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MEMORANDUM

TO: Chief, Threatened and Endangered Species, Ecological Services, Minneapolis, Minnesota

THROUGH: Field Supervisor, Ecological Services, Rock Island, IL

THROUGH: Field Supervisor, Ecological Services, East Lansing, MI

FROM: USFWS WNS Coordination Team

SUBJECT: Biological Opinion for Effects to Three Species of Threatened and Endangered Bats in Association With White-nose Syndrome Treatment Studies and Trials Occurring in the Service's Midwest Region (Region 3) Under the Purview of the Service's Section 10(a)(1)(A) Recovery Permit Program.

This document represents the Fish and Wildlife Service's (Service) biological opinion pursuant to section 7 of the Endangered Species Act (Act) of 1973, as amended (16 U.S.C. 1531 et seq.) regarding our intra-Service review of Section 10(a)(1)(A) permit applications to conduct experimental treatment trials to combat the fungal disease white nose syndrome (WNS), and associated effects to: (1) endangered Indiana bat (*Myotis sodalis*); (2) threatened northern long-eared bat (*Myotis septentrionalis*); and (3) endangered gray bat (*Myotis grisescens*). Although the objective of these trials is to provide long-term, beneficial effects to WNS-affected bat species through reduction of mortality caused by WNS, certain aspects of treatment trials and studies require: (1) capture and handling of bats; (2) acute disturbance on both the individual level and colony level; and (3) short-term modifications to hibernacula that exceed the typical actions permitted through section 10(a)(1)(A) recovery permits. While all Reasonable and Prudent Measures, with their implementing Terms and Conditions, shall be incorporated into Federal action constituting each sub-activity, (i.e., permit amendment, individual site treatment plan and/or trial study), to reduce by all practicable means the extent of take, as defined by the Act, harm and/or harassment and/or low levels of mortality to Indiana and northern long-eared bat could occur as a result of trials and treatments.

The proposed action is expected to provide results advancing the development of a chitosan as a treatment that may ultimately provide for long-term benefits to many of our threatened and endangered bat species affected by WNS. To date, USFWS, Federal and State partners have expended a minimum of +/- 60 million dollars in WNS surveillance, research, and outreach to support a collaborative national response to this crisis. Research and development of chitosan as a treatment for WNS was awarded research funding during two competitive opportunities for funding offered by the USFWS. The proposed action continues to advance this line of research and development. The proposed chitosan treatments may offer a cost-effective treatment that, in laboratory and small-scale in-site field trials, can significantly improve the survival rate of WNS-affected bat species, including endangered Indiana bat, threatened northern long-eared bat, little brown bat, and tri-colored bat.

Although the summer range of the gray bat overlaps with the action area in Illinois, the action is not likely to adversely affect the endangered gray bat because no gray bats have been observed during regular winter census efforts at the proposed treatment sites within the action area. The subject hibernacula in Michigan are far from the known range of gray bats.

The Service is currently in receipt of a section 10(a)(1)(A) permit amendment application from Timothy Carter, Ph.D., of Ball State University, Muncie, Indiana. Dr. Carter, remains in good-standing and currently holds a section 10(a)(1)(A) recovery permit (#TE02560A-4). Dr. Carter submitted a request for a permit modification to perform chitosan treatment trials under an awarded Bat Conservation International and The Nature Conservancy funded grant for a project entitled: *Field Trial to Test the Efficacy of Chitosan Application to Reduce Mortality from White-nose Syndrome (*see Appendix A). The chitosan trials will be performed by Dr. Carter and co-principal investigators Maarten J. Vonhof, Ph.D and Richard R. Eversole, Ph.D, both of Western Michigan University. The proposed chitosan treatment trials are the subject of this Federal action and its attendant effects on the Indiana bat and the northern long-eared bat are assessed herein.

§ 1.0 DESCRIPTION OF THE PROPOSED ACTION

As defined in the ESA section 7 regulations (50 CFR 402.02), "action" means "all activities or programs of any kind authorized, funded, or carried out, in whole or in part, by Federal agencies in the United States or upon the high seas." The "action area" is defined as "all areas to be affected directly or indirectly by the Federal action and not merely the immediate area involved in the action." The direct and indirect effects of the actions and activities must be considered in conjunction with the effects of other past and present Federal, State, or private activities, as well as the cumulative effects of reasonably certain future State or private activities within the action area.

The Federal action being evaluated in this biological opinion is the issuance of a permit under section 10(a)1(A) of the ESA and the possible contribution of funds by USFWS to conduct experimental treatment trials to combat WNS. The research study entitled "*Field Trial to Test the Efficacy of Chitosan Application to Reduce Mortality from White-nose Syndrome"* will be conducted by Timothy Carter, Ph.D., of Ball State University, Muncie, Indiana (Appendix A). The proposed action includes: (1) the construction of a mesh barrier at solitary opening of a hibernaculum, which will remain in place for the winter (November 1, 2016 - March 1, 2017); (2) two entries into each respective hibernaculum for the application and evaluation of the chitosan treatment ; (3)short-term removal of non-listed bats from roosting locations within hibernaculum and arousal from torpor; (3) hand manipulation of bats during application of an appropriately sized and applied aluminum, lipped bat bands; and (4) external application of 6 ml of the 0.1 % non-toxic, polysaccharide chitosan solution to wing membranes and uropatagium (i.e., the membrane that stretches between the legs) of bats in the treatment group. Only non-listed tri-colored bats (*Perimyotis subflavus*) and little brown bat (*Myotis lucifugus*) would be in the treatment groups.. However, threatened northern long-eared bats and endangered Indiana bats may co-occur with the target bats. Research activities have the potential to "take" these non-target species.

In previous research, Carter and Vonhof demonstrated that the application of chitosan, a natural biopolymer with antimicrobial effects, to bats experimentally-infected with the fungus *Pseudogymnoascus destructans* (*Pd*) in the laboratory led to increased survival and significantly reduced fungal invasion into tissues of the nose and sinus cavities (Vonhof et al., unpublished data). Chitosan is derived from chitin, which is naturally occurring, highly abundant, biocompatible, biodegradable, and nontoxic. Importantly, it is an antimicrobial (antibacterial and antifungal) agent, and also has widespread use as a wound-healing accelerant (see reviews in Rabea et al. 2003, Goy et al. 2009, Muzzarelli 2009, Dai et al. 2011, Jayakumar et al. 2011), in addition to

other wide-ranging applications in human and veterinary medicine, water treatment, pulp and paper production, cosmetics, and agriculture (Kumar Dutta et al. 2004, Kaur and Dhillon 2014). The low cost, commercial availability, potential ease of delivery, and combination of antimicrobial and wound healing properties of chitosan make it a promising candidate for treatment to increase survival of bats infected with *Pd*.

As a follow up to lab work, Carter et al. collaborated on a small pilot field study during the 2015-16 hibernation season in Wisconsin. There they simultaneously tested two possible treatments (chitosan and the bacterium *Pseudomonas fluorescens*; see Hoyt et al. 2015) on naturally-infected little brown bats in a natural hibernaculum. Carter et al. found that chitosan treatments doubled survival (survival = 53%) relative to bacteria-treated and control bats (survival = 27%; Hoyt et al., unpublished data) in animals that were kept in cages within the mine. Unfortunately, no reliable results could be recovered from free-ranging animals in the study. In both the field (caged animals) and the laboratory chitosan has been shown to increase survival of infected bats, although sample sizes were limited in the pilot study. Carter et al. propose to carry out a larger field trial at suitable sites to validate the results of their pilot study under natural conditions, and to assess whether chitosan treatment can have a meaningful impact on the survival of WNS-affected bat populations.

Threatened and/or Endangered Species Potentially Affected Within the Action Area					
Species	Name	ESA Status	Effect Determination	Beneficial Effects	Potentially deleterious effects
Myotis sodalis	Indiana bat	Endangered	May affect, likely to adversely affect	Increased survival, increased fitness	Mortality, lower fitness through arousal from torpor
Myotis septentrionalis	Northern long-eared bat	Threatened	May affect, likely to adversely affect	Increased survival, increased fitness	Mortality, lower fitness through arousal from torpor

<u>§ 1.1 Incorporation of Conservation Measures into the Proposed Action</u>

The principal investigator incorporated into the action measures to reduce take; these measures are designed to avoid, minimize, and mitigate take of Indiana and northern long-eared bat. Because the measures described below are considered part of the proposed action, they must be undertaken by the principal investigator while conducting the action for this biological opinion to apply.

§ 1.1.A Conservation Measure: Reduce Deleterious Effects to Breeding/Reproduction

1.1.A.1: Treatment application will occur during a single visit to each site on November 18 - 20 in Illinois and October 21 - 23 in Michigan, immediately following primary swarming, minimizing disruption of breeding activity.

1.1.A.2: Final health/study assessments will occur prior to spring staging behavior by making the return visit on March 3 - 5 (Illinois) and April 7 - 9 (Michigan), or when otherwise deemed appropriate and necessary by the Ecological Services Field Office and the appropriate state wildlife agency. Investigators, including local partners, will also monitor weather conditions beginning in February to help determine if prolonged periods of warm weather may warrant earlier than anticipated final assessments and removal of barriers.

§ 1.1.B Conservation Measure: Ensure Continued Suitability of the Hibernaculum

1.1.B.1: The Illinois DNR provided assurance that the site selection in Illinois consistently accommodates a suitable experimental source population, yet retains the lowest number of listed bats of all available "non-cave" sites. Likewise, Michigan DNR also stated that site selection in Michigan was carefully considered by looking for sites with the following characteristics: minimal bat populations to risk, yet sufficient to complete the required research protocol; sites that are relatively simple (mine adits or with minimal tunnel works); sites that have minimal presence of Northern long-eared bats or other bat species with federal and state protections; sites that are in year two or more of infection; sites that have minimal access points (preferably only one) to allow for improved biosecurity and possible containment of treated animals; and sites that would be accessible in the fall, winter and spring and that have cooperating landowners.

1.1.B.2: Alteration of the hibernacula entrances should be temporary and have negligible effect on temperature or air flow. The principal investigator proposes only to use a removable mesh at the entrance of the hibernacula. The mesh barrier will be in place for only one winter from November to March.

1.1.B.3 Researchers will follow all WNS decontamination protocols and recommendations.

§ 1.1.C Conservation Measure: Ensure Low Toxicity of the Selected Treatment Agent

1.1.C.1: The principal investigator shall externally apply 6 ml (approximate) of 0.1% chitosan solution to treated bats. In the laboratory, application of this chitosan concentration yielded no toxic or detrimental effects to bats in treatment groups.

1.1.C.2: Alteration of the hibernacula entrances should be temporary and have negligible effect on temperature or air flow. The principal investigator proposes only to use a removable mesh at the entrance of the hibernacula.

§ 1.1.D Conservation Measure: Minimize Stress Due to Multiple Arousals from Torpor

1.1.D.1: The principal investigator shall only access the hibernacula for two discrete periods during the hibernation season, outside of the inclusive dates of October 15, 2016 through April 9, 2017 (possibly as late as April 30 if weather conditions continue to prevent bats from emerging), when it is expected that natural rhythms of arousing from torpor are more frequent. To the extent possible, preparatory work for the mesh barrier will be completed prior to the arrival of bats at the mine.

1.1.D.2: Researchers are experienced identifying bat species on the roost. Therefore, they will avoid all intentional direct contact with listed species. In addition, federally listed species will not be banded or treated with chitosan. Only non-listed tri-colored bats and little brown bats will be used in the chitosan treatment study. To the extent possible, researchers will hand capture non-listed species from areas with no or fewer Indiana bats and northern long-eared bats.

1.1.D.3 The principal investigators will provide two suitable water sources inside the proposed barrier placed within Jason Mine (Illinois) to facilitate water needs of hibernating bats. The water sources will 4 x 4 feet in size and approximately 6 - 7 inches deep with bat friendly escape ladders at each corner. Bats are capable of drinking on the wing from these pools.

1.1.D.4 Upon visitation to apply chitosan, researchers will enter the site quietly and with the optimal number of researchers to complete activities with the greatest efficiency. Researchers will minimize use of lights and use red-filtered lights when possible. Researchers will be experienced with activities and protocols to minimize noise from oral communications.

1.1.D.5. Disturbance from research activities and the construction of the barrier will be coordinated with the Service's local field office and species lead recovery biologist for the species in question, to ensure that the work is consistent with the species' recovery program and would not be expected to reduce the viability of the population(s) that would be affected.

§ 1.1.E Conservation Measure: Monitor Interaction of Bats with Mesh Barrier

1.1.E.1. Video/pictures (trail cameras) and/or call recordings (data loggers) from increased monitoring activities throughout the project will be analyzed and provided to the species lead recovery biologist for the species in question. Such information will be helpful in evaluating future research and management efforts in listed species hibernacula while they are present.

§ 1.2. Timeline of Activities

September 16 - 18, 2016

- Construct structural framework for barrier at entrance to MI hibernacula
- Deploy 2 trail cameras per site for field testing at sites

September 21 - 23, 2016

- Construct structural framework for barrier at entrance to IL hibernaculum
- Deploy 2 water pools and 4 trail cameras for field testing at site

October 21 - 23, 2016

- Install ¼ hardware cloth mesh barrier at the entrance to MI hibernacula

- Apply treatment to targeted, non-listed bat species in MI hibernacula
- Evaluate trail camera battery life, sd card size, and image quality
- Deploy acoustic monitors[1] at entrance of MI hibernacula, inside mesh barrier
- Conduct initial survey of listed bat species present in each hibernacula

November 18 - 20, 2016

- Install ¼ hardware cloth mesh barrier at the entrance to IL hibernaculum
- Apply treatment to targeted, non-listed bat species in IL hibernaculum
- Evaluate water pool retention and trail camera battery life, sd card size, and image quality
- Deploy acoustic monitor[1] at entrance of IL hibernaculum, inside mesh barrier
- Where feasible, install light sensitive loggers[1] to verify human activity
- Conduct initial survey of listed bat species present in the hibernaculum

November 7, 2016 - December 19, 2016

- Work with the FWS to coordinate bi-weekly monitoring of the mesh barrier for first month from outside the hibernacula in MI and IL by local support staff.
- If any problems with the barrier are observed, report to FWS and DNR offices and initiate discussion on how to proceed prior to entering hibernacula.
- Report to FWS and DNR offices on all other significant changes or developments

December 19, 2016 - March 10, 2017

- Initiate a monthly monitoring, at a minimum, of the barrier until end of experiment from outside the hibernacula in MI and IL by local support staff.
- Conduct bi-weekly monitoring of the barrier within one month of the conclusion of the study.
- If any problems with the barrier are observed, report to FWS and DNR offices and initiate discussion on how to proceed prior to entering hibernacula.
- Report to FWS and DNR offices on all other significant changes or developments

March 3 - 5, 2017

- Return to IL hibernaculum to evaluate efficacy of experiment
- Conduct wrap up survey of listed bat species present in the hibernaculum
- Salvage all dead specimens (entire carcass) for later genetic analysis and identify to species if possible. Store specimens frozen as soon as possible.
- De-construct mesh barrier and associated structural framework and retrieve trail cameras and acoustic monitors at the IL hibernaculum

April 7 - 9, 2017

- Return to MI hibernacula to evaluate efficacy of experiment
- Conduct wrap up survey of listed bat species present in each hibernaculum
- Salvage all dead specimens (entire carcass) and identify to species if possible. Store specimens frozen as soon as possible.
- De-construct mesh barrier and associated structural framework and retrieve trail cameras and acoustic

[1] Acoustic monitors and light sensitive loggers will be loaned to the project by the Service or other partners.

monitors at the IL hibernaculum

April 10 - May 15, 2017

- Provide salvaged bat specimens to FWS.
- Prepare summary of basic project results on barrier, total counts of listed bat species on both trips, total number of dead specimens found throughout hibernacula, and other related project information.
- Provide digital copies of acoustic and trail camera files to the Service along with summary statistics of these files (# of data files by time and date. Provide summary of results for project briefing to agency representatives
- Engaged all FWS (RIFO, ELFO, BFO, TCFO, WNS Coordinators) and DNR (MI, IL) offices and personnel on preliminary results of the experiment and affects to listed species.

May 2017

- If feasible, Carter and Vonhof present study results at annual WNS workshop

§ 2.0 DESCRIPTION OF THE ACTION AREA

The "Action Area" is defined as all areas to be affected directly or indirectly by the Federal action and not merely the immediate area involved in the action (50 CFR § 402.02). The action area is not limited to the "footprint" of the action nor is it limited by the Federal agency's authority. Rather, it is a biological determination of the reach of the proposed action on listed species. For purposes of this biological opinion, the Action Area encompasses three known hibernacula located in: Illinois (Jason Mine); and two sites in Michigan (The Adventure Adit and Young Adit) where potentially deleterious effects could occur. In order to extend the treatment beyond the three known hibernacula cited above, Dr. Carter would also need to request an amendment to his permit. If chitosan proves to be an effective in-situ treatment for white-nose syndrome and increases survival of bats, beneficial effects would extend the action area to summer habitats occupied by migrating bats for miles outside of the treatment sites.

§ 3.0 STATUS OF THE SPECIES

§ 3.1 Species Listing and Critical Habitat

§ 3.1.1 INDIANA BAT

The Indiana bat was listed as an endangered species on March 11, 1967 (Federal Register 32[48]:4001), under the Endangered Species Preservation Act of October 15, 1966 (80 Stat. 926; 16 U.S.C. 668aa[c]). In 1973, the Endangered Species Preservation Act was subsumed by the Endangered Species Act and the Indiana bat was extended full protection under this law. Critical habitat was designated for the species on September 24, 1976 (41 FR 14914). Thirteen hibernacula, including 11 caves and two mines in six states, were listed as critical habitat.

The Indiana bat is a temperate, insectivorous, migratory bat that hibernates in caves and mines in the winter, and spends the summer in wooded areas. A description of the species physical appearance and a discussion of taxonomy can be found in the Indiana Bat Draft Recovery Plan: First Revision (USFWS 2007).

Indiana Bat Life History

The Indiana Bat Draft Recovery Plan: First Revision (USFWS 2007) provides a comprehensive discussion of Indiana bat life history. A summary of the life history follows (citation for information in the summary is USFWS 2007 unless otherwise noted).

Annual Chronology

Indiana bats arrive at their hibernacula in preparation for mating and hibernation as early as late July; usually adult males or nonreproductive females make up most of the early arrivals (Brack et al. 1983). The number of Indiana bats active at hibernacula increases through August and peaks in September and early October (Cope and Humphrey 1977, Hawkins and Brack 2004, Hawkins et al. 2005). Return to the hibernacula begins for some males as early as July, but most females arrive later. After fall migration, females typically do not remain active outside the hibernaculum as long as males. Males may continue swarming through October in what is believed to be an attempt to breed with late arriving females. Swarming is a critical part of the life cycle when Indiana bats converge at hibernacula, mate, and forage until sufficient fat reserves have been deposited to sustain them through the winter (Hall 1962). Swarming behavior typically involves large numbers of bats flying in and out of cave entrances throughout the night, while most of the bats continue to roost in trees during the day.

Swarming continues for several weeks and mating may occur on cave ceilings or near the cave entrance during the latter part of the period. Limited mating activity occurs throughout the winter and in spring before the bats leave hibernation (Hall 1962). Adult females store sperm through the winter and become pregnant via delayed fertilization soon after emergence from hibernation. Young female bats can mate in their first autumn and have offspring the following year (although how many actually do so is variable), whereas males may not mature until the second year.

In winter Indiana bats hibernate in caves or mines, often with other species. The period of hibernation varies across the range of the species, among years, and among individuals. On a rangewide basis, the months of October through April capture the hibernation period of most individuals.

In spring, Indiana bats emerge from hibernation. Female Indiana bats emerge first, generally late March and through April, and most males emerge later. The timing of annual emergence varies, depending in part on latitude and annual weather conditions. Shortly after emerging from hibernation, females become pregnant via delayed fertilization from the sperm that has been stored in their reproductive tracts through the winter. Most reproductive females appear to initiate migration to their summer habitat quickly after emerging from hibernation. Females migrate to their traditional roost sites, where they find other members of their maternity colony. Members of the same maternity colony may come from many different hibernacula. Most documented

maternity colonies have 50 to 100 adult female bats; average colony size of 80 adult females (Whitaker and Brack 2002) is a widely used estimate.

§ 3.1.2. NORTHERN LONG-EARED BAT

The Northern Long-Eared Bat was listed as a threatened species on April 2, 2015 (80 FR 17974) with a special rule under Section 4(d) of the Act on January 14, 2016 (81 FR 1900). No critical habitat has been designated for this species.

Life History and Biology

The northern long-eared bat is a temperate, insectivorous, migratory bat that hibernates in mines and caves in the winter and spends summers in wooded areas. The key stages in its annual cycle are: hibernation, spring staging and migration, pregnancy, lactation, volancy/weaning, fall migration and swarming. Northern long-eared bat generally hibernate between mid-fall through mid-spring each year. The spring migration period likely runs from mid-March to mid-May each year, as females depart shortly after emerging from hibernation and are pregnant when they reach their summer area. Young are born between mid-June and early July, with nursing continuing until weaning, which is shortly after young become volant in mid- to late-July. Fall migration likely occurs between mid-August and mid-October.

Winter Habitat and Ecology

Suitable winter habitat (hibernacula) includes underground caves and cave-like structures (e.g. abandoned or active mines, railroad tunnels). Generally, northern long-eared bat hibernate from October to April depending on local climate (November-December to March in southern areas and as late as mid-May in some northern areas).

Hibernacula for northern long-eared bat typically have significant cracks and crevices for roosting; relatively constant, cool temperatures (0-9 degrees Celsius) and with high humidity and minimal air currents. Specific areas where they hibernate have very high humidity, so much so that droplets of water are often seen on their fur. Within hibernacula, surveyors find them in small crevices or cracks, often with only the nose and ears visible.

Northern long-eared bats tend to roost singly or in small groups (USFWS 2014), with hibernating population sizes ranging from a just few individuals to around 1,000 (Service unpublished data). Northern long-eared bat display more winter activity than other cave species, with individuals often moving between hibernacula throughout the winter (Griffin 1940, Whitaker and Rissler 1992, Caceres and Barclay 2000). Northern long-eared bat have shown a high degree of philopatry to the hibernacula used, returning to the same hibernacula annually.

Summary information on summer habitat use, ecology, and spring/fall swarming is available in the Service's Programmatic Biological Opinion for Section 10(a)(1)(A) permitting (USFWS 2015a). This information has

not been included here since proposed activities will occur solely in hibernacula during the period of hibernation.

§3.2. Species Range and Distribution

3.2.1 Indiana Bat

Indiana bats are found over most of the eastern half of the United States. The recovery program for the Indiana bat delineates four Recovery Units (RUs): the Ozark-Central, Midwest, Appalachian Mountains, and Northeast RUs (see USFWS 2007 for explanation of RU boundaries). The proposed project would be completed within the Ozark-Central RU.

In 2015, approximately 10.7% of Indiana bats (56,055 of 523,636) in the Ozark-Central RU hibernated in caves in southern Illinois. Other states which supported populations of over 50,000 hibernating Indiana bats included Indiana, Missouri, and Kentucky. Other states within the current winter range of the Indiana bat include Alabama, Arkansas, Michigan, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Tennessee, Vermont, Virginia, and West Virginia. Approximately 46% of the population hibernated in the Ozark-Central Recovery Unit (Table 1). The 2015 population estimate (523,636) is almost 400,000 bats less than when the species was listed as endangered in 1967 (approximately 900,000).

TABLE 1. 2015 POPULATION ESTIMATES FOR THE INDIANA BAT BY RECOVERY UNIT

IBat Recovery Unit	State	2007	2009	2011	2013	2015	% Change from 2013	% of 2015 Total
Ozark-Central	Illinois	53,824	53,342	61,239	58,840	56,055	-4.7%	10.7%
	Missouri	183,304	181,097	182,852	184,245	185,693	0.8%	35.5%
	Arkansas	1,821	1,480	1,206	856	1,389	62.3%	0.3%
	Oklahoma	0	0	13	5	5	0.0%	0.0%
	Total	**238,949**	**235,919**	**245,310**	**243,946**	**243,142**	**-0.3%**	**46.4%**
Midwest	Indiana	238,068	213,244	225,477	226,572	185,720	-18.0%	35.5%
	Kentucky	71,250	57,325	70,598	62,233	66,024	6.1%	12.6%
	Ohio	7,629	9,261	9,870	9,259	4,809	-48.1%	0.9%
	Tennessee	2,929	1,657	1,791	2,369	2,551	7.7%	0.5%
	Alabama	258	253	261	247	247	0.0%	0.0%
	SW Virginia	188	217	307	214	137	-36.0%	0.0%
	Michigan	20	20	20	20	20	0.0%	0.0%
	Total	**320,342**	**281,977**	**308,324**	**300,914**	**259,508**	**-13.8%**	**49.6%**
Appalachia	West Virginia	14,745	17,965	20,296	3,845	2,373	-38.3%	0.5%
	E Tennessee	5,977	11,058	11,096	13,200	2,401	-81.8%	0.5%
	Pennsylvania	1,038	1,035	516	120	24	-80.0%	0.0%
	Virginia	535	514	556	418	460	10.0%	0.1%
	North Carolina	0	1	1	1	0	-100.0%	0.0%
	Total	**22,295**	**30,573**	**32,465**	**17,584**	**5,258**	**-70.1%**	**1.0%**
Northeast	New York	52,779	33,172	15,654	17,772	15,564	-12.4%	3.0%
	New Jersey	659	619	409	448	111	-75.2%	0.0%
	Vermont	325	64	61	53	53	0.0%	0.0%
	Total	**53,763**	**33,855**	**16,124**	**18,273**	**15,728**	**-13.9%**	**3.0%**
Rangewide Total:		**635,349**	**582,324**	**602,223**	**580,717**	**523,636**	**-9.8%**	**100%**

The known summer distribution of the Indiana bat covers a broader geographic area than its winter distribution, but is not relevant to the scope of this opinion.

§3.2.2 Northern Long-Eared Bat Range and Distribution

The northern long-eared bat ranges across much of the eastern and north central United States, and all Canadian provinces west to the southern Yukon Territory and eastern British Columbia (Nagorsen and Brigham 1993; Caceres and Pybus 1997; Environment Yukon 2011)(Figure 1). In the United States, the species' range reaches from Maine west to Montana, south to eastern Kansas, eastern Oklahoma, Arkansas, and east through the Gulf States to the Atlantic Coast (Whitaker and Hamilton 1998; Caceres and Barclay

2000; Amelon and Burhans 2006). The species' range includes the following 37 States (plus the District of Columbia): Alabama, Arkansas, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Vermont, Virginia, West Virginia, Wisconsin, and Wyoming.

Historically, the species has been most frequently observed in the northeastern United States and in Canadian Provinces, Quebec and Ontario, with sightings increasing during swarming and hibernation (Caceres and Barclay 2000). However, throughout the majority of the species' range it is patchily distributed, and historically was less common in the southern and western portions of the range than in the northern portion of the range (Amelon and Burhans 2006).

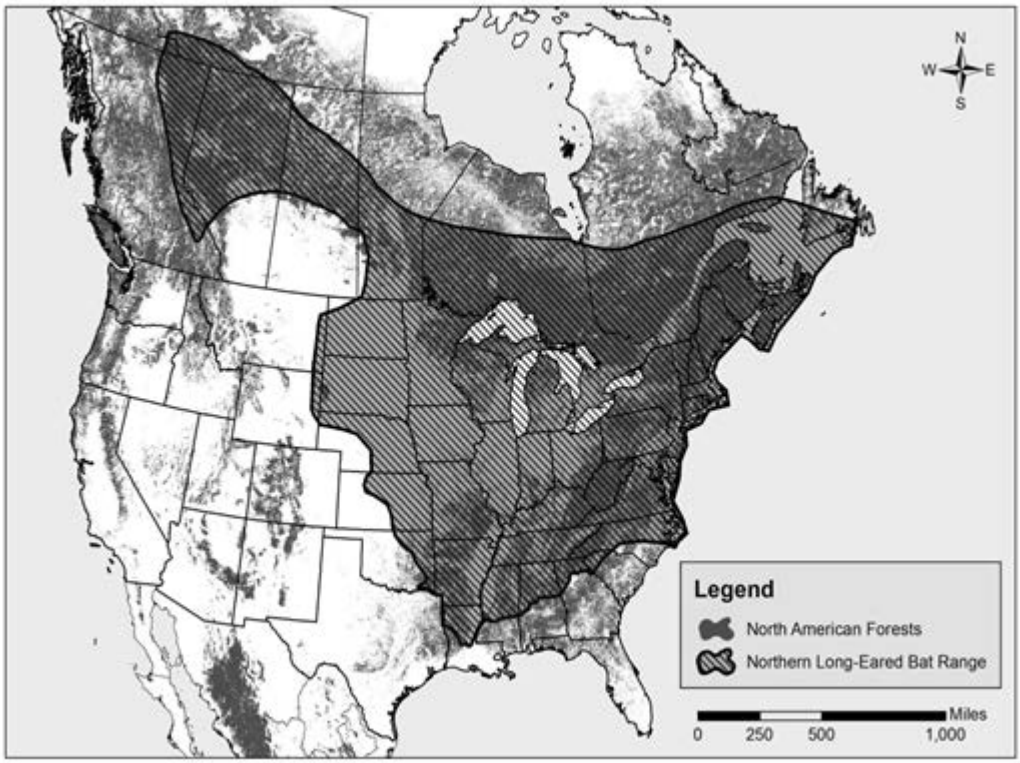

Figure 1. Species range of northern long-eared bat (*Myotis septentrionalis*).

§ 3.3 Population Status and Threats

This section will discuss the status of the Indiana bat and the northern long-eared bat and threats to the species rangewide.

§ 3.3.1.1 Indiana Bat Population Status

The 2015 rangewide population estimate of Indiana bats was 523,636 individuals, based on winter hibernacula survey information compiled by the Service (USFWS unpublished data, 2015). Generally, the Indiana bat

population (rangewide) has decreased from the time of listing through the 1990s, but saw a temporary increase from 2001 through 2007.

§ 3.3.1.2 Northern Long-Eared Bat Population Status

Although they are typically found in low numbers in inconspicuous roosts, most records of northern long-eared bat are from winter hibernacula surveys (Caceres and Pybus 1997). More than 780 hibernacula have been identified throughout the species' range in the United States, although many hibernacula contain only a few (1 to 3) individuals (Whitaker and Hamilton 1998). Known hibernacula (sites with one or more winter records of northern long-eared bats) include: Alabama (2), Arkansas (41), Connecticut (8), Delaware (2), Georgia (3), Illinois (21), Indiana (25), Kentucky (119), Maine (3), Maryland (8), Massachusetts (7), Michigan (103), Minnesota (11), Missouri (more than 269), Nebraska (2), New Hampshire (11), New Jersey (7), New York (90), North Carolina (22), Oklahoma (9), Ohio (7), Pennsylvania (112), South Carolina (2), South Dakota (21), Tennessee (58), Vermont (16), Virginia (8), West Virginia (104), and Wisconsin (67). northern long-eared bat are documented in hibernacula in 29 of the 37 States in the species' range. Other States within the species' range have no known hibernacula (due to no suitable hibernacula present, lack of survey effort, or existence of unknown retreats).

The current range and distribution of northern long-eared bat must be described and understood within the context of the impacts of WNS. Prior to the onset of WNS, the best available information on northern long-eared bat came primarily from surveys (primarily focused on Indiana bat or other bat species) and some targeted research projects. In these efforts, northern long-eared bat was very frequently encountered and was considered the most common myotid bat in many areas. Overall, the species was considered to be widespread and abundant throughout its historic range (Caceres and Barclay 2000).

WNS has been particularly devastating for northern long-eared bat in the northeast, where the species was believed to be the most abundant. There are data supporting substantial declines in northern long-eared bat populations in portions of the Midwest due to WNS. In addition, WNS has been documented at more than 100 northern long-eared bat hibernacula in the southeast, with apparent population declines at most sites. WNS has not been found in any of the western states to date and the species is considered rarer in the western extremes of its range. We expect further declines as the disease continues to spread across the species' range.

§ 3.3.2 Threats

Under the Endangered Species Act, threats to a species are categorized based on five key factors:

A. The present or threatened destruction, modification, or curtailment of its habitat or range.
B. Overutilization for commercial, recreational, scientific, or educational purposes.
C. Disease or predation.
D. The inadequacy of existing regulatory mechanisms.
E. Other natural or man-made factors affecting its continued existence.

The draft revised Indiana Bat Recovery Plan (USFWS 2007) includes a detailed discussion of threats to the Indiana bat. The rulemaking for the Northern long-eared bat (80 FR 17974) and the Environmental Assessment (USFWS 2015b) on the 4(d) Rule for the northern long-eared bat clearly state that the predominant threat to the species is the progression of WNS across its range. For the purpose of this biological opinion, we will limit our discussion to the threat of WNS, or Factor C. While we acknowledge other threats exist, the purpose of the Federal action to issue a permit to conduct WNS Treatment Trials narrows the scope to Factor C.

In the past, disease and predation have generally not been considered major threats to bat populations in general, or Indiana bats specifically (USFWS 2007). The emergence of white-nose syndrome (WNS) has changed that. WNS has caused recent catastrophic declines among multiple species of bats in eastern North America (Lorch et al. 2011, Cryan et al. 2013a) including large declines in Indiana bat populations (Turner et al. 2011). WNS is now recognized as the most significant threat to the Indiana bat. Dead bats were first documented at four sites in eastern New York in the winter of 2006-2007. At the time, the cause of mortality was unknown but white fungus was observed on muzzles of many of the dead bats and the term "white-nose syndrome" was coined. WNS has since caused the death of an estimated 5.7 – 6.7 million bats of seven species, including the Indiana bat and northern long-eared bat, across the eastern North America. Bat population declines due to WNS are one of the fastest declines of wild mammal populations ever observed (Cryan et al. 2010; Frick et al. 2010). The disease is devastating to the Northern long-eared bat and is cited as the primary reason for its decline (USFWS 2015c). In fact, if it were not for the progression of WNS, the Northern long-eared bat would not be a Federally-threatened species.

Associated with the fungus *Pseudogymnoascus destructans* (Minnis and Lindner 2013), the disease is named after the most obvious visible symptom of WNS which is the presence of a white fungus on the face, wing, or tail membranes of some affected animals (some do not exhibit visible fungus). [Note that when first identified the fungus was named *Geomyces destructans* (Gargas et al. 2009), but more recent phylogenetic analyses have demonstrated that the WNS fungus should be placed in the genus Pseudogymnoascus (Minnis and Lindner 2013) and it has been reclassified].

WNS may affect behavioral changes of infected individuals. For example, at some WNS-affected sites a shift of hibernating bats from traditional winter roosts to roosts unusually close to hibernacula entrances has been observed. Bats have also been observed flying outside of hibernacula during winter (often during the day) at some affected sites. At some sites, bat carcasses (particularly of *Myotis lucifugus*, the little brown bat) have been found outside affected hibernacula. Many infected bats do not survive the winter. The exact processes by which the fungal skin infection lead to death are not known, but depleted fat reserves (i.e., starvation) contribute to mortality (Reeder et al. 2012, Warnecke et al. 2012) and dehydration may also have a role (Willis et al. 2011, Cryan et al. 2013b, Ehlman et al. 2013). It is also suspected that some of the affected bats that survive hibernation emerge in such poor condition that they do not survive the summer. Among those bats that do survive, it appears that productivity of female survivors may be negatively affected (Francl et al. 2012).

At the end of the 2012-2013 hibernating season, bats with WNS were confirmed in 29 states and five Canadian provinces (see http://www.whitenosesyndrome.org/ for most recent information). Turner et al. (2011) summarized mortality rates from WNS for six species of bats for five states (NY, PA, VT, VA, WV) at sites where WNS had been present for at least two years. They summarized data from 42 sites and saw an overall decline of 88% in the number of hibernating bats at WNS-affected sites, from a total of 412,340 bats (pre-WNS) to 49,579. Mortality varied among sites and among species (Turner et al. 2011). If current trends for spread and mortality at affected sites continue, WNS will drastically reduce the abundance of many species of hibernating bats in much of North America. We anticipate that WNS will continue to spread rapidly, moving through the Midwest, South and into the Western US.

Impacts of WNS on Indiana Bats

The Indiana bat, which is closely-related to the little brown bat, has also declined due to WNS. Turner et al. (2011) summarized data from 15 Indiana bat hibernation sites in five states (NY, PA, VT, VA, WV) (11 of the sites were in New York) where WNS had been present for at least two years. They documented an overall decline of 72% in the number of hibernating Indiana bats at those sites.

Impacts to Indiana bats have been variable among affected hibernacula. The following is an example of population counts in New York (at the sites with largest Indiana bat populations) when comparing the most recent counts to the last count conducted prior to signs of WNS at any given site, generally 2005 or 2007 counts (USFWS data):

- Haile's Cave 100% decline from 685 bats in 2005 to 0 in 2010, and remained at 0 in 2015
- Williams Preserve Mine 98.5% decline from 13,014 in 2007 to 190 in 2010 and 191 in 2015
- Williams Lake Mine >99% decline from 1,003 in 2007 to 26 in 2010 and down to 1 in 2015
- Glen Park >87% decline from 1,928 in 2007 to 509 in 2010 and 239 in 2015
- Williams Hotel Mine >96% decline from 24,317 in 2007 to 8,152 in 2010 and 918 in 2015
- Jamesville >98% decline from 2,932 in 2007 to 2,324 in 2009 and 40 in 2015
- Barton Hill Mine 13.7% increase from 9,393 in 2007 to 10,678 in 2010, but rebounded to 14,253 in 2015

The Northeast Recovery Unit, where WNS was first observed in the winter of 2006-2007, lost over 70% of its Indiana bats between 2007 and 2015. At the time dead bats were first observed in the winter of 2006-2007, we do not know how long the (previously unidentified) fungus, *Pseudogymnoascus destructans*, had been present in affected sites. Based on subsequent observations as WNS spread, it appears that the arrival of the fungus in an area may precede large-scale fatality of bats by several years. Between 2011 and 2015 the Appalachian Recovery Unit, where WNS was confirmed in the winter of 2008-2009, declined by 84%. The Midwest Recovery Unit, where WNS was confirmed in the winter of 2010-2011, declined by 16% between 2011 and 2015. The Ozark-Central Recovery Unit, where WNS was confirmed in the winter of 2011-2012, declined by less than 1% between 2013 and 2015.

Thogmartin et al.'s (2013) model of the impacts of WNS on Indiana bat populations suggested that WNS will cause local and regional extirpation of some wintering populations of Indiana bats, and overall population declines exceeding 86%. However, they note a number of important limitations and sources of uncertainty that could result in actual declines being less or more severe compared to projections. One uncertainty is whether or not Indiana bats will develop any degree of immunity, genetic resistance, or behavioral tolerance to WNS.

Langwig et al. (2012) found that in Indiana bats and little brown bats, species that cluster in tight aggregations during hibernation, the declines due to WNS were equally severe across a large range of colony sizes, suggesting that WNS transmission is not density-dependent in these species. In little brown bats, after populations had declined they found an increase in the proportion of little brown bats that were roosting individually. This change in behavior could potentially reduce transmission of WNS among surviving little brown bats. Changes in sociality (i.e., clustering behavior) were less apparent in Indiana bats, possibly putting this species at higher continued risk of WNS transmission (i.e., impacts of WNS may be less likely to abate over time). Thogmartin et al. (2012a) suggested that all hibernating populations of Indiana bats are currently susceptible to WNS; throughout the range of the species there are infected source populations within the known migration distance for individual Indiana bats. They projected that all sizeable complexes of hibernating Indiana bat populations may be affected by WNS as early as 2016. Observed spread (see map at http://www.whitenosesyndrome.org/about/where-is-it-now) suggests that Thogmartin et al.'s (2012a) prediction has been largely accurate. WNS now has been confirmed in all Indiana bat RUs and we anticipate that WNS will continue to radiate out to new sites within the more recently affected RUs, eventually reaching all major hibernacula for the species. Based on observations in the Northeast, the area that has been affected the longest and has the best data on mortality, we anticipate that all RUs will eventually experience the level of decline that has been documented in the Northeast.

Ultimately, how WNS will impact Indiana bat populations in the long term is not known, although current data suggest that those impacts will be severe. The impacts of WNS in the Northeast and models of spread and impacts (e.g. Thogmartin et al 2012a, 2012b, 2013) suggest that local and regional extirpations of some populations of Indiana bats should be expected. However, Thogmartin et al. (2012a) noted that the causative processes associated with WNS spread and associated impacts are not well understood. WNS may not cause the same consequences on wintering bat populations (e.g., mortality may be less) as the disease moves west and south. Ehlman et al. (2013) suggested that bat populations experiencing shorter southern winters could persist longer than their northern counterparts when faced with WNS; modeling by Flory et al. (2012) also suggested that mortality may be lower in some areas due to different environmental conditions, however support for these hypotheses has been limited. It has been documented that bats held in captivity and given supportive care can recover from the wing damage caused by *P. destructans* (Meteyer et al. 2011). Healing of wing membranes has also been observed in free-ranging bats caught during the active season (following WNS infection during hibernation) (Dobony et al. 2011, Fuller et al. 2011). However, the recovery process is physiologically challenging (Cryan et al. 2013a). Current thinking is that it is likely that *P. destructans*, the fungus that causes WNS, was accidentally translocated from Europe to the U.S. (Blehert 2012). Although the fungus is widespread among bats in Europe, bat mortality events similar to those in North America have not been observed in Europe (Wibbelt et al. 2010). Researchers hypothesize that bats in Europe may be more

immunologically or behaviorally resistant to the fungus than their congeners in North America because they potentially coevolved with the fungus. Whether or not European bats have immunological resistance to WNS has not been determined. Likewise it is unknown if North American bats will develop resistance, although immunologically resistant individuals have not been detected to date (Moore et al. 2013).

Impacts of WNS on Northern Long-Eared Bats

WNS has been particularly devastating for northern long-eared bat in the northeast, where the species was believed to be the most abundant. There are data supporting substantial declines in northern long-eared bat populations in portions of the Midwest due to WNS. In addition, WNS has been documented at more than 100 northern long-eared bat hibernacula in the southeast, with apparent population declines at most sites. Within the species' range WNS has not been found in any western states to date and the species is considered rarer in the western extremes of its range. We expect further declines as the disease continues to spread across the species' range.

Winter population sizes based on colony surveys conducted through 2014 have revealed an overall decline of 96% of northern long-eared bats in 147 colonies where WNS had been confirmed (USFWS, unpublished data). Although northern long-eared bats are often underrepresented in winter survey estimates, the following examples indicate decreases in some of the larger hibernating colonies since the arrival of WNS.

- One site in Fairfield county, CT - mean 80 pre WNS to 0 in the two most recent counts. 100% decrease.
- One site in Litchfield County, CT - mean 408 pre WNS to 0 in most recent count. 100% decrease.
- Two sites in LaSalle County, IL - mean 993 pre WNS to mean 145 in four most recent surveys. 95% decrease.

In addition to these declines documented for larger winter colonies, many smaller colonies with few documented northern long-eared bats observed prior to WNS have had no northern long-eared bats observed after the arrival of WNS. Northern long-eared bats are no longer observed at about 69% of WNS affected sites where this species was historically present (Frick et al. 2015).

§ 4.0 ENVIRONMENTAL BASELINE

Under section 7(a)(2) of the ESA, when considering the "effects of the action" on federally listed species, the Service is required to take into consideration the environmental baseline. The environmental baseline includes past and ongoing natural factors and the past and present impacts of all Federal, State, or private actions and other activities in the action area (50 CFR 402.02), including Federal actions in the area that have already undergone section 7 consultation, and the impacts of State or private actions that are contemporaneous with the consultation in process. As such, the environmental baseline is "an analysis of the effects of past and ongoing human and natural factors leading to the current status of the species, its habitat (including critical habitat), and ecosystem, within the action area" (USFWS and NMFS 1998, page 4-22).

§ 4.1 Status of the Indiana Bat and Northern Long-eared Bat in the Action Area

Currently, the Action Area is known to support wintering habitat for the Indiana bat and Northern Long-eared bat. All three hibernacula are mines. There will be no effect to designated Indiana bat critical habitat because these are not designated as critical habitat. There is no designated critical habitat for the northern long-eared bat.

Recent surveys of listed species indicate the presence of Indiana bat and Northern Long-eared Bat at the Jason Mine, IL. Most recent survey estimates documented 36 Indiana bats and two Northern Long-eared bats. While 20 Indiana bats are known to hibernate in the across all of Michigan hibernacula; the proposed location of the study is not within the known range and will not be considered in later discussion. Both The Adventure Mine Adit and Young's Adit are only known to support wintering habitat for one federally listed species, the Northern Long-eared bat. The Michigan Department of Natural Resources, in their letter of support, provided the following assurances that both sites had minimal presence of Northern long-eared bats.

§ 4.1.A Factors Affecting Indiana Bat Environment within the Action Area

This analysis describes factors affecting the environment of the Indiana bat in the Action Area. The baseline includes the past, present and future impacts from federal, state, tribal, local, and private actions that have occurred or are presently occurring. This section of a biological opinion also incorporates impacts from future federal actions in the Action Area that have undergone section 7 consultation; in this case there are none.

The factors affecting Indiana bats in the Action Area are a subset of the threats affecting the species rangewide and in the Ozark-Central Recovery Unit, as discussed above in the Population Status and Threats section of this document. To characterize the environmental baseline for these bats we must consider the other stressors to these same bats that utilize the Action Area. The main threat to bats at these 3 hibernacula identified in the Action Area is the disease WNS.

§ 4.1.B White-Nose Syndrome

WNS is a devastating disease affecting many eastern U.S. bats including Indiana bats and northern long-eared bats. The disease was first documented in the Midwest (specifically the Ozark-Central RU) in 2011. By the end of 2013-2014 hibernating season, WNS has spread to multiple hibernacula in both Illinois and Michigan. All three hibernacula in the proposed action were recently confirmed with WNS (See http://www.whitenosesyndrome.org/about/where-is-it-now for a current map of where WNS has been found). A Priority 1 hibernacula for Indiana bats is located in Union County, IL, only miles away from the Jason Mine in southern Illinois.

As noted previously, according to 2015 rangewide population estimates, the Northeast Recovery Unit lost approximately 70% of its Indiana bats since the onset of WNS. The Appalachian RU, first affected in 2008, had declined approximately 83% and the Midwest RU, affected in 2010, declined 19%. The Ozark-Central RU, where WNS was confirmed in 2011, has experienced population declines of less than 1% by 2015. As

previously discussed, we expect declines to continue in the coming years as WNS has now been documented in all recovery units.

There are no firm population estimates for the northern long-eared bat rangewide; nor do we have the benefit of a viability analysis; however, a rough estimate of the population size in a portion of the Midwest has been calculated. That estimate shows there may have been more than four million bats in the six-State area that includes the States of Illinois, Indiana, Iowa, Ohio, Michigan, and Missouri (Meinke pers. comm.). This population size estimate (for the northern long-eared bat) was developed for the Midwest Wind Energy Multi-Species Habitat Conservation Plan (MSHCP) and was calculated by adjusting the 2013 Indiana bat winter population size (within the 6 States) based on the ratio of northern long-eared bats compared to Indiana bats in summer mist-net surveys. This estimate has limitations, however. The amount and quality of data available to estimate the population result in substantial uncertainty. The principal limitation is that the estimate is based on data that were primarily gathered prior to the onset of WNS in the Midwest; thus declines that have occurred in WNS affected States are not reflected in the estimated number. Although declines of over 90% have been documented in some Midwest hibernacula, other hibernacula still show no signs of WNS. The data available are too limited to estimate declines across the six-State area.

§ 5.0 EFFECTS OF THE ACTION

This section includes an analysis of the direct and indirect effects of the proposed action, and interrelated and interdependent activities, on the Indiana bat and/or critical habitat. For the proposed project, effects will be analyzed for Indiana bats that roost and forage in the Action Areas. Effects of proposed mitigation, which has been incorporated into the project, will also be assessed. All effects will be evaluated as they pertain to the Indiana bat population within the local, regional, and rangewide populations (summering or wintering populations to which impacted bats belong).

Note that there is no designated critical habitat for the Indiana bat in or near the Action Area. Critical habitat has not been designated for the northern long-eared bat. There is no potential for the project to affect critical habitat.

EFFECTS OF THE ACTION

This section includes an analysis of the direct and indirect effects of the proposed action, and interrelated and interdependent activities, on the Indiana bat and northern long-eared bat and/or their critical habitat. For the proposed project, effects will be analyzed for Indiana bats and northern long-eared bats that hibernate in the Action Area. All effects will be evaluated as they pertain to the overall bat populations.

Beneficial Effects

Although the chitosan treatment will not be tested directly on listed species, the findings from this study will provide evidence to understand the efficacy of this treatment to improve survival of two genera (*Myotis &*

Perimyotis) of bats affected by WNS, one of which (*Myotis*) is shared with both the Indiana bat and Northern long-eared bat. The potential benefit of a safe and effective treatment is pivotal in the management of WNS as it can reduce lethal and sub-lethal effects of the disease, providing long-term benefits to all WNS-affected species.

Analysis of the Effects of the Action

The purpose of the action will be to conduct an experiment that evaluates the survival of two non-listed, WNS affected bat species treated with chitosan. Principal investigators of the chitosan study have proposed actions that could involve the incidental handling of listed species during research collection and treatment of non-listed bat species, construction a temporary mesh barrier at solitary openings of all three proposed hibernacula and two entries into each site during the course of the winter.

Chitosan treatment will not be directly applied to listed species and has not been documented or known to transfer in noticeable amounts from a treated bat to others or the environment. However, listed species will be affected by disturbance associated with two planned visits to each mine, making it possible that individual bats may be physically harmed during these activities. The conservation measures in the Description of the Action will be enacted to minimize harm and harassment.

Incidental handling, multiple entries into, and construction of a mesh barrier at an Indiana bat and/or northern long-eared bat hibernacula when bats are present (approximately October 15 - April 30) may be likely to result in take of both listed species . Considerable planning and some preparatory work will be conducted prior to the arrival of bats at the project site to mitigate additional disturbance. The primary actions under consideration will be conducted after bats have initiated hibernation in November 2016 and will continue through March 2017. Furthermore, experienced handlers and bat experts will be part of the entry and processing of bats to minimize the incidental handling and duration of activities. Duration of the proposed actions involving listed species will range from a short period (<1 day) for application of the treatment to non-listed species to a longer period (>1 day) during which a temporary mesh barrier will prevent free movements by bats into and out of the mine.

The mesh barrier will be in place during the hibernation season and will prevent bats from leaving or entering the sites between October and April. This study period coincides with the weeks when Indiana bats and northern long-eared bats employ behavioral and physiological adaptations to remain within hibernacula for extended periods. Thus, although hibernating bats have been documented to leave caves and mines during winter, such mid-winter emergences performed by Indiana bats and northern long-eared bats are unlikely to be requirements for survival. Moreover, although the currently proposed actions are preferred to maximize the reliability of results to determine the efficacy and feasibility of chitosan as a treatment for WNS, future implementation of this treatment, if warranted, would not require barricading hibernacula.

Hibernating bats arouse from torpor periodically during hibernation to perform a variety of activities. The proposed action will not affect activities associated with these arousals that occur within the mines, e.g. urinating, flying, sleeping, mating, grooming, etc. However, bats may attempt to leave hibernation sites during winter to a) drink, b) carry out social behaviors such as mating, c) relocate to other roost locations, or d) forage.

a) Leaving to drink is a primary reason bats will leave a site during the winter (Speakman and Racey 1989, Thomas 1995, Thomas and Geiser, 1997, Boyles et al. 2006). If it is not already present in the site (the sites in Michigan contain water during the winter), 'kiddie pools' will be installed with exit ramps to ensure that bats have access to drinking water ad libitum throughout the study period.. A similar approach was used for a captive study in a bunker in Northern Maine, and bats were observed on camera drinking from pools numerous times during the winter (J. Reichard, pers. comm.).

b) It is well known that bats arouse throughout the hibernation season and perhaps may leave the hibernaculum for short periods, and one hypothesis is that, beyond looking for water or food, they use that time to maintain social relationships or to carry out mating (Thomas et al. 1979, Boyles et al. 2006). Bats are long-lived mammals and while being unable to pursue social activities outside of the hibernaculum for part of a single winter can be considered a negative effect of this experiment, the proposed site in Illinois (Jason Mine) is large enough to permit activity and social behaviors inside the mine. The two proposed study sites in Michigan experience far more severe winter weather conditions and it is unlikely weather conditions outside the mines will be suitable for mid-winter emergences.

c) Hibernating bats, especially northern long-eared bats, may move between hibernacula during the winter (see Boyles et al. 2006). Given the cold climate of the proposed sites in Michigan, it is unlikely bats commonly emerge from these sites and risk flights in the cold to relocate under normal circumstances. In Illinois, however, warmer winter climate may provide ample opportunity for bats to emerge from one site and move to another. Bats inhabiting Jason Mine safely interact with the permanent, bat-friendly gate installed at this site and it is likely bats will experience some level of harm and harassment. Bats have the ability to detect the mesh barrier visually and acoustically, and there is space on both sides of the barrier within which bats can safely approach and maneuver. Britzke et al. (2014) documented the ability of bats, including Indiana bats, to largely avoid contacting netting held in place by 2x4 framing at entrances to their cave roosts during spring emergence. In their study of bats' interactions with these barriers, they documented 4301 interactions with barriers where only 3 involved contact with the barrier causing bats to change course and fly away. Barriers have also been used in studies at hibernacula in several studies where investigators did not report injury or mortality caused by bats colliding with the barriers (A. Hicks, pers comm., C. HErzog, pers.comm.). Dr. Carter will monitor bat activity around the barrier using cameras and an acoustic detector to observe any potential interaction, related injuries, and/or associated mortality. While the mesh barrier would prevent this movement from happening, bats that are prevented from emerging would simply remain within the mine that is suitable for continuing hibernation.

It is more likely that the mesh barrier could have a negative effect by preventing bats from entering the site. Although it is unlikely entering individuals would collide with the mesh barrier and become injured, these immigrating individuals would not gain access to the internal environment that is suitable for hibernation. On occasions when bats are turned away

from the barricaded mines and are incapable of finding an alternate roosting location, they may succumb to exposure, exhaustion, or starvation. Installing the barricades after swarming and migration is expected to be complete (mid October in Michigan and early November in Illinois) will reduce risk of interfering with continuing immigration to these sites.

d) There are no data to suggest that healthy Indiana bats in the proposed IL study site regularly leave their hibernation sites during mid hibernation, although there are records of northern long-eared bats changing hibernacula during winter (see Boyles et al. 2006). Whitaker and Rissler (1992) documented winter activity of little brown bats, tri-colored bats, and northern long-eared bats outside of a hibernaculum in winter, but determined these bats did not forage during those forays. Thus, the best available evidence indicates that feeding is not the prime reason for leaving sites during the winter (see also Boyles et al. 2006, Lausen and Barclay 2008). There are no data to suggest that any departures during mid-hibernation lead to greater survival for these species. The purpose of hibernation is to allow these animals to survive during the winter months when there is no food supply. There are many sites, especially northern sites (such as those in Michigan and any sites further north), where the northern long-eared bat is naturally barricaded into the hibernation sites by snow and ice for long periods.

The expected date for removal of the barrier may be adjusted if forecasted weather for the local area would subject additional harm onto the bats. The dates will vary given the geographic variability among the 1 IL and 2 MI mines. Principal investigators will work with USFWS local field offices, State DNRs and other bat specialists that have experience with spring emergence and predicting the timing of spring emergence of hibernating bats at each of the sites.

Proximity and distribution of the action: The action will be conducted in three occupied hibernacula. One hibernacula is located in Illinois and the remaining two hibernacula are located in Michigan.

Disturbance frequency, intensity, and severity: The applications of chitosan and recovery of data will be completed on two separate visits to each site in the winter of 2016-2017. The intensity of the disturbance for individual bats will vary, ranging from zero (no arousal) to high (reduced opportunity to leave site if aroused). To the greatest extent possible, project investigators and state agencies have limited the duration, intensity, and severity of the trials to ensure habitats and/or bats subjected to more than short-term or seasonal disturbance and conditions return to normal after study completion.

§ 5.1 Effects Analysis:

Direct effects: Individual listed bats may be disturbed during the two planned visits to each mine. Disturbance to bats associated with human activity in hibernacula has been identified in numerous studies. Furthermore, listed species may be unintentionally captured during one of two planned entries to the hibernacula. Furthermore, bats may be prevented from leaving the hibernacula or harmed by the mesh barrier constructed to facilitate the project purpose.

Indirect effects: Indirect effects are caused by or result from the proposed action, are later in time and reasonably certain to occur because of the proposed action. No indirect effects are anticipated. Bats will be identified on the wall and no federally-listed bats will be handled or treated with chitosan.

Species' response to a proposed action: We estimate Indiana bats and northern long-eared bats may be directly or indirectly affected by the proposed activity.

In summary, the following effects are anticipated for the hibernacula within the Action Area:

- Individual listed bats may be disturbed during the two planned visits to each mine. Disturbance to bats associated with human activity in hibernacula has been identified in numerous studies. When hibernating bats are disturbed, they may arouse from torpor and remain euthermic for a period of time. During this time, individuals may fly around the mine, roost in other locations within the mine, or even vacate the site. Alternatively, human activity inside the mines may not result in any additional activity by the bats. Periodic arousals from torpor are a natural part of hibernation behavior. In the proposed study, one disturbance in each site will occur near the beginning of the hibernation season, after which bats are expected to resume the natural cycles of torpor and arousal for the remainder of the winter. At the completion of the study in March, human caused disturbance will take place at a time when hibernating bats are employing more frequent arousal bouts in preparation for emerging from hibernation. Additional arousals and subsequent activity during hibernation may result in additional consumption of body fat reserves for disturbed individuals. Fat reserves at the completion of hibernation and during early spring are critical for females to initiate ovulation and early gestation. Therefore, disturbance may indirectly lead to lower reproductive output in disturbed populations. This negative effect on reproductive output would not continue later than a single summer after the proposed actions. We do not anticipate disturbance to result in mortality.
- Listed species may be unintentionally captured during one of two planned entries to the hibernacula, and this may result in arousal and subsequent behavior similar to that caused by disturbance described above. It is highly unlikely, but possible that handling of a bat could lead to mortality. The experience of the researchers to avoid handling listed species and to replace these bats safely on roosting surfaces if they are unintentionally captured will further reduce the potential for the actions to cause harm.
- Bats may be prevented from leaving the hibernacula or harmed by the mesh barrier constructed to facilitate project purpose. Bats that are prevented from leaving the mines will be expected to resume normal hibernation behavior with negligible disruption of their activities, especially because they will have access to water and social interactions within the site. Harm caused by the mesh barrier is unlikely, but may result in injury followed by predation, lasting injury, or mortality.

We anticipate non-lethal effects in the form of harm and/or harassment to occur to all Indiana and northern long-eared bats within the three mines. The most recent surveys counted 36 Indiana bats and two Northern Long-eared bats in Jason Mine, IL. The maximum count at Jason Mine, IL was 87 Indiana bats in 2004 and 29 northern long-eared bats in 2011. The most recent surveys at the two sites in Michigan yielded counts of 21 to 1 (Young Adit, 2015 and 2016, respectively) and 127 (Adventure Adit, 2012) northern long-eared bats. Historic estimates of northern long-eared bats at the Michigan sites are not available because Myotis species

were not distinguished during routine surveys, rather species composition was estimated based on a subset of identifiable Myotis species (Kurta and Smith, 2016). Based on historic winter census counts, the number of Indiana bats that could be affected accounted for only 0.002% (87 of 56,055 bats) of the total hibernating Indiana bat population known to winter within Illinois. Approximately 10.7% of Indiana bats (56,055 of 523,636) hibernated in caves in southern Illinois. The 2015 rangewide Indiana bat population was estimated at 523,636. The current population of northern long-eared bats is not as well known, but the 2016 Programmatic Biological Opinion for the northern long-eared bat 4(d) rule estimates that the population may be around six million individuals.

Although there may be some short-term loss and impacts to individuals from these actions, these impacts are not likely to affect the species' long-term reproduction and survival. Under no likely scenarios, is the estimated amount of loss/take of reproductive individuals likely to cause an appreciable long-term change in viability of the local RU or range-wide status. At worst, only short-term reproductive loss and reduction in numbers of this hibernation colony is anticipated as a result of the proposed action. In none of the Action area is the amount of proposed activities believed to be extensive enough to cause the species to be permanently displaced from its traditional hibernation range. Thus, all currently extant Indiana bat northern long-eared bat hibernacula are likely to persist within the Action Area following implementation of the proposed action, potentially benefitting in the long-term.

Any Incidental Effects to Other Species or Critical Habitats: No effects to other species or critical habitats are anticipated as the localized efforts and methods are specific to the hibernacula.

Interrelated and interdependent actions: No interrelated and interdependent actions are associated with the proposed action.

Cumulative Effects: Cumulative effects include the effects of future state, tribal, local or private actions that are reasonably certain to occur in the Action Area considered in this biological opinion. Future federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the Act.

The Service is unaware of any future state, tribal, local or private actions, other than the proposed project, which would impose significant cumulative effects on the Indiana bats that use the area.

§ 6.0 CONCLUSION

After reviewing the current status of the Indiana bat and the northern long-eared bat and the effects anticipated from implementing actions described in the 10(a)(1)(A) permit application, it is the Service's biological opinion that the Section 10(a)(1)(A) permit, as proposed, is not likely to jeopardize the continued existence of these species.

We reached this opinion based on the following factors:

1. Chitosan appears to be one of the safest and more effective laboratory approved treatments for application to bats. Preliminary results from the principal investigators suggest that chitosan is effective at reducing germination and increasing bat survival. With demonstrated efficacy in

multiple non-listed species, including those within the same genus (i.e., *M. lucifugus*) of the listed species in question, we are hopeful such an application of chitosan would be useful as a treatment for listed species as well, if deemed necessary. This could result in beneficial effects to listed bats.

2. The potential benefit is large, as increasing survival is critical to the maintenance of all WNS-affected species. We will work with species leads and recovery teams (including the WNS multi-species recovery team) to assess the population-level implications of any demonstrated increases in survival that result from our experiments.
3. Disturbance from research activities and the construction of the barrier will be coordinated with the Service's local field office and species lead recovery biologist for the species in question, to ensure that the work is consistent with the species' recovery program and would not be expected to reduce the viability of the population(s) that would be affected.
4. While short-term movement and reproductive output of the listed species may be compromised for one winter, long-term advancements in WNS treatment could provide much greater recovery benefit for the populations of listed bats already and those soon to be affected by WNS.
5. Video/pictures (trail cameras) and/or call recordings (data loggers) from increased monitoring activities throughout the project will be analyzed and provided to the species lead recovery biologist for the species in question. Such information will be helpful in evaluating future research and management efforts in listed species hibernacula while they are present.

§ 7.0 CONSERVATION RECOMMENDATIONS

Section 7 (a) (1) of the Act directs Federal agencies to use their authorities to further the purposes of the Act by carrying out conservation programs for the benefit of endangered and threatened species. Conservation recommendations are discretionary agency activities to minimize or avoid adverse effects of a proposed action on listed species or critical habitat, to help carry out recovery plans, or to develop information.

The Service provides the following conservation recommendations for the PI(s). These activities should be conducted at the discretion of PI as time and funding allow:

1. Working with the Service, develop national guidelines for addressing outstanding issues associated with WNS treatment projects within the range of the Indiana bat and northern long-eared bat.

2. Conduct research on understanding/controlling and mitigating the effects of White-Nose Syndrome.

3. Expand on scientific research and educational outreach efforts on Indiana bats and Northern Long-eared bats in coordination with the Service’s WNS Coordinator and Field Offices.

In order for the Service to be kept informed of actions for minimizing or avoiding adverse effects or benefiting listed species or their habitats, the Service requests notification of the implementation of any conservation recommendations.

§ 8.0 INCIDENTAL TAKE STATEMENT

Section 9 of the Act and Federal regulation pursuant to section 4(d) of the Act prohibit the take of endangered and threatened species, respectively, without special exemption. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. Harm is further defined by the U.S. Fish and Wildlife Service (Service) to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing essential behavioral patterns, including breeding, feeding, or sheltering. Harass is defined by the Service as intentional or negligent actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding or sheltering. Incidental take is defined as take that is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity. Under the terms of section 7(b)(4) and section 7(o)(2), taking that is incidental to and not intended as part of the agency action is not considered to be prohibited taking under the Act provided that such taking is in compliance with the terms and conditions of this Incidental Take Statement.

The measures described below are nondiscretionary, and must be undertaken by the Service so that they become binding conditions of any permit issued for the exemption in section 7(o)(2) to apply. The Service has a continuing duty to regulate the activity covered by this incidental take statement. If the Service (1) fails to require the permittee to adhere to the terms and conditions of the incidental take statement through enforceable terms that are added to the permit or grant document, or (2) fails to retain oversight to ensure compliance with these terms and conditions, the protective coverage of section 7(o)(2) may lapse. In order to monitor the impact of incidental take, the permittee must report the progress of the action and its impact on the species to the Service as specified in the incidental take statement [50 CFR 402.14(i)(3)].

AMOUNT OR EXTENT OF TAKE

The Service anticipates that all Indiana bats and northern long-eared bats in the three mines will be incidentally taken as a result of the activities associated with the proposed action. Current surveys indicate that there were 36 Indiana bats and two Northern Long-eared bats in Jason Mine, IL. The most recent surveys at the two sites in Michigan yielded counts of 21 to 1 (Young Adit, 2015 and 2016, respectively) and 127 (Adventure Adit, 2012) northern long-eared bats. The pre-WNS surveys conducted in Michigan demonstrates that northern bats represent 10% of the total Myotis count (Kurta and Smith, 2014a). More recently, Kurta and Smith (2016) estimate that northern's may now represent only 1.5% of the identified Myotis species after the latest counts from the Winter of 2015-2016. Given the inherent variability in post-WNS hibernacula population estimates, the Service anticipates that no more than 87 Indiana bats and 177 northern long-eared bats, the historic maximum total count per species for all three mines, will be incidentally taken. Incidental take is expected in the form of harm (not mortality) and/or harassment to occur to all Indiana and northern long-eared bats within the three mines.

The Service realizes that the incidental taking of Indiana and northern long-eared bats may be difficult to detect and assess in real-time without adding additional harm or harassment to the listed species. Due to the difficulty of detecting take of these species, the Service will monitor the amount and extent of the take using the population survey conducted at the beginning and end of the field season, acoustic monitoring devices, and trail cameras in all 3 locations. If the initial survey indicates that more than 87 Indiana bats and 177

northern long-eared bats are present, then the PI cannot erect the barrier, and the Service will reinitiate consultation as described below.

EFFECT OF THE TAKE

In the preceding BO, the Service has determined that the anticipated level of incidental take is not likely to jeopardize the continued existence of the Indiana bat or the northern long-eared bat.

REASONABLE AND PRUDENT MEASURES

We believe the following reasonable and prudent measure is necessary and appropriate to minimize impacts of incidental take of the northern long-eared bat:

1. The Service will ensure the section 10(a)(1)(A) permit for this proposed action adheres to permit conditions for amended or renewed permits.

TERMS AND CONDITIONS

In order to be exempt from the prohibitions of section 9 of the Act, the Service must comply with the following terms and conditions, which carry out the reasonable and prudent measure described above and outline required reporting/monitoring requirements. These terms and conditions are non-discretionary.

1. The permit will include conditions containing contingency procedures in the event that mortality to a listed bat occurs during the performance of proposed activities. Given the history of the ongoing activities authorized by the Service, we do not anticipate injury or mortality to individuals of listed bat species. If unintended mortality or injury should occur, authorized activities will stop after completion of the current day's activities and the permittee will contact the appropriate Service office noted in the permit conditions by the next workday. Based on discussions between the Service office and the Service's Law Enforcement Division, a decision will be made as to whether or not the authorized activities will be allowed to continue as stated in the Timeline of Activities of this Biological Opinion. A decision will also be made as to the disposition of any dead or injured bats.

2. All current and subsequent white nose syndrome-related decontamination protocols approved by the Service and deemed necessary to address the disease, will be followed by permittee.

3. The Service will ensure that all minimization measures discussed in the description of the proposed activities are incorporated as permit conditions for any permits proposing to implement those activities.

4. The Service will ensure the permittee provide reports on all covered activities that include estimates and/or actual amounts of take (permitted (i.e., purposeful) take activities and incidental take) that occurs as a result of permitted activities. Such reports must be received on an annual or more frequent basis.

The reasonable and prudent measure, with its implementing terms and conditions, is designed to minimize the impact of incidental take that might otherwise result from the proposed action. If the Service or permittees observe or become aware of incidental take that occurs during implementation of permitted activities that exceeds or is different from the incidental take authorized by this biological opinion, such incidental take

represents new information requiring reinitiation of consultation and review of the reasonable and prudent measure provided. The Service and/or permittee must immediately provide an explanation of the causes of the taking and review, and the Service must evaluate the need for possible modification of the reasonable and prudent measure.

§ 9.0 REINITIATION NOTICE

This concludes formal consultation on the actions outlined in the consultation. As written in 50 CFR 402.16, reinitiation of formal consultation is required where discretionary Service involvement or control over the action has been retained (or is authorized by law) and if: (1) the amount or extent of incidental take is exceeded; (2) new information reveals effects of the Service action that may affect listed species or critical habitat in a manner or to an extent not considered in this opinion; (3) the Service action is later modified in a manner that causes an effect to the listed species or critical habitat not considered in this opinion; or (4) a new species is listed or critical habitat designated that may be affected by the action. In instances where the amount or extent of incidental take is exceeded, any operations causing such take must cease until reinitiation.

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