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ENVIRONMENTAL ASSESSMENT

FIELD AND CAPTIVE STUDIES TO ASSESS THE SAFETY AND EFFECTIVENESS OF TREATMENT DELIVERY METHODS IN BATS

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Table 1: Selected Wisconsin county – Human Environment

Table 2: Selected Minnesota counties – Human Environment

ACRONYMS

ACUC	Animal Care and Use Committee
BSL	Biosafety level
DNA	Deoxyribonucleic acid
EA	Environmental assessment
EIS	Environmental impact statement
FEA	Filament Extension Atomization (FEA)
NWHC	National Wildlife Health Center
PARC	Palo Alto Research Center
<i>Pd</i>	<i>Pseudogymnoascus destructans</i>
RCN	Raccoon poxvirus
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
WNS	White-nose syndrome

ENVIRONMENTAL ASSESSMENT OF FIELD AND CAPTIVE STUDIES TO ASSESS THE SAFETY AND EFFICACY OF TREATMENT DELIVERY METHODS IN BATS

1. INTRODUCTION

The U.S. Geological Survey (USGS) National Wildlife Health Center (NWHC) in Madison, WI, is proposing to conduct field and captive animal studies to assess the safety and efficacy of treatment delivery methods to allow for the treatment of wild bats against diseases such as white-nose syndrome (WNS). A combination of field and captive studies will be done to assess mediums in which to deliver oral vaccines, as well as devices to administer treatments in the field. The goal of these studies will be to produce safe, effective delivery methods for treating wild bats while producing the least possible distribution to wildlife, the environment and humans.

Captive studies to assess delivery mediums and volumes will take place at the NWHC with wild caught and captive-bred big brown bats (*Eptesicus fuscus*), as well as wild-caught little brown bats (*Myotis lucifugus*). Field studies will take place in restricted sites in Minnesota and Wisconsin (Figures 1 and 2) with species of bats known to be susceptible to WNS to test the efficacy of aerosol devices for the non-invasive delivery of WNS treatments to wild bats. The specific sites will be selected just prior to field work based on the presence of bats and the total population.



Little brown bat
Photo courtesy of BatWorlds



Big brown bat
Photographer: Phil Myers, University of Michigan

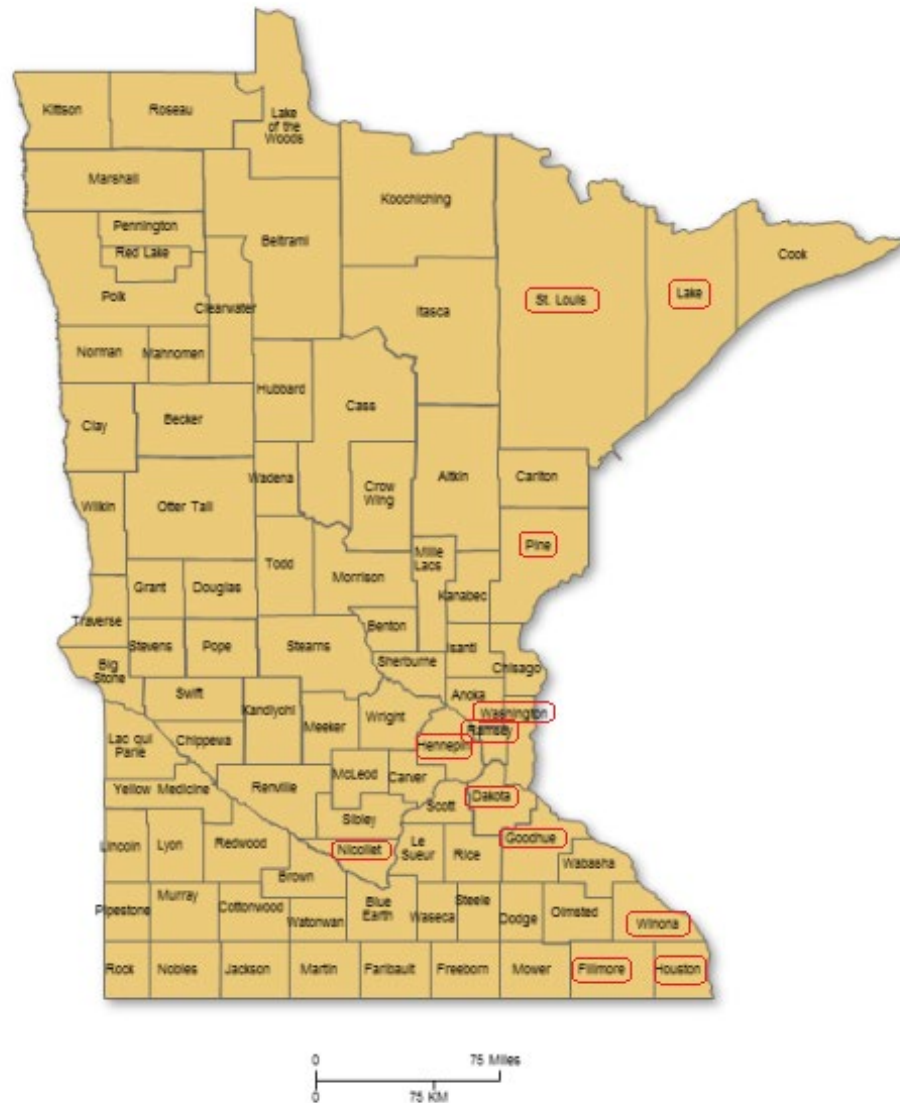


Figure 1: Selected Minnesota counties for field studies – Dakota, Fillmore, Goodhue, Hennepin, Houston, Lake, Nicollet, Pine, Ramsey, St. Louis, Washington, and Winona

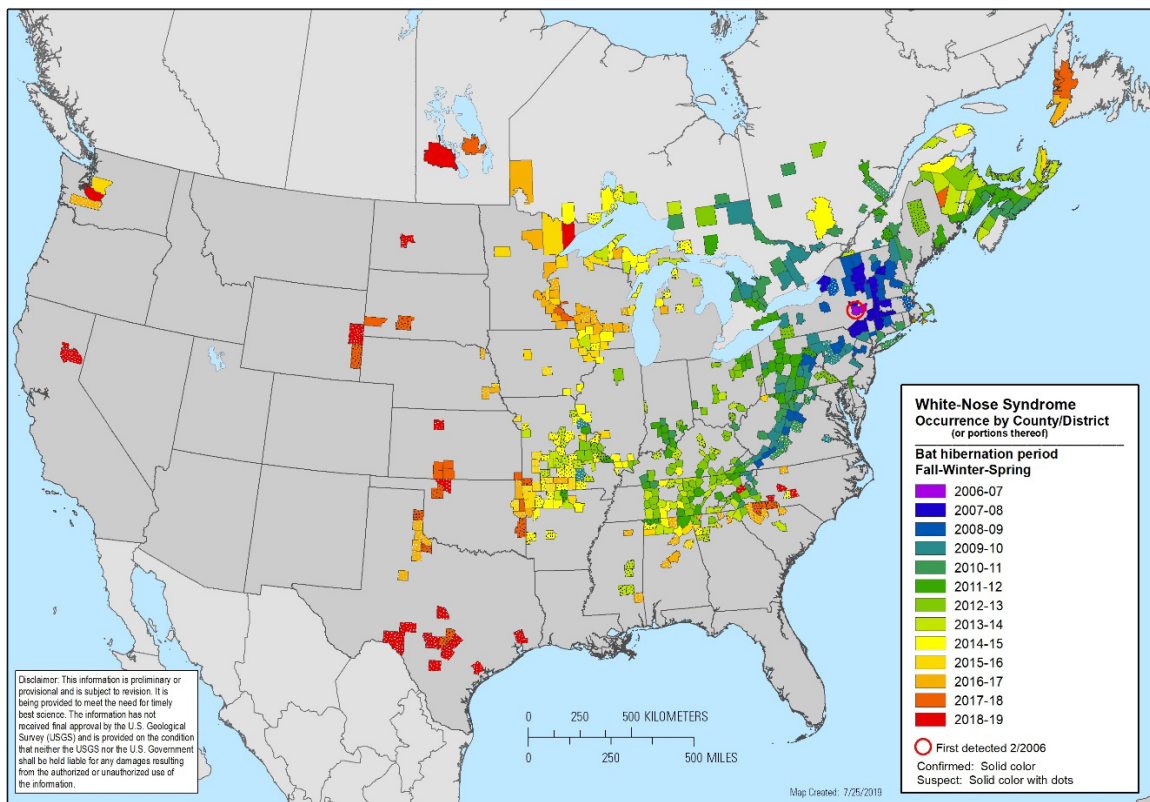


Figure 2: Selected Wisconsin county for field studies – Pierce

2. BACKGROUND

White-nose syndrome, caused by the fungus *Pseudogymnoascus destructans* (Pd) (Lorch et al. 2011), was first discovered to be affecting hibernating North American bats in 2006 (Blehert et al. 2009). Since its emergence, WNS has been responsible for wide-spread declines in bat populations across eastern and central regions of North America (Turner et al. 2011). While Pd infects multiple species of hibernating bats, four species have suffered the greatest declines; these include little brown bat (*Myotis lucifugus*), Indiana bat (*Myotis sodalist*), tricolored bat (*Perimyotis subflavus*), and northern long-eared bat (*Myotis septentrionalis*) (Langwig et al. 2012; Langwig et al. 2015). The fungus is well adapted to hibernacula, as it grows in dark, cold and moist environments and grows easily on the skin of hibernating bats (Verant et al. 2012). Fungal growth of Pd on the wings of bats alters their ability to regulate body temperature, hydration, and other physiological processes critical for hibernation (Reeder et al. 2012; Cryan et al. 2013; Verant et al. 2014). These physiological alterations during hibernation lead bats to arouse more frequently from torpor, reducing vital fat stores potentially leading to emaciation and death (Reeder et al. 2012).

Since its initial emergence in New York, Pd has continued to spread westward across North America, threatening the persistence of hibernating bat species (Fig. 3). Due to the severity of the disease and precipitous declines in populations where the disease has emerged, there is great interest in producing management tools to prevent or mitigate WNS infections in the wild.



Citation: White-nose syndrome occurrence map - by year (2019). Data Last Updated: 7/25/2019. Available at: <https://www.whitenosesyndrome.org/static-page/wns-spread-maps>.

*Figure 3. Spread and occurrence of *Pseudogymnoascus destructans* (Pd) throughout North America (updated 8/30/19). Source www.whitenosesyndrome.org*

3. PURPOSE AND NEED FOR THE ACTION

The purpose of the proposed action is to assess the safety and efficacy of treatment delivery methods in wild bats. The need for these actions is explained below.

As previously mentioned, (see Background), WNS poses a significant threat to the persistence and survival of hibernating wild bats in North America. There is significant interest in the development of tools to manage WNS to help prevent the spread of the pathogen while mitigating losses in affected populations. Tools currently being developed and tested include volatile compounds released by bacteria, chemical anti-fungals, probiotic microbes, and vaccines (Hoyt et al. 2019).

Oral vaccine candidates have been developed and jointly tested by the USGS NWHC and the University of Wisconsin (Madison, WI), and are intended as a preventative tool for controlling WNS in wild North American bats. These vaccine candidates use a viral vector (attenuated raccoon poxvirus, RCN) genetically modified to express highly-conserved fungal and specific Pd antigens. These vaccine candidates have been shown to elicit antifungal immunity, reducing weight loss, and the risk of mortality from WNS in captive, experimentally challenged little brown bats (*M. lucifugus*) (Rocke et al. 2019). While these vaccines and other potential treatments continue to be developed, there is a need for safe and effective methods of treatment delivery. Establishing methods for the mass delivery of treatment options in bats will allow for the immediate application of tools against WNS, such as vaccines, once they are fully developed.

Specifically, there is a need to determine the application volume for topical administration of delivery mediums. Topical application is a viable and practical method for treatment delivery in bats, as they are known to self and/or socially groom allowing for the ingestion of administered treatments. The high rate of self and mutual grooming in bats has been previously exploited in Central and South America to deliver poisons to eliminate vampire bat colonies. Only a small number of bats may need to be administered a treatment, as contact and mutual grooming allows for the transfer of treatment among a colony. Additionally, topical application may allow for the local absorption of treatment through the skin, on areas susceptible to colonization by Pd.

Rhodamine B (RB; <0.5% concentration) will be used as a biomarker to assess medium uptake. Biomarkers are regularly incorporated into baits to evaluate the success of bait distribution or to identify animals that have consumed vaccine-laden bait (Southey et al. 2002; Fernandez and Rocke 2011). 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. Preliminary results from various studies in bats have demonstrated a high success rate in the administration and uptake of RB-laden delivery medium, both through direct administration and subsequent social grooming, as well as environmental application to entries of bat houses (Rocke et al. unpublished data). Captive studies are needed to monitor uptake and transfer between bats through topical application with RB-laden medium. Captive studies of individually housed bats would allow for the determination of both the minimum volume of medium required for RB marker detection, while studies with co-housed bats would determine rates of medium transfer among bats.

Glycerin jelly, a semi-solid paste that is easily applied to surfaces, readily ingested by bats, and stable for the vaccine, is a potential medium for treatment delivery. 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), big brown bats (*Eptesicus fuscus*) (Stading et al. 2017), vampire bats (*Desmodus rotundus*), or little brown bats (*Myotis lucifugus*) (Rocke, unpublished data). While this medium has proven to be safely administered to various bat species, there is still a need to determine if this medium is the most effective for broad treatment delivery in bats susceptible to WNS.

USGS NWHC, in partnership with Palo Alto Research Center (PARC), is in the process of developing scalable, universal delivery devices. These devices implement novel aerosol technology allowing for the delivery of viscous mediums through a high-quality spray. Filament Extension Atomization (FEA) has a unique application over a wide-range of fluid viscosities, ranging from 1 mPa-s (the viscosity of water) to 600 Pa-s (the viscosity of peanut butter). FEA technology (Fig. 5) allows the generation of narrowly-dispersed micron-sized droplets from fluids with this range of viscosities. The small aerosol droplets can then be inhaled or ingested by the bats (mucosal uptake in the mouth or nose) as well as absorbed through their skin (transdermal uptake) with the viscosity contributing significantly to bioretention in both cases.

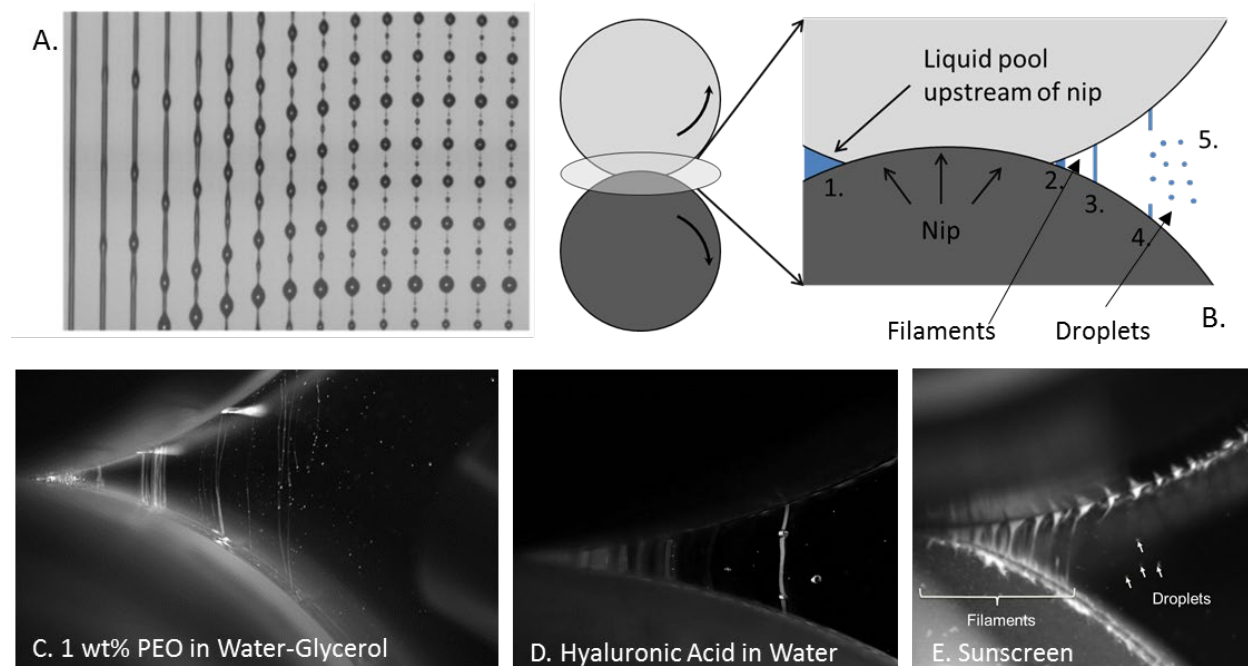


Fig. 4. PARC FEA Technology: A. Beads-on-a-string structures in viscoelastic fluids; B. Highly parallelized FEA technology in which fluid is 1) dispensed onto a roller, 2) rapidly turned into a large number of filaments that 3) thin and eventually 4) break into droplets; C.-E. Images from high speed videos of representative fluids sprayed with FEA (PEO – Polyethylene Oxide in Water-Glycerol, Hyaluronic Acid, and Sunscreen).

Prototype delivery devices produced by PARC will need to be tested in controlled laboratory settings by NWHC personnel to assure proper volume delivery. Once uptake is confirmed in laboratory studies, the mass scalable delivery methods in field settings will need to be tested. Devices will be mounted at cave entrances to allow for delivery as bats fly through (Fig. 6). The ideal field-deployable system will be motion-actuated and on a timer so bats will be targeted primarily during early morning fly-in, when grooming behavior and contact with other bats would be maximized (Fig. 6). Coupled with the on-going work at NWHC in developing vaccine treatments for WNS, PARC's aerosol delivery solution will allow wider distribution of the formulations across bat colonies with a clear path to scaling up for massive field-deployment to potentially limit or reduce the occurrence of WNS without significant disruption of bat behavior.

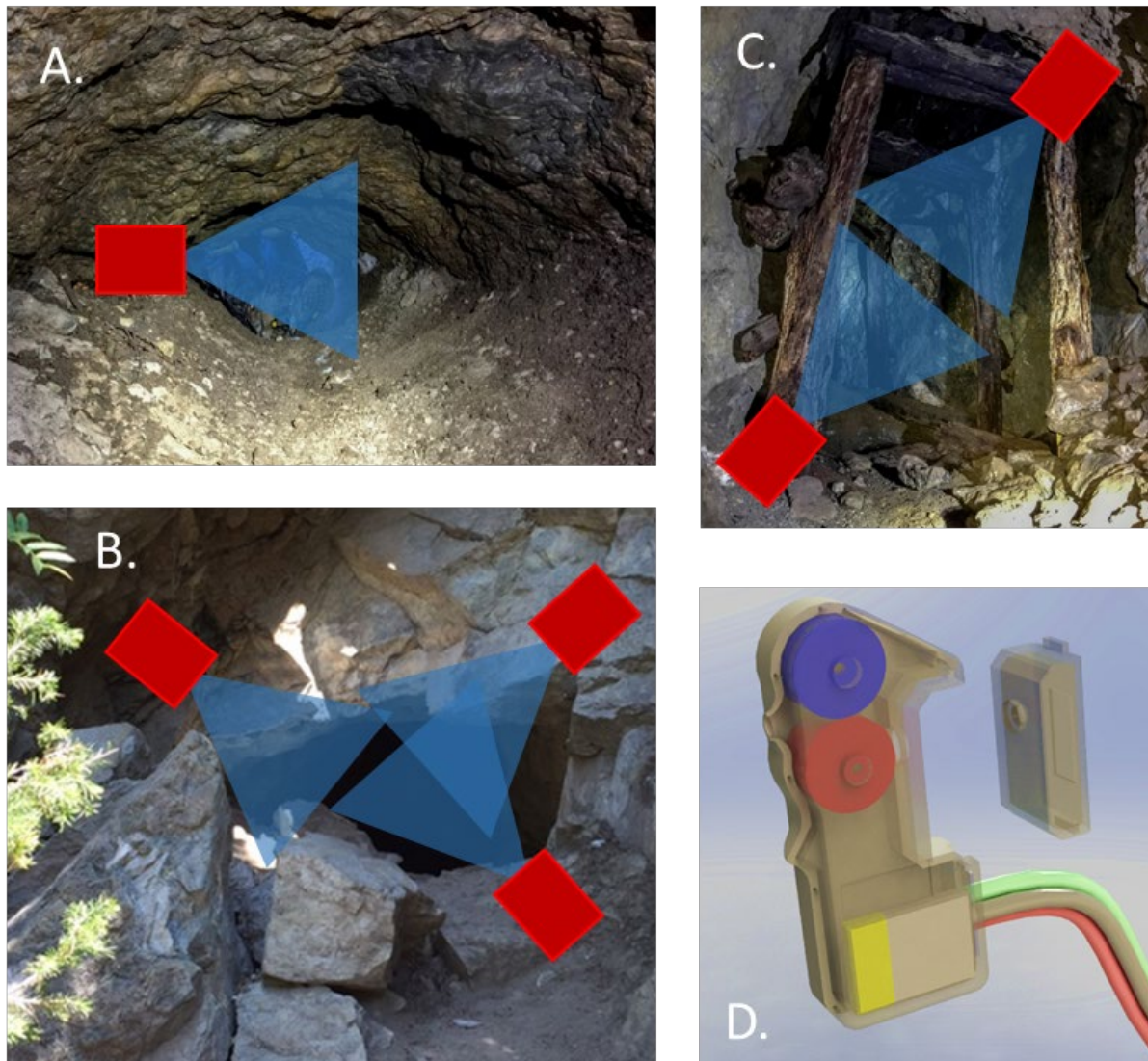


Figure 5. FEA Device Field-Deployment: A. Single device covering an entrance to a small cave/mine, B. Multiple FEA devices covering a large cave entrance, C. Multiple FEA devices covering an internal contraction inside a mine, D. Potential device form factor.

Bats provide valuable economic and ecosystem services which help to maintain agricultural productivity and ecological health (Kasso and Balakrishnan 2013). As WNS continues to spread across North America it is important to develop methods for preventing future infections, while mitigating effects in currently infected populations. Mitigation is of particular importance for the recovery of affected populations, since reproductive females of most bat species produce only one pup each year. A breakthrough in the fight against WNS is vital in ensuring bat populations do not continue to decline, which would create losses in not only bat numbers but also in various sectors of our economy and in our natural systems.

3.1 Decisions to be made

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

- Should the USGS undertake captive studies to determine the safety and efficacy of treatment delivery mediums and devices?
 - If not, should the USGS implement another alternative?
- Should the USGS undertake field studies to determine the safety and efficacy of treatment delivery devices?
 - If not, should the 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?

3.2 Scoping and issues

3.2.1 Actions analyzed

This Environmental Assessment evaluates the environmental effects of the application of studies to assess the safety and effectiveness of the treatment delivery methods in wild bats.

3.2.2 Site specificity

The analysis of alternatives is limited to potential study sites in Minnesota 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 delivery device and related information, examines the potential effects that field testing this universal delivery device 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 delivery device 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 supplies a description of reasonable alternative actions that address the Purpose and Need in enough detail to show 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 five (5) captive trials at the NWHC will be conducted over a one-to-two (1 to 2) year period to evaluate the safety and efficacy of various treatment delivery mediums and devices. These studies will provide important information regarding the uptake and safety of the mediums in wild bats in addition to identifying the volumes necessary for efficacy. It will also provide an opportunity to test aerosol spray devices, determine their safety, efficacy, and optimization for use in free-living bats in up to five (5) field trials.

4.1.2 Proposed activities

The USGS-NWHC is proposing to conduct both captive and field trials to assess the safety and efficacy of treatment delivery methods in wild bats. Cooperating agencies include USFWS, Wisconsin Department of Natural Resources, Minnesota Department of Natural Resources, and Texas Parks and Wildlife Department. Captive studies to optimize delivery mediums and volumes will begin in the fall/winter 2019; additional captive studies will begin in summer 2020 to test prototype spray devices for oral and topical delivery of treatments. After the completion of captive studies, field trials will begin to test delivery treatment in free-flying wild bats. In all these studies, rate of uptake and delivery will be determined by use of a biomarker. The primary objective of these studies will be to produce a mass delivery system for treatment of wild bats that could be used to mitigate the effects of white-nose syndrome in North America.

Initial captive trials will be done using wild-caught and captive-bred big brown bats (*Eptesicus fuscus*) (max of 75), with follow-up studies in a smaller group of little brown bats (*Myotis lucifugus*) (<25). Bats will be caught locally in Wisconsin or other states as needed. Bats will be treated topically, either by hand or by aerosol device, with test formulations (volumes ranging from 0.2 – 1ml) that include the biomarker Rhodamine B. Bats will either be housed individually or co-housed with untreated bats. Uptake and transfer between bats will be done by collecting hair for biomarker analysis.

Field trials will be done at selected sites (3-4) that are limited in size (e.g. <3 acres). Access will be amenable to contingency management in the unlikely event of an adverse outcome of treatment medium uptake in bats or non-target species. Application rates of medium via aerosol device will be determined from previous studies and employed to allow maximum contact and uptake by individual animals. At each site selected for study, the timing of application and the application period will vary from 1-3 days, depending on the location as well as the type and size of the roost. Sites will be selected where little brown bats (*Myotis lucifugus*) or big brown bats (*Eptesicus fuscus*) are roosting, including hibernacula and maternity roosts after the young are flying. Bats captured after treatment will be examined for evidence of medium uptake and general health. Any carcasses found will be submitted for full diagnostic testing. Animals will be released or euthanized depending on health status, as is 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 effects of mediums and delivery devices. Field studies would be suspended until laboratory studies were completed.

Elements of the design for these studies will include:

- 1) Assessment of medium uptake via Rhodamine B biomarker and estimate of topical medium removal rate.
- 2) Post-treatment monitoring for morbidity and/or mortality in bats.

4.1.3 Monitoring and mitigation activities

4.1.3.1 Monitoring

After topical application of medium to captive bats, either by direct application or via aerosol device, observers will check the presence or absence of medium daily. Uptake will be measured by incorporating a biomarker, Rhodamine B (RB), into the medium (Fernandez and Rocke 2011). Medium containing RB is a bright red color that can be visualized 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.

For field studies, bats will be trapped 5-7 days after medium delivery to collect hair samples for biomarker analysis. Each captured bat will be inspected for outward signs of a negative response to the delivery medium, 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 RB-laden medium 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 and harp traps.
- Animals in captive studies that cannot be re-released will be used in other studies to mitigate the number of animals needed to be caught in the future.
- Animals caught 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.
- 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.
- All animal handling, capture and use protocols will be approved by the NWHC Animal Care and Use Committee.

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 treatment delivery methods in captivity and in the field prior to the mass delivery of WNS treatment 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 (2019-2020) is the earliest time when these studies would be possible. 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 delivery methods would occur. This delay would impact future applications of WNS treatments 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.

4.2.3 No action (Alternative 3)

No RB-laden medium would be applied to bats. USGS would not conduct research for WNS control or use resources available. Field studies mass delivering successful vaccine candidates, or other treatments against WNS as management tools for bat conservation

would be prevented. WNS would continue to pose an unregulated threat to existing populations of bats.

4.2.4 Alternatives considered but eliminated from detailed analysis

There are no practical alternatives to captive studies for the assessment of treatment delivery to wild bats. The only alternative to field studies would be more laboratory studies although they would not be able to provide an assessment of delivery success in free-flying bats, which is critical for optimizing a system for mass delivery as a management tool. Preliminary laboratory and field studies have been performed with RB-laden glycerin jelly in multiple bat species. These studies demonstrated safety of the topical application and oral ingestion of the RB-laden medium (Rocke et al. unpublished). Numerous studies have shown the safety of RB as a marker in treatment laden baits (e.g. Fernandez & Rocke, 2011; Southey et al., 2002).

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 and big brown bats in Minnesota and Wisconsin 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 Wisconsin county – Human Environment

County	Pierce, WI
Total Population	42,555
Housing units	16,693
Veterans	2,434
Racial Makeup	
White	95.8%
Black/African American	0.9%
Native American	0.5%
Asian	1.3%
Pacific Islander	0.0%
Two or more races	1.5%
Hispanic or Latino of any race	2.1%
Median Income and Poverty	
Household	\$66,772
Per Capita Income	\$31,109
Persons in poverty	7.8%
Education	
High School Graduate	94.5%
Bachelor's degree or higher	27.7%

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 and big 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 little brown bats and big 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 big brown 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, buildings, wood piles and rock crevices. 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 predominantly in caves or mines, but bats can be found in warm man-made structures.

5.3.2 Terrestrial Mammals

Bat species include little brown bats (*Myotis lucifugus*) and big brown bats (*Eptesicus fuscus*).

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 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.

Threatened, endangered, and candidate species in Pierce County, Wisconsin

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Higgins Eye (pearly mussel)	<i>Lampsilis higginsii</i>	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Northern long-eared bat	<i>Myotis septentrionalis</i>	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	<i>Lespedeza leptostachya</i>	Threatened	No open land or wetlands are present in the project area which would support this species.	None
Sheepnose Mussel	<i>Plethobasus cyphus</i>	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
American Bittern	<i>Botaurus lentiginosus</i>	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	<i>Pluvialis dominica</i>	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	<i>Haliaeetus leucocephalus</i>	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	<i>Chlidonias niger</i>	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

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Black-billed Cuckoo	<i>Coccyzus erythrophthalmus</i>	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	<i>Dolichonyx oryzivorus</i>	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.

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	<i>Lynx canadensis</i>	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	<i>Cardellina canadensis</i>	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	<i>Setophaga tigrine</i>	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	<i>Dendroica cerulea</i>	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	<i>Calidris alpina arctica</i>	Breeds elsewhere	No tidal flats, beaches, or bodies of water are present in the project area which would support this species.	None

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Eastern Whip-poor-will	<i>Antrostomus vociferous</i>	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
Evening Grosbeak	<i>Coccothraustes vespertinus</i>	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	<i>Aquila chrysaetos</i>	Breeds elsewhere	This species is not present in the state of Minnesota.	None
Golden-winged Warbler	<i>Vermivora chrysoptera</i>	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	<i>Canis lupus</i>	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	<i>Ammodramus henslowii</i>	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.

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Higgins Eye (pearly mussel)	<i>Lampsilis higginsii</i>	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Hudsonian Godwit	<i>Limosa haemastica</i>	Breeds elsewhere	This species is not present in the state of Minnesota.	None
Karner Blue Butterfly	<i>Lycaeides Melissa samuelis</i>	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 (<i>Lupinus perennis</i>) in the project area.	None
Kentucky Warbler	<i>Oporornis formosus</i>	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	<i>Ixobrychus exilis</i>	Breeds Aug 16 to Oct 31	This species is not present in the state of Minnesota.	None
Leedy's Roseroot	<i>Rhodiola integrifolia ssp. leedyi</i>	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	<i>Tringa flavipes</i>	Breeds elsewhere	No marshes, mudflats, shores, or ponds are present in the project area which would support this species.	None
Long-eared Owl	<i>Asio otus</i>	Breeds Mar 1 to Jul 15	No dense forests with open meadows are present in the project area which would support this species.	None

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Northern Long-eared Bat	<i>Myotis septentrionalis</i>	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	<i>Contopus cooperi</i>	Breeds May 20 to Aug 31	This species is not present in the state of Minnesota.	None
Prairie Bush-clover	<i>Lespedeza leptostachya</i>	Threatened	No open land or wetlands are present in the project area which would support this species.	None
Prothonotary Warbler	<i>Protonotaria citrea</i>	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	<i>Melanerpes erythrocephalus</i>	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	<i>Arenaria interpres morinella</i>	Breeds elsewhere	No beaches, mudflats, jetties, or rocky shores are present in the project area which would support this species.	None
Rusty Blackbird	<i>Euphagus carolinus</i>	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	<i>Bombus affinis</i>	Endangered	No open land or native prairie forbs are present in the project area which would support this species	None

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Semipalmated Sandpiper	<i>Calidris pusilla</i>	Breeds elsewhere	No beaches, mudflats, or lakes are present in the project area which would support this species.	None
Sheepnose Mussel	<i>Plethobasus cyphus</i>	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Short-billed Dowitcher	<i>Limnodromus griseus</i>	Breeds elsewhere	No mudflats, tidal marshes, or freshwater ponds are present in the project area which would support this species.	None
Snuffbox Mussel	<i>Epioblasma triquetra</i>	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Spectaclecase (mussel)	<i>Cumberlandia monodonta</i>	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None
Whooping Crane	<i>Grus Americana</i>	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	<i>Empidonax traillii</i>	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	<i>Quadrula fragosa</i>	Endangered	No bodies of water or rivers are present in the project area which would support this species.	None

Species Name	Scientific Name	Status	Potential Impact	Potential Mitigation
Wood Thrush	<i>Hylocichla mustelina</i>	Breeds May 10 to Aug 31	The project is not found 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.

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 34). The Texas State Historic Preservation Officer (June 2018) has determined that no historic properties will be affected at the study sites (Appendix B, page 50)

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 biomarker, delivery devices, capture/handling of animals and placing animals in captivity.

- **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 (Fisher et al. 1999; Southey et al. 2002; Fernandez and Rocke 2011). 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.

- Delivery device

Aerosol spray devices supply a promising method of delivering treatment to wild bats, particularly on dose control and scalability. After thorough testing in captivity, in a controlled setting, delivery devices be used in the field. The safe implementation of automated delivery devices for use in wildlife has been done in other systems (Müller et al. 2012; Smyser et al. 2015). Captive experiments will determine safe and effective doses, as well as distance of spray and other logistical details which will ensure the specific targeting of free-flying bats at cave entrances. Additionally, these devices will only be in place for short windows of time and will be monitored throughout their use in the field.

- 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. Big brown bats used in captive studies may be caught locally in houses by pest control operators and brought to the NWHC, they may also be acquired from local rehabbers, or bred in captivity.

Bats will be examined for injuries and evidence of WNS. For field trials, hair samples will be collected for biomarker analysis and bats will be released at the point of capture after processing. 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).

- Bats in captivity

A limited number of bats will be removed from the wild, including little brown (<25) and big brown bats (up to 75). Initial captive trials will be done with big brown bats who are common and who, among hibernating bats, have suffered some of the smallest declines from WNS (Langwig et al. 2012). Big brown bats are often removed as pests from residential and other man-made structures, providing opportunistic trapping opportunities. Additionally, they adapt well to captivity, making them a valuable model for the study of WNS and treatment delivery. Unlike big brown bats, little brown bats have suffered significant declines in Eastern and Central North America due to the emergence of WNS (Frick et al. 2010), and are more difficult to maintain in captivity. It should be noted despite the challenges with little brown bats, they have been successfully housed at the NWHC in past studies. Little brown bats will only be

used for follow-up captive studies to optimize delivery methods for this and other similar species. It is essential that any delivery method developed from these trials is safe and effective in little brown bats, as they are highly susceptible to WNS and have suffered precipitous declines from the disease. For both species, the number of bats that will be removed from the wild is not anticipated to have any significant negative impacts on population sizes.

6.1.2 Issues considered in detail with rationale

- Medium 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). Other jelly or delivery mediums may be tested in captivity but will not be used in field trials until captive studies confirm their safety and efficacy.

- 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 big brown 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.

6.1.3 Effects of climate change, 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 C, page 38) 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 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 2007).

6.2.1.2 Potential impacts of capture/handling methods 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 treatment delivery methods would impact future studies on vaccine candidates, and other treatments, 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 delivery medium, biomarker, 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 and small-scale captive studies will not result in risk of cumulative adverse impacts on the quality of the human environment.

6.4 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
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 often, and animals released at once after sample collection.	Same as Alternative 1.	Same as Alternative 1.	No impact.

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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.

Appendix A

Agency Coordination

ROUT 9-6-17

HP-05-07 (8/15/02)

For SHPO Use Only, Class 3

17-1208/PI

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:

I. GENERAL INFORMATION

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

AUG 28 2017

- a. Federal Agency Jurisdiction (Agency providing funds, assistance, license, permit): U.S. Geological Survey
 b. Federal Agency Contact Person: Eva J. Bryson Phone: 303-236-6172
 c. Project Contact Person: Eva J. Bryson Phone: 303-236-6172
 d. Return Address: P.O. Box 25046, MS 206, 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: W5000 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, WI Zip Code: 54723 and 54750
 i. Project Location: Township 24N, Range 17W, B/W (circle one), Section 9, Quarter Section _____
 Township 24N, Range 16W, Section 15

j. Project Narrative Description—Attach Information as Necessary

- k. Attach of Potential Effect (APE). Attach Copy of U.S. U.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 800.7. Attach supporting materials.

III. FINDINGS

1. 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.
 2. The proposed undertaking will have no adverse effect on one or more historic properties located within the project APE under 36 CFR 800.5. Attach necessary documentation, as described at 36 CFR 800.11.
 3. 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 -0600 Date: August 29, 2017Type 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.
☐ Comment review and information is sought follows: _____

Authorized Signature: [Signature] Date: SEP 11 2017



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,

A handwritten signature in black ink that reads 'Sarah J. Beimers'.

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 cyphus*), 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_utrump@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 statutes 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: <i>“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).”</i>

Endangered Species	Endangered Species Act of 1973 (16 USC 1531 et seq.)	Correspondence from the USFWS (9-21-2017) notes <i>“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 cyphus), and Prairie Bush-clover (Lespedeza leptostachya) as the proposed project does not occur within or impact suitable habitat for these species.”</i>
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 amended, 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