.5% concentration) will be used as a biomarker. Biomarkers are regularly incorporated into baits to evaluate the success of bait distribution studies or to identify animals that have consumed vaccine-laden bait. Rhodamine B is an analytical dye that has been widely used as a marker and tracer in animal studies that marks hair, feces, or blood (Evans and Griffith, 1973; Johns and Pans, 1981; Lindsey, 1983; Fisher, et al., 1999). In preliminary field trials using glycerin jelly with Rhodamine B in vampire bats, jelly has been readily consumed and transferred between bats by grooming activities (Rocke, unpublished data).

Experimental field trials are needed to evaluate the effectiveness of the vaccine candidates in wild bats. Identification of an effective WNS vaccine for wild bats will enable wildlife managers to combat the threat of WNS in hibernating bats with the goal of enhancing the conservation of bats. Without a breakthrough in the fight against WNS, bat populations will most likely continue to decline. Recovery of bat populations affected by WNS is expected to be slow because reproductive females of most bat species produce only one pup each year. Bats play important ecological roles with economic and agricultural impacts. Without bats, farmers and foresters would spend billions of dollars each year to combat insect pests (Kasso and Balakrishnan, 2013). Bats also play a role in protecting human health by consuming vast numbers of mosquitoes. By depositing guano in caves, bats play an important role in maintaining cave ecosystems. As WNS continues to spread across North America and infecting additional bat species, it is important to develop an effective method to prevent additional deaths due to WNS in bats.

3.1 Decision to be made

Based on the scope of this Environmental Assessment, the following questions must be answered:

- Should USGS undertake field trials to determine the safety and effectiveness of WNS vaccine candidates in wild bats?
- If not, should USGS implement another alternative?

- Would implementing the proposed action or an alternative action have significant adverse impacts on the quality of the human environment requiring the preparation of an Environmental Impact Statement?
- When to conduct the trial?

3.2 Scoping and issues

3.2.1 Actions analyzed

This Environmental Assessment evaluates the environmental effects of application of WNS vaccine candidates to assess the safety and effectiveness of the vaccine candidates in wild bats.

3.2.2 Site specificity

The analysis of alternatives is limited to potential study sites in Minnesota, Texas, and Wisconsin and the associated species and habitats, as described in Section 5.

3.3 Summary of public involvement

The environmental assessment, which is based on a risk analysis prepared to assess the risks associated with the field testing of this vaccine and related information, examines the potential effects that field testing this veterinary vaccine could have on the quality of the human environment. Based on the risk analysis and other relevant data, the Responsible Official has reached a preliminary determination that field testing this veterinary vaccine will not have a significant impact on the quality of the human environment, and that a public notice and comment period need not be prepared.

The EA has been prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 et seq.) and (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508). The draft environmental assessment is located at the USGS National Wildlife Health Center website: https://www.usgs.gov/centers/nwhc. The draft environmental assessment will be available on the National Wildlife Health Center public website for 30 days after the date of publication. Unless substantial issues identifying adverse environmental impacts are raised in response to this publication, the USGS intends to issue a finding of no significant impact (FONSI) based on the environmental assessment and initiate the field tests.

4. ALTERNATIVES INCLUDING THE PROPOSED ACTION

This section provides a description of reasonable alternative actions that address the Purpose and Need in enough detail to identify potential environmental impacts. The No-Action Alternative is included as a baseline and for comparison (40 CFR 1508.9(b)).

4.1 Proposed action (Alternative 1)

4.1.1 Project objective and context

Up to three small field trials per state will be conducted over a one-to-two (1 to 2) year period to evaluate the safety and effectiveness of the vaccine candidates for bats under field conditions at selected sites in Minnesota, Texas, and Wisconsin. These studies will provide important information regarding the uptake and safety of the vaccine candidates in wild bats in addition to identifying the most effective vaccine. It will also provide a framework and foundation for future studies examining the use and effectiveness of vaccines to prevent wide-spread WNS-induced mortality of bats.

4.1.2 Proposed activities

The USGS-NWHC is proposing to conduct small field trials to assess the safety and effectiveness of the vaccine candidates in wild bats under field conditions. Cooperating agencies include USFWS, Wisconsin Department of Natural Resources, Minnesota Department of Natural Resources, Texas Parks and Wildlife Department, and Bat Conservation International. Field trials will begin in free-ranging bats (little brown bats and tri-colored bats) in the fall of 2018. These trials will be designed to confirm biosafety and effectiveness of the vaccine candidates in the field. The primary objectives are to evaluate the survival rate and occurrence of WNS in vaccinated bats compared to non-vaccinated bats and to demonstrate "safety" (i.e. absence of vaccine-associated pathology) in wild bats.

Selected sites will be limited in size (e.g., <3 acres) and access and will be amenable to contingency management in the unlikely event of an adverse outcome of vaccine uptake in bats or non-target species. In pilot studies, bats will be hand captured and the vaccine or placebo will be delivered directly into the mouth at the appropriate dosage. In follow-up studies, it will be applied to the bats topically. Relatively high vaccine-laden glycerin jelly application rates (0.5 ml/bat) will be employed to allow maximum contact and uptake by individual animals. Bats captured after vaccine/jelly application will be examined for evidence of vaccine uptake, signs of pox lesions and/or morbidity, and any carcasses found will be submitted for full diagnostic testing. Animals will be released or euthanized depending on their health status, as detailed in Section 4.1.3.1. If adverse effects are found or suspected in any species, laboratory studies will be conducted to more fully assess and characterize the health effects of the vaccine in the species involved, and the field study will be suspended until laboratory studies are completed.

Elements of the design for these studies will include:

1) Assessment of vaccine uptake via Rhodamine B biomarker and estimate of glycerin jelly removal rate;
2) Comparison of bat survival and WNS occurrence at study sites in vaccinated bats compared to non-vaccinated bats;
3) Assessment of exposure to vaccine antigens via serology;
4) Post-vaccination monitoring for pox lesions and mortality in bats;
5) Collection and assessment of feces for vaccine shedding (not anticipated based on laboratory studies—Rocke, unpublished data); and

6) Molecular characterization of any poxvirus isolated to ensure no genetic changes occurred (not anticipated based on laboratory studies—Rocke, unpublished data).

4.1.3 Monitoring and mitigation activities

4.1.3.1 Monitoring

Once vaccine jelly has been applied to bat houses or directly to bats in fall roosting sites or hibernacula, observers will record the presence or absence of vaccine jelly daily to determine the rate of removal. Vaccine jelly uptake can be measured by incorporating a biomarker, Rhodamine B, into the jelly (Fernandez and Rocke, 2011). Jelly containing Rhodamine B is a bright red color making it easily visible in the field. After jelly consumption, Rhodamine B can be visualized under natural light visible as red staining and under ultraviolet light as an orange fluorescence. Using microscopy, fluorescent bands can be detected in hair samples taken from animals that consumed the biomarker-laden jelly.

Bats will be trapped five to seven (5-7) days after vaccine jelly application to collect hair samples for biomarker analysis and mark bats with wing bands and Passive Integrated Transponder tags for individual identification. For pilot studies, where bats will be administered vaccine via the oral route by pipette, they will be marked immediately with wing bands and Passive Integrated Transponder tags for individual identification. There will be no need for recapture. Bat health will also be evaluated. Passive Integrated Transponder tag readers will be placed on bat houses and in hibernacula to assess survival by recording bat entry. Once or twice during hibernation, personnel will enter the hibernacula to look for marked bats and to assess WNS occurrence by ultraviolet light examination (NWHC ACUC #ST150418B R1). Bats in torpor will be captured by hand (NWHC ACUC #ST120524A). The following spring, Passive Integrated Transponder tag readers attached to bat houses and in hibernacula will be used to determine the rates of returning and surviving bats.

Each captured bat will be inspected for lesions consistent with poxvirus infection as well as other outward signs of a negative response to the vaccine candidates, such as lethargy, ataxia, tremors, nasal or ocular discharge, and unkempt appearance. Any bat with these signs or suffering severe injury or morbidity will be humanely euthanized as detailed in Section 6.1.1 and their carcasses submitted to NWHC for necropsy and complete virologic and histologic examination.

4.1.3.2 Mitigation activities

Mitigation measures are any features of an action that serve to prevent, reduce, or compensate for impacts that otherwise might result from that action. Mitigation activities would include:

- Public information and education actions and media announcements to inform the public about application of vaccine candidates in the field before they occur;
- Study description, including telephone numbers to call for more information, will be posted on signs at the study sites;
- Methods used to capture bats would be limited to mist nets for the most part. Animals caught in mist nets that must be sacrificed (killed) for testing would be euthanized in accordance with recommendations by Animal Care and Use Committee protocols;

- All drug use in capturing and handling animals would be under the direction and authority of the NWHC veterinarian; and
- A contingency management plan will be in place in the unlikely case of an adverse event defined as widespread mortality or morbidity of bats or non-target species.

4.2 Alternatives

4.2.1 Rationale behind selection of alternatives

Viable alternatives must enable collection of data to assess the safety and effectiveness of vaccine candidates in the field prior to further studies on successful vaccine candidates as management tools.

4.2.2 Alternative action—another time (Alternative 2)

This action would be to conduct the proposed studies at an alternative (later) time. The proposed time (fall 2018) is the earliest time when these studies would be possible, pending vaccine candidate regulatory approval. Participating scientists are currently prepared to undertake the studies at the proposed times. If the studies are postponed until a future time, considerable delays in obtaining data assessing field safety and effectiveness of vaccine candidates would occur. This delay would impact future studies on successful vaccine candidates as management tools for bat conservation. WNS would remain a threat to these populations of animals during the intervening time with the potential for species of bats to become listed as threatened or endangered species.

4.2.3 Alternative action—other locations (Alternative 3)

If the bat populations identified in Alternative 1 are unavailable for use due to reasons such as WNS mortalities, lack of approval by landowners, or other explanations, substitute locations of suitable bat populations would need to be identified. This action would delay the field studies resulting in additional time spent identifying bat populations, obtaining permission from landowners, and holding public meetings to inform the public in the area. As in Alternative 2, delays in the proposed studies would impact future studies on successful vaccine candidates as management tools for bat conservation.

4.2.4 No action (Alternative 4)

No vaccine-laden jelly would be applied to bat houses or individual bats. USGS would not conduct research for WNS control or use resources available. Field studies assessing successful vaccine candidates as management tools for bat conservation would be prevented. WNS would continue to pose an unregulated threat to existing populations of bats.

4.2.5 Alternatives considered but eliminated from detailed analysis

The only alternative to field studies would be laboratory studies to assess the safety and efficacy of the vaccine candidates. Preliminary laboratory studies have been performed with two vaccine candidates (RCN-cal and RCN-sp) in little brown bats. These studies demonstrated safety of the

raccoon pox vaccines and suggested that vaccine-induced immunity to WNS may be possible. However, little brown bats are extremely difficult to maintain in a laboratory setting and no laboratory animal can serve as a model for WNS. Thus, additional laboratory studies to assess the efficacy of RCN-cal and RCN-sp are not considered further.

Numerous laboratory studies have shown that RCN-vectored vaccines are safe in a wide variety of animal species (Esposito et al., 1988, 1989, 1992; Fekadu et al., 1991; DeMartini et al., 1993; Osorio et al., 2003a; Mencher et al., 2004; Rocke et al., 2008b, 2010a, unpublished data; Tripp et al., 2015). Additional laboratory studies of vaccine candidates would not assess safety of the vaccine candidates under field conditions and are, therefore, not considered further.

5. AFFECTED ENVIRONMENT

This section presents descriptive information on the environment of the areas that would be affected by the proposed action. Bat populations selected for the field studies would be in isolated areas with restricted access. Prospective study areas for little brown bats in Minnesota, and Wisconsin and for tri-colored bats in Texas include populations that inhabit caves and mines, tree hollows, and under buildings or bridges on privately-owned or state-owned properties.

The proposed action does not involve construction, major ground disturbance, or habitat modification. Therefore, the following resource values are not expected to be affected by the proposed action: soils, geology, minerals, water quality/quantity, visual resources, air quality, prime and unique farmlands, aquatic resources, vegetation, and range. These resources will not be analyzed further.

5.1 Human Environment

The proposed action will have negligible, if any, effects on the surrounding communities, including minority and low-income populations. Field studies will be conducted on isolated sites closed or restricted to the public. For sites on privately-owned land, studies will be undertaken with landowner permission.

Table 1: Selected Texas, and Wisconsin counties – Human Environment

County	Pierce, WI		Freestone, TX	Leon, TX
Total Population	42,555		19,808	17,270
Housing units	16,693		9,476	9,813
Veterans	2,434		1,337	1,482
Racial Makeup				
White	95.8%		80.0%	89.7%
Black/African American	0.9%		15.8%	7.2%
Native American	0.5%		1.5%	0.9%
Asian	1.3%		0.8%	0.7%
Pacific Islander	0.0%		0.0%	0.0%
Two or more races	1.5%		12.0%	1.4%
Hispanic or Latino of any race	2.1%		15.8%	15.0%
Median Income and Poverty				
Household	$66,772		$45,890	$44,875
Per Capita Income	$31,109		$24,060	$27,096
Persons in poverty	7.8%		16.1%	16.1%
Education				
High School Graduate	94.5%		81.8%	82.8%
Bachelor's degree or higher	27.7%		11.7%	15.9%

Census data from the U.S. Department of Commerce, United States Census Bureau.
https://www.census.gov/data.html Data Retrieved October 2019

Table 2: Selected Minnesota counties – Human Environment

County	Dakota, MN	Fillmore, MN	Goodhue, MN	Hennepin, MN	Houston, MN	Lake, MN
Total Population	425,423	21,058	46,403	1,259,428	18,578	10,658
Housing units	168,117	10,028	20,692	537,756	8,777	7,995
Veterans	23,298	1,407	3,619	56,662	1.369	948
Racial Makeup						
White	84.1%	97.7%	94.5%	74.4%	97.0%	96.5%
Black/African American	7.0%	0.5%	1.4%	13.6%	0.7%	0.8%
Native American	0.6%	0.2%	1.5%	1.1%	0.3%	0.7%
Asian	5.2%	0.6%	0.7%	7.5%	0.6%	0.5%
Pacific Islander	0.1%	0.0%	0.1%	0.1%	0.0%	0.0%
Two or more races	2.9%	1.0%	1.7%	3.2%	1.5%	1.5%
Hispanic or Latino of any race	7.4%	1.8%	3.5%	7.0%	1.2%	1.7%
Median Income and Poverty						
Household	$79,995	$57,093	$62,431	$71,154	$56,837	$56,078
Per Capita Income	$38,863	$28,441	$33,477	$41,794	$30,150	$32,319
Persons in poverty	5.8%	10.3%	8.0%	10.5%	7.6%	8.4%
Education						
High School Graduate	94.7%	91.2%	93.8%	93.0%	94.2%	95.8%
Bachelor's degree or higher	41.1%	20.7%	24.7%	48.2%	23.2%	29.0%

Census data from the U.S. Department of Commerce, United States Census Bureau. https://www.census.gov/data.html Data Retrieved October 2019

Table 2 Cont.: Selected Minnesota counties – Human Environment

County	Nicollet, MN	Pine, MN	Ramsey, MN	St. Louis, MN	Washington, MN	Winona, MN
Total Population	34,220	29,483	550,210	199,754	259,201	50,825
Housing units	13,621	17,635	220,680	105,002	99,459	21,237
Veterans	1,851	2,247	23,910	15,061	14,261	2,942
Racial Makeup						
White	92.3%	91.4%	67.4%	92.3%	85.9%	93.7%
Black/African American	3.7%	2.3%	12.6%	1.6%	4.9%	1.9%
Native American	0.5%	3.5%	1.0%	2.4%	0.5%	0.5%
Asian	1.7%	0.7%	15.3%	1.2%	6.2%	2.7%
Pacific Islander	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%
Two or more races	1.7%	2.1%	3.6%	2.5%	2.4%	1.3%
Hispanic or Latino of any race	4.7%	3.0%	7.6%	1.8%	4.3%	3.1%
Median Income and Poverty						
Household	$62,593	$47,285	$60,301	$50,936	$89,598	$53,975
Per Capita Income	$29,722	$24,044	$32,544	$29,197	$41,591	$27,200
Persons in poverty	8.8%	12.2%	14.0%	14.5%	4.2%	13.8%
Education						
High School Graduate	93.5%	89.4%	90.1%	93.6%	96.1%	92.8%
Bachelor's degree or higher	32.2%	13.9%	41.5%	28.4%	42.3%	29.6%

Census data from the U.S. Department of Commerce, United States Census Bureau. https://www.census.gov/data.html Data Retrieved October 2019

5.2 Physical Description and Climate

Prospective study areas for little brown bats in Wisconsin include populations on privately-owned land used for underground sand mining operations.

Wisconsin County	Lowest Elevation	Highest Elevation	Average Rain per Year	Average Snowfall per Year	High July Temp	Low January Temp
Pierce	692 feet	1,325 feet	34 inches	46 inches	81°F	4°F

Prospective study areas for tri-colored bats in Texas include populations in the Texas rights-of-way along interstate highways in Freestone and Leon counties.

Texas Counties	Lowest Elevation	Highest Elevation	Average Rain per Year	Average Snowfall per Year	High July Temp	Low January Temp
Freestone	388 feet	608 feet	41 inches	None	95°F	37°F
Leon	354 feet	630 feet	43 inches	None	94°F	37°F

Prospective study areas for little brown bats in Minnesota include populations in caves, hollow trees, under bridges and under building eaves.

Minnesota Counties	Lowest Elevation	Highest Elevation	Average Rain per Year	Average Snowfall per Year	High July Temp	Low January Temp
Dakota	640 feet	1,257 feet	32 inches	42 inches	83°F	6°F
Fillmore	594 feet	1,407 feet	35 inches	43 inches	81°F	7°F
Goodhue	640 feet	1,286 feet	32 inches	40 inches	83°F	6°F
Hennepin	656 feet	1,188 feet	32 inches	52 inches	83°F	6°F
Houston	725 feet	1,421 feet	34 inches	41 inches	82°F	9°F
Lake	602 feet	2,067 feet	30 inches	75 inches	74°F	4°F
Nicollet	705 feet	1,283 feet	31 inches	39 inches	82°F	6°F
Pine	768 feet	1,385 feet	30 inches	48 inches	80°F	0°F
Ramsey	659 feet	1,329 feet	33 inches	50 inches	83°F	8°F
St. Louis	577 feet	2,034 feet	28 inches	65 inches	77°F	-4°F
Washington	656 feet	1,339 feet	33 inches	46 inches	83°F	7°F
Winona	614 feet	1,362 feet	34 inches	35 inches	81°F	8°F

5.3 Biological Resources

5.3.1 Terrestrial Vegetation

Little brown bats and tri-colored bats are found in old-growth forests often close to water, their preferred foraging grounds. The bats tend to roost in dead or dying trees, such as oak or maple. Edge habitat, the transition zone between two types of vegetation, is important for bats as they forage and migrate at the change of seasons. Hibernation occurs mainly in caves or mines.

5.3.2 Terrestrial Mammals

Bat species include little brown bats (*Myotis lucifugus*) and tri-colored bats (*Perimyotis subflavus*).

Other animal species in forested areas include squirrels, deer mice, chipmunks, voles, shrews, rabbits, and skunks. Predators include badgers, raccoons, foxes, coyotes, weasels, and bobcats. Ungulates include deer.

No mammals are expected to inhabit the caves and mines where bats hibernate. Barriers will be put up to exclude predators, such as raccoons.

5.3.3 Birds

Numerous species of birds are found in the forested study sites, including raptors, woodpeckers, ground birds, and passerines. No birds are expected to inhabit the caves and mines where bats hibernate.

5.3.4 Arthropods

Numerous species of insects are found on the study sites including, among others, fleas, flies, ants, butterflies and moths, beetles, bugs, bees, grasshoppers, and crickets. Non-insect arthropods include spiders and ticks.

5.3.5 Reptiles and Amphibians

Reptiles found in the study sites include snakes and lizards. Toads and frogs may be found near water.

5.4 Federal Threatened and Endangered Species and Critical Habitat

The bat species included in the field studies are currently not listed by the USFWS, although they are listed as threatened or of special concern in specific states.

The USFWS has indicated that no critical habitats under jurisdiction of the USFWS are known to occur in the proposed project areas in Minnesota (Appendix A, page 49), Texas, or Wisconsin. Species have been identified as threatened, endangered, or candidate species through the USFWS Information, Planning and Consultation System. Based on the known distributions for the species of concern and the habitats that these species may occupy, this project has the possibility to overlap with endangered or threatened species. Potential impact and avoidance procedures for these and other species are further discussed in the tables below. The following threatened, endangered, and candidate species may be present during bat capture:

Table 3: Threatened, endangered, and candidate species in Pierce County, Wisconsin

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Higgins Eye (pearly mussel)	*Lampsilis higginsii*	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Northern long-eared bat	*Myotis septentrionalis*	Threatened	The project is not located in any critical habitat. The species may be present in the project area.	If captured during the field studies, the species will be released.
Prairie Bush-clover	*Lespedeza leptostachya*	Threatened	No open land or wetlands are present in the project area which would support this species.	None
Sheepnose Mussel	*Plethobasus cyphyus*	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None

Table 3: Threatened, endangered, and candidate species in Freestone and Leon Counties, Texas

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Bald Eagle	*Haliaeetus leucocephalus*	Breeds Sep 1 to July 31	No bodies of water or nesting areas are present in the project area which would support this species.	None
Harris's Sparrow	*Zonotrichia querula*	Breeds elsewhere	There are no nesting areas present in the project area which would support this species.	None
Houston Toad	*Bufo houstonensis*	Endangered	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations.
Large-fruited Sand-verbena	*Abronia macrocarpa*	Endangered	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations.

Table 3: Threatened, endangered, and candidate species in Freestone and Leon Counties, Texas

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Least Tern	*Sterna antillarum*	Endangered	No bodies of water or nesting areas are present in the project area which would support this species.	None
Lesser Yellowlegs	*Tringa flavipes*	Breeds elsewhere	No marshes, mudflats, shores, or ponds are present in the project area which would support this species.	None
Navasota Ladies- tresses	*Spiranthes parksii*	Endangered	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations.
Piping Plover	*Charadrius melodus*	Threatened	No bodies of water or nesting areas are present in the project area which would support this species.	None
Red Knot	*Calidris canutus rufa*	Threatened	No bodies of water or nesting areas are present in the project area which would support this species.	None
Red-headed Woodpecker	*Melanerpes erythrocephalus*	Breeds May 10 to Sep 10	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.
Whooping Crane	*Grus Americana*	Endangered	No marshes, open water, or nesting areas are present in the project area which would support this species.	None

Table 3: Threatened, endangered, and candidate species in Dakota, Fillmore, Goodhue, Hennepin, Houston, Lake, Nicollet, Pine, Ramsey, St. Louis, Washington, and Winona counties, Minnesota

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
American Bittern	*Botaurus lentiginosus*	Breeds Apr 1 to Aug 31	No freshwater wetlands dominated by tall dense vegetation are present in the project area which would support this species.	None
American Golden-plover	*Pluvialis dominica*	Breeds elsewhere	No short-grass prairies, flooded pastures, mudflats, or shores are present in the project area which would support this species.	None
Bald Eagle	*Haliaeetus leucocephalus*	Breeds Oct 15 to August 31	No forested areas or large bodies of water are present in the project area which would support this species.	None
Black Tern	*Chlidonias niger*	Breeds May 15 to Aug 20	No fresh marshes, lakes, or coastal waters are present in the project area which would support this species.	None
Black-billed Cuckoo	*Coccyzus erythropthalmus*	Breeds May 15 to Oct 10	No mixed deciduous-coniferous woods, bogs, or marshes are present in the project area which would support this species.	None
Bobolink	*Dolichonyx oryzivorus*	Breeds May 20 to Jul 31	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.

Table 3 Cont.: Threatened, endangered, and candidate species in Dakota, Fillmore, Goodhue, Hennepin, Houston, Lake, Nicollet, Pine, Ramsey, St. Louis, Washington, and Winona counties, Minnesota

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Canada Lynx	*Lynx canadensis*	Threatened	The project partially overlaps the final critical habitat. The species may be present in the project area. (Ref. Federal Register, Vol. 79, No. 177, pages 54782-54846)	The field studies will take place in mines or caves. (Ref. USFWS Canada Lynx 5-Year Review, dated Nov. 13, 2017)
Canada Warbler	*Cardellina canadensis*	Breeds May 20 to Aug 10	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.
Cape May Warbler	*Setophaga tigrine*	Breeds Jun 1 to Jul 31	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.
Cerulean Warbler	*Dendroica cerulea*	Breeds Apr 21 to Jul 20	No mature deciduous forests or heavily forested landscapes are present in the project area which would support this species.	None
Dunlin	*Calidris alpine arcticola*	Breeds elsewhere	No tidal flats, beaches, or bodies of water are present in the project area which would support this species.	None
Eastern Whip-poor-will	*Antrostomus vociferous*	Breeds May 1 to Aug 20	No open understory, sparse ground cover, or shaded habitats are present in the project area which would support this species.	None

Table 3 Cont.: Threatened, endangered, and candidate species in Dakota, Fillmore, Goodhue, Hennepin, Houston, Lake, Nicollet, Pine, Ramsey, St. Louis, Washington, and Winona counties, Minnesota

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Evening Grosbeak	*Coccothraustes vespertinus*	Breeds May 15 to Aug 10	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.
Golden Eagle	*Aquila chrysaetos*	Breeds elsewhere	This species is not present in the state of Minnesota.	None
Golden-winged Warbler	*Vermivora chrysoptera*	Breeds May 1 to July 20	No brushy areas with patches of weeds or shrubs and scattered trees are present in the project area which would support this species.	None
Gray Wolf	*Canis lupus*	Endangered	The project partially overlaps the final critical habitat. The species may be present in the project area. (Ref. Federal Register, Vol. 43, No. 47, pages 9607-9615 and Federal Register, Vol. 80, No. 34, pages 9218-9229)	The field studies will take place in mines or caves not known to provide habitat for the species or their primary prey item(s).
Henslow's Sparrow	*Ammodramus henslowii*	Breeds May 1 to Aug 31	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.
Higgins Eye (pearly mussel)	*Lampsilis higginsii*	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Hudsonian Godwit	*Limosa haemastica*	Breeds elsewhere	This species is not present in the state of Minnesota.	None

Table 3 Cont.: Threatened, endangered, and candidate species in Dakota, Fillmore, Goodhue, Hennepin, Houston, Lake, Nicollet, Pine, Ramsey, St. Louis, Washington, and Winona counties, Minnesota

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Hudsonian Godwit	*Limosa haemastica*	Breeds elsewhere	This species is not present in the state of Minnesota.	None
Karner Blue Butterfly	*Lycaeides Melissa samuelis*	Endangered	No oak savannas or pine barren ecosystems are present in the project area which would support this species. Further there are no wild blue lupine (*Lupinus perennis*) in the project area.	None
Kentucky Warbler	*Oporornis formosus*	Breeds Apr 20 to Aug 20	No shaded woods with dense humid thickets are present in the project area which would support this species.	None
Least Bittern	*Ixobrychus exilis*	Breeds Aug 16 to Oct 31	This species is not present in the state of Minnesota.	None
Leedy's Roseroot	*Rhodiola integrifolia ssp. leedyi*	Threatened	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations.
Lesser Yellowlegs	*Tringa flavipes*	Breeds elsewhere	No marshes, mudflats, shores, or ponds are present in the project area which would support this species.	None
Long-eared Owl	*Asio otus*	Breeds Mar 1 to Jul 15	No dense forests with open meadows are present in the project area which would support this species.	None
Northern Long-eared Bat	*Myotis septentrionalis*	Threatened	The project is not located in any critical habitat. The species may be present in the project area.	If captured during the field studies, the species will be released.
Olive-sided Flycatcher	*Contopus cooperi*	Breeds May 20 to Aug 31	This species is not present in the state of Minnesota.	None

Table 3 Cont.: Threatened, endangered, and candidate species in Dakota, Fillmore, Goodhue, Hennepin, Houston, Lake, Nicollet, Pine, Ramsey, St. Louis, Washington, and Winona counties, Minnesota

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Prairie Bush-clover	*Lespedeza leptostachya*	Threatened	No open land or wetlands are present in the project area which would support this species.	None
Prothonotary Warbler	*Protonotaria citrea*	Breeds Apr 1 to July 31	No slow moving or standing water bodies or flooded river bottom hardwoods are present in the project area which would support this species.	None
Red-headed Woodpecker	*Melanerpes erythrocephalus*	Breeds May 10 to Sep 10	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.
Ruddy Turnstone	*Arenaria interpres morinella*	Breeds elsewhere	No beaches, mudflats, jetties, or rocky shores are present in the project area which would support this species.	None
Rusty Blackbird	*Euphagus carolinus*	Breeds May 10 to Jul 20	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.
Rusty Patched Bumble Bee	*Bombus affinis*	Endangered	No open land or native prairie forbs are present in the project area which would support this species	None
Semipalmated Sandpiper	*Calidris pusilla*	Breeds elsewhere	No beaches, mudflats, or lakes are present in the project area which would support this species.	None
Sheepnose Mussel	*Plethobasus cyphyus*	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None

Table 3 Cont.: Threatened, endangered, and candidate species in Dakota, Fillmore, Goodhue, Hennepin, Houston, Lake, Nicollet, Pine, Ramsey, St. Louis, Washington, and Winona counties, Minnesota

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Short-billed Dowitcher	*Limmodromus griseus*	Breeds elsewhere	No mudflats, tidal marshes, or freshwater ponds are present in the project area which would support this species.	None
Snuffbox Mussel	*Epioblasma triquetra*	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Spectaclecase (mussel)	*Cumberlandia monodonta*	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Whooping Crane	*Grus Americana*	Experimental Population Non-Essential	No marshes, open water, or nesting areas are present in the project area which would support this species.	None
Willow Flycatcher	*Empidonax traillii*	Breeds May 20 to Aug 31	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.
Winged Mapleleaf	*Quadrula fragosa*	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Wood Thrush	*Hylocichla mustelina*	Breeds May 10 to Aug 31	The project is not located in any critical habitat. The species may be present in the project area.	Personnel will be trained to identify the species and to avoid disturbing any populations especially during breeding and nesting seasons.

Endangered Species data from the U.S. Fish and Wildlife Service, https://www.fws.gov/ipac/, data retrieved October 22, 2019

5.5 Cultural Resources

Cultural resources in the study sites relate to historic occupation of these areas by Native Americans and may include human remains and associated artifacts. The Wisconsin State Historic Preservation Officer (August 2017) has determined that no historic properties will be affected at the study sites (Appendix B, page 46). The Texas State Historic Preservation Officer (June 2018) has determined that no historic properties will be affected at the study sites (Appendix B, page 47). The Minnesota State Historic Preservation Officer (July 2018) has determined that no historic properties will be affected at the study sites (Appendix B, page 48).

5.6 Human Uses

5.6.1 Subsistence Uses

The study sites are not used for subsistence purposes.

5.6.2 Other Public Uses

Study forested areas may have recreational uses, such as hiking. Caves and mines will be closed to public access.

5.7 Designated Wilderness

There are no designated wilderness areas in the study sites.

6. ENVIRONMENTAL EFFECTS

6.1 Issues considered

6.1.1 Issues considered in detail

The impacts of the four (4) alternatives on the natural environment of the study sites are analyzed with respect to the vaccine candidates and capture/handling of animals.

- Vaccine candidates

Vaccine candidates use a recombinant raccoon poxvirus incorporating genes of *Pseudogymnoascus destructans*. The vaccine candidates (RCN-cal, RCN-sp, and RCN-cal-sp) are orally delivered to target animals via glycerin jelly. The vaccine virus produces proteins of *Pseudogymnoascus destructans* (cal, sp) in infected cells to stimulate an immune response by bats that consume vaccine-laden glycerin jelly.

- Biomarker

Biomarkers are distinctive biological indicators used to identify, often through indirect means, when an event or physiologic process of interest has occurred in an animal. Biomarkers are normally incorporated into the baits to identify animals that have consumed vaccine-laden bait. Glycerin jelly will contain Rhodamine B, an industrial and analytical dye that has been widely used as a marker and tracer in animal studies that marks hair, feces, or blood (Evans and Griffith,

1973; Johns and Pans, 1981; Lindsey, 1983; Fisher et al., 1999). After bait consumption, Rhodamine B can be visualized under natural light (red staining) and under ultraviolet light (orange fluorescence). Using a fluorescence microscope, fluorescent bands can be detected in hair removed from captured bats. Glycerin jelly will contain 0.16% Rhodamine B.

- Capture and handling

Bats will be captured using mist nets or harp traps (NWHC ACUC #ST120524A). Nets or traps will be placed near the entrances of caves/mines or roost sites in the evening to capture bats that emerge for nighttime feeding. Bats will be removed from nets/traps within 15 minutes of capture to minimize stress and potential injuries. Field technicians will wear sturdy gloves to remove bats from traps and place bats into cloth or paper bags for holding until processing. Trapping will not occur during inclement weather such as rain or high winds. Field technicians will remain at or near the trapping site while nets/traps are in place to ensure animals are released.

Bats will be examined for injuries and evidence of WNS. Hair samples will be collected for biomarker analysis. Bats will be marked with an arm band and Passive Integrated Transponder tags. After processing, bats will be released at the point of capture. If an animal is severely injured and cannot be released or is obviously suffering from severe disease (WNS), it will be euthanized by anesthetic overdose followed by cervical dislocation or decapitation while under anesthesia (NWHC ACUC #ST100407B).

6.1.2 Issues considered in detail with rationale

- Bait composition

Glycerin jelly, used as the vaccine vehicle, is composed of 46% glycerin, 46% water, 7% gelatin, and 1% phenol. Glycerin, also known as glycerol, naturally occurs in foods and animals as a component of triglycerides. It is a common food additive recognized as generally safe by the Food and Drug Administration with no known carcinogenic, mutagenic, or teratogenic effects. The amount of jelly to be ingested by each bat is estimated to be well below the LD50 amount for rats, 512mg/kg (Carolina Biological Supply Company Safety Data Sheet, http://www.carolina.com/teacher-resources/Document/msds-glycerin-jelly/tr-msds-glycerinjellghs.tr). Previous work using glycerin jelly as an oral vaccine vehicle showed no adverse effects in Brazilian free-tailed bats (Stading et al., 2016). In addition, use of glycerin jelly in vampire bats as part of preliminary work for a rabies vaccine showed no adverse effects (Rocke, unpublished data).

- Potential impacts on threatened and endangered species

Although the bats in this study are not federally listed as threatened or endangered, little brown bats and tri-colored bats are listed as threatened or of special concern in some of the states involved. Efforts will be made to avoid the unnecessary capture of and to minimize disturbance to these species. All work will be conducted on foot. Field crews will be trained to identify all threatened and endangered plants, mammals, and birds and to avoid them if discovered. Field crews will take precautions to avoid spreading *Pseudogymnoascus destructans* between study

sites and other areas by using disposable personal protective equipment and thoroughly decontaminating footwear, clothing, and equipment.

- Potential impacts on cultural resources

The proposed action would not cause major ground disturbance, would not cause any physical destruction or damage to property, or any alterations of property, wildlife habitat, or landscapes, and does not involve the sale, lease, or transfer of ownership of any property. Also, the proposed methods do not have the potential to introduce visual, atmospheric, or audible elements to areas in which they are used that could result in effects on the character or use of historic properties. Any cultural artifacts discovered during the study will be left undisturbed.

- Human subsistence and other uses

Study sites will be in remote forested areas or in caves/mines. Sites will not be used for human subsistence and caves/mines will be closed to recreational use.

- Potential human health impacts in the event of human consumption of vaccinated wildlife

The issue expressed here is the potential to develop a raccoon pox infection from eating a vaccinated bat or some other animal that has eaten vaccine-laden glycerin jelly. Bats are not usually eaten by people in the United States. Non-target animals that may ingest the vaccine-laden jelly could include rodents which are usually not eaten by people. Biophotonic imaging studies showed that raccoon poxvirus exposure in prairie dogs resulted in a localized infection that did not progress systemically (Berlier et al., 2010). The raccoon poxvirus from vaccine candidates would most likely only bind to animal tissues in the mucous membranes of the oral cavity, pharynx, and esophagus when orally ingested since raccoon poxvirus does not spread throughout the body of the animal. Those tissues are rarely consumed by humans, but if they were, they would most likely be cooked which would kill the virus. In addition, public access to study sites will be restricted. Therefore, the potential for adverse health effects from consuming animals that have eaten vaccine candidates in glycerin jelly is low.

6.1.3 Effects of Global Warming, Habitat Loss, and Pollution on Wildlife Populations

Program activities likely to result from the proposed action would have a negligible effect on atmospheric conditions including the global climate. Meaningful direct or indirect emissions of greenhouse gasses would not occur because of the proposed action. The proposed action would meet the requirements of applicable Federal laws, regulations, and Executive Orders (See Appendix B, page 52) including the Clean Air Act and Executive Order 13514. Other than minor uses of fuels for motor vehicles and other materials, there are no irreversible or irretrievable commitments of resources. The contribution of the proposed action to the emission of gases that potentially contribute to global warming will be like the other alternatives and is expected to be minimal. Thus, these will not be analyzed further.

6.2 Issues analyzed by alternative

6.2.1 Proposed Action (Alternative 1)

6.2.1.1 Potential impacts of vaccine

Based on previous laboratory and field studies (Stading et al., 2016; Rocke, unpublished data) using glycerin jelly on bats, the probability of exposure to the vaccine by ingestion of vaccine-laden jelly is high for bats; they are known to consume the jelly by self- and mutual-grooming activity.

Non-target animals that may encounter vaccine-laden bait include rodents, felids, raccoons, birds, and reptiles. For these animals, the probability of ingesting jelly is low. The jelly does not contain any attractant that might appeal to these animals. Predators may accidentally ingest jelly by consuming a bat with jelly on its fur.

The likelihood of inadvertent human exposure to vaccine-laden jelly during conduct of this field trial is extremely limited. Study personnel applying the jelly will be trained in proper handling of the jelly and will wear personal protective equipment (latex gloves). Human exposure will be further limited by the restricted access to the remote study sites, the anticipated pre-field trial publicity, and the short time the jelly will remain in the field.

The virulence of raccoon poxvirus is highly attenuated by inactivation of the thymidine kinase (*tk*) gene by insertional recombination. Recombinant raccoon poxvirus has been used in several oral vaccines in raccoons, prairie dogs, mice, cotton rats, rabbits, striped skunks, dogs, bobcats, non-human primates, cats, and sheep with no harmful effects (Esposito et al., 1988, 1989, 1992; Fekadu et al., 1991; DeMartini et al., 1993; Osorio et al., 2003a; Tripp et al., 2015).

6.2.1.1.1 Potential to cause white-nose syndrome

The nature of the recombinant virus used as vaccine candidates is such that it cannot cause WNS. The vaccine candidates carry two genes of *Pseudogymnoascus destructans* (cal and sp) associated with its secretome. The vaccine candidates do not contain the full set of genes necessary for production of the fungus which would need to occur to produce WNS.

6.2.1.1.2 Potential to cause raccoon pox

Raccoon poxvirus is considered a Biosafety Level-2 pathogen. The deletion of the *tk* gene considerably attenuates the virus. The virulence of the recombinant raccoon poxvirus is expected to be low in bats, non-target animals, and humans. Recombinant raccoon poxviruses have been developed as vaccines for rabies (Esposito et al., 1988, 1989, 1992; Stading et al., 2016, 2017), feline panleukopenia virus (Hu et al., 1996), plague (Osorio et al., 2003b; Mencher et al., 2004; Rocke et al., 2008a, 2010ab; Rocke, unpublished), and WNS (Rocke, unpublished data) using the *tk* gene as the site for insertional recombination. Recombinant raccoon poxvirus has been used in several oral vaccines in raccoons, mice, cotton rats, rabbits, striped skunks, dogs, bobcats, non-human primates, cats, and sheep with no harmful effects (Esposito et al., 1988, 1989, 1992; Fekadu et al., 1991; DeMartini et al., 1993; Osorio et al., 2003a). Based on

outcomes of other raccoon poxvirus vaccine studies, ingestion of vaccine-laden jelly is not expected to cause adverse reactions in non-target animals and humans.

In experimental studies, there have been no adverse effects associated with the use of oral raccoon poxvirus-vectored WNS vaccines in bats (Rocke, unpublished data). USGS has inoculated bats intramuscularly. Intranasally, and orally with raccoon poxvirus WNS vaccine constructs and compared results with negative control animals. No morbidity or mortality of bats has been observed due to infection with these viruses (Rocke, unpublished data). No mortality has been observed even in severe combined immune deficient mice or pregnant mice upon injection of raccoon poxvirus-based vaccines (Jones et al., 2014).

The only documented case of human exposure to recombinant raccoon poxvirus occurred because of a laboratory needle stick accident with an experimental plague vaccine using recombinant raccoon poxvirus as a vaccine vector for *Yersinia pestis* antigen F1 (Rocke et al., 2004b). Within nine (9) days, the patient developed a small blister at the injection site that healed within four (4) weeks; no other systemic symptoms were reported during this period. Raccoon poxvirus was cultured from the lesion, and the patient developed antibody to plague antigen (F1) and raccoon poxvirus suggesting infection of the patient with RCN-F1. The blister was possibly due to an inflammatory response to limited viral replication.

6.2.1.1.3 Potential for recombinant raccoon poxvirus to revert to virulence or to recombine with other viruses in the wild and result in a virus that could cause disease in humans or animals

The concern here is whether the recombinant raccoon poxvirus used in the vaccine is genetically stable so that it would not become virulent after it replicates in animals that eat vaccine-laden jelly with the potential of being passed on to other animals. The *tk* gene (approximately 844 bp) of raccoon poxvirus has been inactivated by insertional recombination with IRES/tPA sequences and the calnexin or serine protease genes of *Pseudogymnoascus destructans*. The presence of these genes is not known to promote any homologous recombination or DNA insertion. Because of the large insertion made into the *tk* gene, it is unlikely that the recombinant raccoon poxvirus would regain its *tk* gene to become fully virulent. In studies of tk^{-} strains of vaccinia, a related pox virus, no evidence of reversion was detected (Buller et al., 1985). In addition, there is no evidence to suggest that the donor DNA sequences (cal and sp) enhance the virulence of raccoon poxvirus or its ability to survive in target animals.

For a different raccoon poxvirus vaccine, a back-passage study involving an initial passage of RCN-F1-V307 and two back-passages of pooled virus isolates in prairie dogs demonstrated that RCN-F1-V307 can be shed orally by inoculated prairie dogs but does not pass between prairie dogs (Rocke, unpublished data). None of the prairie dogs developed clinical signs associated with infection of raccoon poxvirus showing the virus did not revert to virulence. These results indicate that although RCN-F1-V307 is replicative in prairie dogs it is not pathogenic. It is expected that the WNS vaccine candidates would act similarly.

6.2.1.2 Potential impacts of biomarker

The lethal dose 50% (LD50) of Rhodamine B in orally inoculated laboratory mice is 887 mg/kg (Rhodamine B; MSDS, 2007). Each milliliter (ml) of glycerin jelly used in this study would contain 0.16% Rhodamine B (RB) (1.6 mg RB/ml jelly). If a 10-gram (g) bat consumes 1 ml of jelly, the dose would be 0.016 mg/kg. A 10-gram bat would have to consume over 5 ml of jelly to reach the LD50. In preliminary trials with hand application, bats groomed and consumed the jelly within 24 hours (and probably immediately). Because jelly will not be applied to every bat within a colony and because bats are mutual groomers, it is unlikely that an individual bat will consume more than 5 ml of jelly.

Raccoons and other predators may consume bats that have ingested jelly, although it is unlikely that this source will lead to a dangerous level of Rhodamine B ingestion; feces from raptors and coyotes became dyed by Rhodamine B after feeding on prey that were exposed to concentrations of Rhodamine B of at least 1% but no adverse effects were noted (Evans and Griffith, 1973).

6.2.1.3 Potential impacts of capture/handling methods used in monitoring and surveillance actions

Trapping and handling of bats will be conducted by experienced personnel. Traps will be checked frequently, and animals released immediately after sample collection, resulting in little impact. Personnel entering hibernacula to monitor bats during winter will be experienced in minimizing disturbance to hibernating bats.

6.2.2 Alternative action—another time (Alternative 2)

This action would be to conduct the proposed studies at an alternative (later) time. Delaying the timing of the proposed project would not result in benefits for bats. Delay would potentially harm the bat populations if WNS were to occur during the intervening time. Alternative study sites would need to be selected if WNS-associated bat population declines occurred in the proposed sites. Delays in obtaining data assessing the field safety and effectiveness of vaccine candidates would impact future studies on vaccine candidates found to be effective and their subsequent use as management tools for conservation of bats. WNS would remain a threat to these populations of animals with the potential for species of bats to become listed as threatened or endangered.

6.2.3 Alternative action—other locations (Alternative 3)

Alternative sites identified would be like those described in Section 5, in that they would have restricted access and comparable biological resources, cultural resources, and human activity. Thus, the potential impacts of the vaccine, biomarker, and capture and handling methods used in monitoring and surveillance actions on the alternative sites would be like those described for Alternative 1, the preferred option. As mentioned previously, this action would delay the field studies leading to the negative effects associated with Alternative 2.

6.2.4 No action alternative (Alternative 4)

Under the no action alternative, no proposed actions would take place and would have no impact on terrestrial wildlife or humans as a direct result. No adverse effects from vaccine or biomarker would occur. However, bat populations would be negatively affected by outbreaks of WNS with subsequent repercussions. Vaccine candidates would be unavailable as management tools to combat WNS.

6.3 Cumulative Impacts

No cumulative environmental impacts are expected from any alternative, except for Alternative 4—No Action, which might lead to increased WNS activity in bats. The analysis in this Environmental Assessment indicates that the proposed short-term field trials will not result in risk of cumulative adverse impacts on the quality of the human environment.

6.5 Summary of impacts of alternatives for each issue

Issue/Impact	Alternative 1 Proposed Action	Alternative 2 Another Time	Alternative 3 Other Locations	Alternative 4 No Action
Potential to cause WNS.	No probable risk from vaccine candidates for humans or animals.	No probable risk from vaccine candidates for humans or animals. Risks of naturally occurring WNS may be higher in bats during time before postponed use of vaccine candidates.	No probable risk from vaccine candidates for humans or animals. Risks of naturally occurring WNS may be higher in bats during time required to identify and coordinate alternative locations.	No risk from vaccine candidates. Risk of naturally occurring WNS occurring in bats may be higher without protection from vaccine candidates.
Potential to cause raccoon pox.	Possible, but risk is low. The virulence of the recombinant raccoon pox is expected to be low in bats, non-target animals, and humans. Infections that may occur are expected to be mild and self-limiting.	Same as Alternative 1.	Same as Alternative 1.	No risk from vaccine candidates.
Potential for recombinant raccoon pox to revert to virulence or to recombine with other viruses in the wild and result in a virus that could cause disease in humans or animals	Very low risk. The recombinant raccoon pox has been inactivated.	Same as Alternative 1.	Same as Alternative 1.	No risk from vaccine candidates.
Impacts of biomarker.	Low risk of toxicity. Animals are highly unlikely to ingest enough glycerin jelly to reach the LD50.	Same as Alternative 1.	Same as Alternative 1.	No risk from vaccine candidates.
Impact of methods used to collect wild animal specimens critical for timely program evaluation.	Low impact. Collections will be conducted by experienced personnel. Traps will be checked frequently, and animals released immediately after sample collection.	Same as Alternative 1.	Same as Alternative 1.	No impact.

7. AGENCIES, ORGANIZATIONS, AND INDIVIDUALS CONSULTED

Jeremy Coleman, U.S. Fish and Wildlife Service, Hadley, MA
Jonathan Reichard, U.S. Fish and Wildlife Service, Hadley, MA
Richard Geboy, U.S. Fish and Wildlife Service, Bloomington, IN
Paul White, Wisconsin Department of Natural Resources, Madison, WI

8. LIST OF PREPARERS AND REVIEWERS

Preparers
Rachel Abbott, Biologist, USGS NWHC, Madison, WI
Tonie Rocke, Epizootiologist, USGS NWHC, Madison, WI

Reviewers
Eva Bryson, Environmental Manager, USGS, Lakewood, CO
Diane Lynch, U.S. Fish and Wildlife Service, Ecological Services, Hadley, MA

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10. GLOSSARY

Hibernaculum	A shelter occupied during the winter by hibernating bats.
LD50	(Lethal dose 50%). The dose of a substance that would kill one half of the test animals.
Recombinant	Produced by the combining of genetic material from more than one origin.
Secretome	Totality of secreted organic molecules and inorganic elements by biological cells, tissues, organs, and organisms.
Vector	In molecular cloning, a vector is a virus or DNA molecule used as a vehicle to carry foreign genetic material into another cell, where it can be replicated and/or expressed.

Photo courtesy of Nancy Heaslip, New York Department of Environmental Conservation, April 2009

Appendix A

Agency Coordination

RCVD 9-6-17

HP-03-07 (8/15/02)

For SHPO Use Only: Date R 17-1208/PE

REQUEST FOR SHPO COMMENT AND CONSULTATION ON A FEDERAL UNDERTAKING

Submit one copy with each undertaking for which our comment is requested. Please print or type. Return to:

Wisconsin Historical Society, Division of Historic Preservation, Office of Preservation Planning, 816 State Street, Madison, WI 53706

Please Check All Boxes and Include All of the Following Information, as Applicable:

AUG 2 8 2017

I. GENERAL INFORMATION

☑ This is a new submittal.
☐ This is supplemental information relating to Case #: _____ and title: _____
☐ This project is being undertaken pursuant to the terms and conditions of a programmatic or other interagency agreement.
The title of the agreement is _____

a. Federal Agency Jurisdiction (Agency providing funds, assistance, license, permit): U.S. Geological Survey

b. Federal Agency Contact Person: Eva J. Bryson — Phone: 303 236 9172

c. Project Contact Person: Eva J. Bryson — Phone: 303-236-9172

d. Return Address: P.O. Box 25046, MS 205, Lakewood, CO — Zip Code: 80225

e. Email Address: ebryson@usgs.gov

f. Project Name: USGS White Nose Syndrome Vaccine Field Studies

g. Project Street Address: W5900 State Road 35, Bay City, WI 54723 and W3302 Highway 35 S, Maiden Rock, WI 54750

h. County: Pierce — City: Bay City and Maiden Rock, W — Zip Code: 54723 and 54750

i. Project Location: Township 24N, Range 17W, (E/W circle one), Section 3 ; Quarter Section
Township 24N, Range 16W, Section 15

j. Project Narrative Description—Attach Information as Necessary

k. Attach a Potential Effect (APE). Attach Copy of U.S.G.S. 7.5 Minute Topographic Quadrangle Showing APE.

II. IDENTIFICATION OF HISTORIC PROPERTIES

☐ Historic Properties are located within the project APE per 36 CFR 800.4. Attach supporting materials.
☑ Historic Properties are not located within the project APE per 36 CFR 800.4. Attach supporting materials.

III. FINDINGS

☑ No historic properties will be affected (i.e., none is present or there are historic properties present but the project will have no effect upon them). Attach necessary documentation, as described at 36 CFR 800.11.
☐ The proposed undertaking will have no adverse effect on site or more historic properties located within the project APE under 36 CFR 800.5. Attach necessary documentation, as described at 36 CFR 800.11.
☐ The proposed undertaking will result in an adverse effect to one or more historic properties and the applicant, or other federally authorized representative, will consult with the SHPO and other consulting parties to resolve the adverse effect per 36 CFR 800.6. Attach necessary documentation, as described at 36 CFR 800.11, with a proposed plan to resolve adverse effects.

Authorized Signature: EVA BRYSON Date: 2017.08.29 09:15:19 -06'00' Date: August 29, 2017

Type or print name: Eva J. Bryson, Environmental Manager

IV. STATE HISTORIC PRESERVATION OFFICE COMMENTS

☒ Agree with the finding in section III above.
☐ Object to the finding for reasons indicated in attached letter.
☐ Cannot review until information is sought as follows: _____

Authorized Signature: _____ Date: 30 Aug. 2017

[EXTERNAL] Section 106 Submission

noreply@thc.state.tx.us Jun 11, 2018 (1 day ago)

To Eva Bryson, USGS

TEXAS HISTORICAL COMMISSION
real places telling real stories

Re: Project Review under Section 106 of the National Historic Preservation Act and/or the Antiquities Code of Texas
201810347
USGS White-Nose Syndrome Bat Vaccine ProjectNA
Buffalo Gap,TX

Dear Eva Bryson:
Thank you for your submittal regarding the above-referenced project. This response represents the comments of the State Historic Preservation Officer, the Executive Director of the Texas Historical Commission (THC), pursuant to review under Section 106 of the National Historic Preservation Act and the Antiquities Code of Texas.

The review staff, led by Justin Kockritz, has completed its review and has made the following determinations based on the information submitted for review:

Above-Ground Resources

&#8226 THC/SHPO concurs with information provided .
&#8226 No historic properties are present or affected by the project as proposed. However, if historic properties are discovered or unanticipated effects on historic properties are found, work should cease in the immediate area; work can continue where no historic properties are present. Please contact the THC's History Programs Division at 512-463-5853 to consult on further actions that may be necessary to protect historic properties.

Archeology Comments

&#8226 THC/SHPO concurs with information provided

We look forward to further consultation with your office and hope to maintain a partnership that will foster effective historic preservation. Thank you for your cooperation in this review process, and for your efforts to preserve the irreplaceable heritage of Texas. If the project changes, or if new historic properties are found, please contact the review staff. If you have any questions concerning our review or if we can be of further assistance, please email the following reviewers: justin.kockritz@thc.texas.gov.

Sincerely,

for Mark Wolfe, State Historic Preservation Officer
Executive Director, Texas Historical Commission

DEPARTMENT OF ADMINISTRATION
STATE HISTORIC PRESERVATION OFFICE

July 10, 2018

Ms. Eva Bryson
Environmental Manager
United State Dept of the Interior
U.S. Geological Survey
Box 25046 M.S. 205
Denver Federal Center
Denver, CO 80225

RE: Field Studies to Assess the Safety and Effectiveness of White Nose Syndrome Vaccine Candidates in Bats
Multiple locations throughout Minnesota
SHPO Number: 2018-2250

Dear Ms. Bryson:

Thank you for initiating consultation on the above project. Information received in our office on 16 June 2018 has been reviewed pursuant to the responsibilities given the State Historic Preservation Officer by Section 106 of the National Historic Preservation Act of 1966 and implementing federal regulations at 36 CFR 800.

We have reviewed the documentation included with your June 8, 2018 cover letter. As we understand it, the federal undertaking is to perform field studies to help assess the safety and effectiveness of White Nose Syndrome vaccine candidates in bats at multiple locations throughout Minnesota. One of the field sites chosen for this undertaking is the Soudan Iron Mine, a historic property which is listed in the National Register of Historic Places and is also a National Historic Landmark. Based on the nature of the proposed undertaking as well as information that has been provided in your submittal, we concur with your agency's determination that this project will have **no effect** on historic properties.

Please contact Kelly Gragg-Johnson, Environmental Review Program Specialist, at (651) 201-3285 or kelly.graggjohnson@state.mn.us if you have any questions regarding our review of this project.

Sincerely,

Sarah J. Beimers
Environmental Review Program Manager

United States Department of the Interior
FISH AND WILDLIFE SERVICE
Ecological Services
Minnesota-Wisconsin Field Office
4101 American Boulevard East
Bloomington, Minnesota 55425-1665

September 21, 2017

Ms. Eva Bryson
Environmental Manager
U.S. Geological Survey, Office of Administration
P.O. Box 25046 M.S. 205
Denver Federal Center
Lakewood, Colorado 80225

Dear Ms. Bryson:

The U.S. Fish and Wildlife Service (Service) has received your letter dated August 29, 2017 (received September 7, 2017) and additional email communications dated September 14 and 20, 2017 (from Ms. Rachel Abbott also from your agency), requesting concurrence with your determination that the activities associated with the proposed *Field Studies to Assess the Safety and Effectiveness of White Nose Syndrome Vaccine Candidates in Bats* project may affect, but are not likely to adversely affect the northern long-eared bat (*Myotis septentrionalis*). The project area includes two privately owned mines in Pierce County, Wisconsin.

The U.S. Geological Survey (USGS) National Wildlife Health Center (Madison, WI) is proposing to conduct field studies to assess the effectiveness of vaccine candidates that have been developed for oral use in bats and are designed to control white-nose syndrome (WNS) in free-ranging bats. The experimental vaccine uses raccoon poxvirus to carry genes of *Pseudogymnoascus destructans (Pd)*, the causative agent of WNS. The field studies will assess the ability of the vaccine to confer protective immunity against WNS to bats that ingest the vaccines. The field studies will be performed outside two restricted sites in Wisconsin in areas where bats are known to overwinter to assess the effectiveness of the vaccine candidate. USGS plans to apply the vaccine treatment to 225-300 little brown bats (*Myotis lucifugus*) total between the two sites. The vaccine treatment will be contained within a glycerin jelly. After each captured little brown bat is treated it will be released and likely will ingest the vaccine contained within the jelly while grooming and there is potential ingestion of the vaccine treatment by other bats roosting nearby (although likely small amount).

Northern long-eared bat

The northern long-eared bat occurs in hibernacula (including the mines where proposed activities will occur) outside of the active time period for the species (approximately April 1 to September

30). During the active period for the species, it may inhabit forested habitats nearby the project area from approximately April 1 to September 30.

The proposed project will include the use of mist nets or harp nets outside the entrance to both mines to capture little brown bats that will receive the treatment prior to hibernation. The Wisconsin Department of Natural Resources (WDNR) will be in charge of conducting the capture of bats using the traps/nets. The USGS will be handling and treating only little brown bats. If any northern long-eared bats are captured by the WDNR, they will be promptly released without receiving the treatment. Captured little brown bats will be treated with the vaccine and released by USGS personnel. Also, all USGS researchers will observe all cave and mine closures and decontamination protocols as required by section NR 40.07 (8) (b) 1., 2., and 3. Wisconsin Administrative Code as well as the Service's 6/29/09 Disinfection Protocol.

There is potential for northern long-eared bats that roost in close proximity to treated little brown bat(s) within the hibernaculum to ingest a very small amount of the glycerin jelly with the vaccine treatment within it. The raccoon poxvirus that is being used for the vaccine treatment has been tested in a laboratory setting on little brown bats with no adverse effects found. Also, the glycerin jelly that the treatment is contained within has also proven non-harmful to bats. We would expect a negligible or insignificant affect to northern long-eared bats from the application of the experimental vaccine on little brown bats. Therefore, we concur that the proposed project may affect, but is not likely to adversely affect the northern long-eared bat.

Although concurrence with our office is not required for "no effect" determinations, we agree with your determination of "no effect" for Higgins Eye (pearlymussel) (*Lampsilis higginsii),* Sheepnose Mussel (*Plethobasus cyphyus), and* Prairie Bush-clover (*Lespedeza leptostachya)* as the proposed project does not occur within or impact suitable habitat for these species.

Thank you for your coordination on this issue. This concludes Section 7 consultation on the proposed action. Please contact us if plans change in a manner that may lead to effects not previously considered. For any further coordination, please contact Jill Utrup at 952-252-0092 (extension 207) or via email at *jill_utrup@fws.gov*.

Sincerely,

Betsy M. Galbraith

for:

Peter J. Fasbender
Field Supervisor

cc: Rachel Abbott, USGS (email only)
Tonie Rocke, USGS (email only)
Jonathan Reichard, USFWS(email only)
Richard Geboy, USFWS (email only)
Paul White, WDNR (email only)

Appendix B

Compliance with Environmental Statutes

From the ***US Geological Survey Manual*** (2002) http://www.usgs.gov/usgs-manual/handbook/hb/445-1-h/ch1.html

> Chapter 1 Authority, Purpose, and General Policies:
>
> 1. Scope: This Handbook established the US Geological Survey (USGS or Bureau) policy for compliance with both statutory and regulatory requirements and the management of USGS environmental programs.
>
> A. Applicability.
>
> (1) This manual applies to all USGS facilities and organizations.
>
> (2) The major Federal environmental statues contain waivers for sovereign immunity that require USGS facilities to comply not only with Federal, but also State and local substantive and procedural requirements. Applicable Federal, State, and local requirements or Executive Orders (EO) which are more stringent than this Handbook will be followed.
>
> (3) State and local regulatory programs may establish regulations which are more stringent than the Federal requirements. Each USGS facility should obtain copies of its respective State and local regulations to determine if the facility is subject to requirements that go beyond the Federal laws and regulations.

The following table lists some of the Federal legal mandates that are pertinent to the proposed action. This list is representative, not exhaustive, and is compiled for information, not for legal purposes.

Pertinent Federal Legal Mandates – representative, not exhaustive

Element	Authority	Compliance
Air Quality	The Clean Air Act of 1970, as amended (42 USC 7401 et seq.) National Emissions Standards for Hazardous Air Pollutants (40 CFR Parts 61 and 63)	Proposed action does not require air quality permitting.
Bald Eagles	Bald Eagle Protection Act (16 USC 668).	Response from USFWS analysis found that no endangered or threatened species are known to occupy the project area. (9-21-2017).
Cultural, Archeological and Historical Resources	National Historic Preservation Act, as amended (16 USC 470); Antiquities Act of 1906 (16 USC 431-433); Archeological and Historic Preservation Act (AHPA) of 1974 (16 USC 469 et seq.); Archaeological Resources Protection Act of 1979 (16 USC 470(aa) et seq.); Historic Sites, Buildings and Antiquities Act of 1935 (16 USC 461-462, 424-467; 49 Stat.666), as amended National Register of Historic Places (36 CFR 60) Protection of Historic and Cultural Properties (35 CFR 700)	Correspondence with the WI SHPO concerning Cultural Resource Assessment Section 106 Review (8-28-2017) states: *"No historic properties will be affected (i.e., none is present or there are historic properties present, but the project will have no effect upon them)."*

Endangered Species	Endangered Species Act of 1973 (16 USC 1531 et seq.)	Correspondence from the USFWS (9-21-2017) notes *"Although concurrence with our office is not required for "no effect" determinations, we agree with your determination of "no effect" for Higgins Eye Pearlymussel (Lampsilis higginsii), Sheepnose Mussel (Plethobasus cyphyus), and Prairie Bush-clover (Lespedeza leptostachya) as. the proposed project does not occur within or impact suitable habitat for these species."*
Energy	Energy Policy Act (EPACT) of 2005 (PL 109-58) National Energy Conservation Policy Act of 1978 (PL 95-619) EO 12759, April 15, 1991, Federal Energy Management EO 12902, March 8, 1994, Energy Efficiency and Water Conservation at Federal Facilities EO 13123, June 3, 1999, Greening the Government Through Energy Efficient Management	Proposed action does not impact energy resources, nor does it produce greenhouse gases.
Environmental Justice	EO 12898, February 11, 1994, Environmental Justice	Proposed action does not impact minority or low-income populations inequitably.
Environmental Protection	National Environmental Policy Act (NEPA) of 1969 as amended (PL 91-190, 42 USC 4321 et seq.)	The proposed action is following all requirements and regulations.
Farmland	Farmland Protection Policy Act (7 U.S.C. 4201, et seq.)	Proposed action will not convert farmland to nonagricultural use.
Floodplains	Watershed Protection and Flood Prevention Act (16 U.S.C. 1101, et seq. 33 U.S.C. 701b) EO 11988, May 24, 1977, Floodplain Management Floodplain Management (42 CFR 26951)	Proposed action does not impact national or local waterways and does not require construction of flood protection measures.

Pertinent Federal Legal Mandates – representative, not exhaustive

Element	Authority	Compliance
Hazardous and Solid Waste	Hazardous and Solid Waste Amendments of 1984 (PL 98-616) Federal Facilities Compliance Act of 1992 (PL 102-386) Hazardous Materials Transportation Uniform Safety Act of 1990 (PL 101-615) Pollution Prevention Act of 1990 (42 USC 13101 et seq.) Resource Conservation and Recovery Act of 1976, as amended (42 USC 2901 et seq.) Toxic Substances Control Act of 1976 (15 USC 2601 et seq.) Solid Waste Disposal Act of 1965, as amended (42 USC 3251 et seq.) EO 12856, August 3, 1993, Federal Compliance with Right-to-Know Laws and Pollution Prevention Requirements EO 12873, October 20, 1993, Federal Acquisition, Recycling and Waste Prevention EO 13101, September 15, 1998, Greening the Government Through Waste Prevention, Recycling, and Federal Acquisition	Bait ingredients are food grade, FDA approved, and do not contain any hazardous substances. Vaccine-laden glycerin jelly will be applied to bat houses in the environment for consumption by bats. Glycerin jelly undergoes natural biodegradation and photo degradation in the environment. Although vaccine-laden jelly is expected to disappear in days, uneaten jelly will be removed from the study sites and disposed of by autoclaving.
Health and Safety	Occupational Safety and Health Act of 1970 (29 USC 651 et seq.) Occupational Safety and Health Standards (29 CFR 1910)	All actions proposed will comply with appropriate health and safety regulations and standards.

Pertinent Federal Legal Mandates – representative, not exhaustive

Element	Authority	Compliance
Migratory Birds	Migratory Bird Treaty Act of 1918, as ameneded,16 USC 703-71	Response from USFWS analysis found that no endangered or threatened species are known to occupy the project area. (9-21-2017).
Noise	Noise Control Act 1972 (42 U.S.C. Sec 4901 et seq.)	All bait distribution will be conducted on foot and transport vehicles will use and remain on established roads.
Noxious Weeds	Federal Noxious Weed Act of 1974 (7 USC 2801 et seq.) Noxious Plant Control Act of 1968 (45 USC 1241 et seq.) Non-indigenous Aquatic Nuisance Prevention and Control Act of 1990 (16 USC 4701, 104 Stat. 4761, Title I of P.L. 101-646) EO 13112, February 3, 1999, Invasive Species Carlson-Foley Act of 1968 (PL 90-583)	The proposed action will not distribute seeds and plants and bait distribution will be conducted on foot to further reduce unintentional transport of seeds. Personnel will be trained to avoid infested areas.
Soil	Soil Conservation Act of 1938 (16 USC 5901 et seq.)	The proposed action will not disturb the soil and bait distribution will not chemically alter the soil composition.
Water Quality	Clean Water Act of 1977, as amended, (PL 95-217, 33 U.S.C. 1251 et seq.) – Section 401 Oil Pollution Act of 1990 (PL 101-380, 33 USC 2701 et seq.) Pollution Prevention Act of 1990 (42 USC 13101 et seq.) Water Quality Act of 1965 (PL 89-234) Safe Drinking Water Act (SDWA) of 1974 (42 USC 3000(f) et seq.)	The proposed action will have no impacts to surface or ground water.

Pertinent Federal Legal Mandates – representative, not exhaustive

Element	Authority	Compliance
Wetlands	Section 404 (USC 1344) Clean Water Act Section 401 (33 USC 1341) Clean Water Act Section 10 (33 USC. 403) Rivers and Harbor Act. North American Wetlands Conservation Act, 16 U.S.C. Sec. 4401 et seq. EO 11990, May 24, 1977, Protection of Wetlands	The proposed action will have no impact to waters of the US including but not limited to; rivers, streams, ditches, coulees, lakes, ponds and their adjacent wetlands.
Wildlife	Fish and Wildlife Conservation Act of 1980 (16 USC 2901 et seq.) Wildlife and Fisheries (40 CFR 1-End)	No additional permits or actions are required for implementation of the proposed project.

Notes:
CFR – Code of Federal Regulations
EO – Executive Order
PL – Public Law
Stat. – Statute
USC – United States Code

South Congress Bridge, Austin, TX – Bats in flight at dusk - Fritz Poelking/Getty Images