

Supporting Decontamination Documentation for Researchers
(WNS Decontamination Supplement 2 of 2)
U.S. Fish and Wildlife Service
Version 01.25.2011

I. GENERAL INFORMATION:

The fungus *Geomyces destructans* (*G.d.*), is believed to cause white-nose syndrome (WNS), and has been discovered in Connecticut, Delaware, Indiana, Maryland, Massachusetts, Missouri, New Hampshire, New Jersey, New York, Oklahoma, Pennsylvania, Tennessee, Vermont, Virginia, West Virginia and the provinces of Ontario and Quebec, Canada. WNS is a rapidly spreading disease of hibernating bats, and is responsible for the death of over 1 million bats in eastern North America since discovery in 2007. The U.S. Fish and Wildlife Service (USFWS) strongly advises the implementation of the decontamination procedures detailed herein for all bat and cave¹-related research to significantly reduce the risk of unintentional, human-assisted spread of *G.d.* Additional regional restrictions may apply for conducting research either under a federal permit or Section 6 authorities (e.g., USFWS Midwest Region – see R3 website at <http://www.fws.gov/midwest/Endangered/mammals/>). States and/or other land management agencies may also require additional precautions for activities on their lands; therefore, researchers should be familiar with all requirements for conducting research at their respective study areas.

Bat-to-bat transmission is believed to be the primary vector for the spread of WNS. Research at WNS-affected hibernacula has demonstrated, however, the propensity for conidia (spores) of *G.d.* to attach to gear used inside affected caves (Okoniewski 2010), supporting concern for anthropogenic, or human-assisted, spread of the fungus. Additionally, research has demonstrated that bats can develop WNS directly from an affected cave environment in the absence of infected bats (Hicks et al. 2010). Therefore, precaution must be taken to ensure that conidia of *G.d.* are not transported or otherwise introduced to unaffected bats or environments through human action.

Any object or substance capable of carrying and transferring infectious organisms that comes in contact with bats, individuals handling bats, or the environments where bats occur has the potential to be a vector for the spread of WNS. Therefore, to help prevent spread of WNS to unaffected caves, **it is important that you should NOT transport or use any exposed clothing or gear outside of a WNS-affected state or region in a WNS-unaffected state or region.** Clothing or gear that has been or is suspected of being exposed to *G.d.* may be reused in other WNS affected caves; however, the WNS decontamination procedures provided in this document should continue to be followed for items used in affected caves prior to entering other affected caves or leaving the affected state or region. Used gear that must be transported out of affected states or regions should be decontaminated, contained, and sealed prior to leaving the affected area and should not be stored or transported in close proximity with unexposed equipment. If gear cannot be decontaminated, either for safety reasons or fear that equipment may be damaged, it should not enter subsequent caves but rather be designated for use in one specific cave.

As stated in the WNS Decontamination Protocol v. 1.25.2011 (Available at: <http://www.fws.gov/WhiteNoseSyndrome/pdf/WNSDecontaminationProtocol v01252011.pdf>), the most effective course of action to guard against the transportation of *G.d.*, or any similar microbe, is to fully decontaminate clothing and gear after exiting each and every cave visited. In areas of high cave density, however, circumstances may require that multiple caves be visited on the same day. Assuming that bat-to-bat transmission will likely account for the rapid spread of the fungus between caves in close proximity, and that only aspects of decontamination is going to be feasible within cave complexes visited on the same day in remote locations, the actions recommended in this protocol can be adjusted to accommodate field activities in these areas. Since limited hibernacula data show that bats easily move upwards of 10 miles in search of resources (i.e., food, mates, roost and hibernation sites) during portions of the fall, winter, and early spring, researchers should, at the very minimum, use full decontamination procedures on a daily basis and between any two caves more than 10 miles apart if surveying/monitoring multiple caves on the same day. Whenever there is a question, biologists and researchers should always choose the conservative approach of decontaminating gear, clothing, and equipment between each individual cave visited.

¹ The use of the word “cave” in this document includes natural caves, man-made mines, or any other site that may harbor *G.d.* spores.

PLEASE REMEMBER: In all cases, decontamination efficacy is based on application to hard, nonporous surfaces and submersible gear/clothing, and the ability to prevent the regrowth of artificial culture media. Tests have been conducted on porous fiber materials such as ropes and harnesses to determine disinfection efficacy for killing fungal spores on these substrates and their effects on gear integrity. The repeated use of decontamination products may compromise the integrity of vertical equipment; therefore, since this equipment cannot currently be decontaminated, it should be dedicated to one cave or not used at all. **The act of implementing these procedures may require a significant change in the way most researchers and biologists conduct research or surveys. Therefore, the purpose and value of cave visits should be thoroughly considered as there will be additional expenses to conducting field work in a safe and responsible way.**

II. RECOMMENDED DECONTAMINATION PRODUCTS:

All necessary and appropriate precautionary, use, storage, and disposal information should be apparent on each of the product labels. It is critical that all researchers and biologists read and follow all label instructions provided on the products mentioned in this protocol. It would be a violation of federal law to use, store, or dispose of a regulated product in any manner not prescribed on the approved label/MSDS.

The following chemical (with a minimum of 0.3% quaternary ammonium compound, unless otherwise denoted) and natural products were tested in the laboratory and found to be effective for killing the conidia of *Geomyces* spp.:

1. Lysol[®] IC Quaternary Disinfectant Cleaner - (A concentrate product effective at 1:128 dilution, or 1 ounce of concentrate per gallon of water.)²
2. Professional Lysol[®] Antibacterial All-purpose Cleaner - (A concentrate product effective at 1:128 dilution, or 1 ounce of concentrate per gallon of water.)²
3. Formula 409[®] Antibacterial All-Purpose Cleaner (use off-the-shelf concentrations as specified by label)²
4. A 10% solution of household bleach - this must be made by measuring 1 part bleach to 9 parts water²
5. Lysol[®] Disinfecting Wipes (0.28 % di-methyl benzyl ammonium chloride)^{2 & 3}
6. Boiling in water for 15 minutes²

III. DECONTAMINATION PROCEDURES:

Any clothing, footwear and/or equipment, including outer clothing, should never be used in subsequent caves unless the following recommended decontamination procedures can be performed between each cave or within the parameters (e.g. within 10 miles, affected vs. unaffected) discussed in Section I are met. Upon entering and exiting any cave, scrub off all dirt and mud from your clothes, boots, and gear. Prior to leaving the site, ensure that clothing, boots, and equipment that were used in the cave are placed in a sealed plastic bag or plastic container with lid to be cleaned and decontaminated off site, if all decontamination is not feasible at cave entrance. In all cases, outer clothing (i.e., Tyvek[®] suits, coveralls, etc.) must be removed prior to entering a vehicle and after/between cave visits. A clean change of clothing is recommended.

As mentioned, the first step of decontamination is to remove all soil and organic material from equipment, clothing, and boots using a brush and preferably water (best done at entrance of cave upon exiting). This is

² Use of some products which contain quaternary ammonia, isopropanol, and other potentially harmful chemicals or boiling water in confined spaces needs to be approached carefully due to inhalation or contact risks of the product. Since products/procedures may also cause damage to clothing, gear, and sensitive electronic equipment, all users should be aware of these risks prior to entering cave environments. Use of personal protective equipment to reduce contact with the product is strongly encouraged, particularly if extended contact is anticipated or as recommended by the manufacturer. Always read and follow the MSDS information and all safety/use criteria for every product used.

³ The active ingredient is considered to be at the effective concentrations known to kill the conidia of *Geomyces* spp.; however, the efficacy of field application remains to be demonstrated. Any equipment decontaminated with this product should be used with extra precaution until laboratory results are finalized.

especially important since organic material (i.e. clay soils) can prevent the chemical products from penetrating clothing, boots, and equipment.

A. Submersible Gear (i.e., clothing and equipment that can be submerged without damage):

Wash all clothing and any appropriate equipment in washing machine or by hand using conventional detergents. Washing can be done in cold, warm or hot water. Laboratory testing has found Woolite® fabric wash to be an effective detergent for this procedure. Rinse thoroughly, and then follow by soaking for a minimum of 10 minutes in one of the recommended decontaminating products listed under Section II, then rinse and air dry. Please notice when boiling water is selected as the decontamination method, all gear must be submersed for 15 minutes, then followed by air drying.

If multiple entries into a single cave are planned, the trip necessitates extended efforts in a remote location, with NO vehicular travel to new or additional caves, and full decontamination is not possible, then researchers/biologists should, at a very minimum, swap out and/or use disposable equipment (i.e., Tyvek® suits, holding bags, punches, latex gloves) between cave visits. Similarly, sensitive equipment (i.e., camera, headgear, lights) should be swapped out or wiped using an appropriate decontamination product (i.e., Lysol® disinfectant wipes) prior to entering each new successive cave. All footwear should be intensively scrubbed to remove all dirt and debris, and then wiped using Lysol disinfectant wipes (if procedures listed below are not feasible).

Tyvek® brand disposable suits are a useful alternative to protect and cover field clothing when conducting surveys in caves. When using Tyvek® suits, they may rip when crawling through tight spaces. A more durable disposable suit (i.e., ProShield®), or use of a caving suit is recommended for surveys of this type. If using a disposable suit, use a new suit for each cave visit. Place suit in a garbage bag when field work is complete, store in plastic container until the garbage bag can be thrown in the trash. It important to understand, outside of situations that allow for multiple cave visits within the same day, **all clothing under Tyvek® should still be decontaminated using one of the appropriate products found in Section II.**

1. Footwear:

Where possible, rubber (wellington-type) caving boots (which withstand harsh decontaminating products and are easily cleaned) are recommended. Boots need to be fully scrubbed and rinsed so that all soil and organic material is removed. The entire boot, including soles, leather uppers and other portions, should then be decontaminated with an appropriate product listed under Section II for a minimum of 10 minutes, then rinse and air dry.

2. Ropes and Harnesses:

It is the responsibility of each researcher/biologist using vertical gear, including caving or life-support equipment (e.g., harnesses, webbing, and ropes), to ensure that the decontamination protocols in use are chemically compatible with their equipment. **To date, only Sterling rope and webbing have been shown not to be damaged by the following decontamination protocol:** Wash rope/webbing in a front loading washing machine on the gentle cycle using Woolite® Extra Delicates detergent. Treat by immersion in a 1:128 dilution of Lysol IC Quaternary Disinfectant Cleaner for 10 minutes. Rinse in fresh, clean water for a minimum of two rinses and allow to air dry.

If you are using other brands of rope and webbing not mentioned above, these products have yet to be tested for integrity after decontamination. In cases where safety following decontamination has not yet been evaluated, then ropes and webbing should be dedicated to one cave or not used at all to prevent the spread of WNS.

3. Nets:

Researchers must use separate nets between confirmed WNS-affected and unaffected states or regions. Furthermore, realizing that some WNS affected states or regions contain both affected and unaffected caves, under no circumstances are any nets used at an affected cave to be used at a known unaffected cave. All nets are required to be decontaminated in boiling water for a minimum of 15 minutes and dried between nights of use. Contact your state wildlife agency for updated information regarding specifics for WNS affected caves by visiting the following webpage <http://www.fws.gov/offices/statelinks.html> .

To avoid unintentional spread of *G.d.* among individual captures, all bats must be separated using new and clean holding bags. Holding cages are not acceptable means for processing bats. All bats must be kept in breathable holding bags for no longer than the specified holding times in your respective federal and/or state permits. Non-disposable holding bags are

only permitted for use once per night of field work or when the respective bags are decontaminated (following procedures above for submersible gear) and dried between nights of use. Disposable paper bags are allowed for holding bats temporarily, but are not permitted for reuse. Regardless of the bag, disposable and non-disposable, each individual bag is limited to only one bat per bag.

Disposable exam gloves must be worn over handling gloves and changed before and after the handling of each bat. Disposable gloves should be one size larger than the handling gloves. Smooth leather gloves may be wiped down with a Lysol® disinfectant wipe in between handling bats. However, if only using leather gloves (w/o latex exam gloves), each handler must have several sets of gloves to interchange in between each bat. This allows time to remove all heavy soil deposits and ample drying time for the disinfectant in between handling individual bats, which ultimately allows for an effective fungal kill. Furthermore, after each night of netting, remove heavy soil deposits from surface of bags and gloves, soak in an appropriate decontamination product in Section II, rinse and then dry completely. Researchers may also find it necessary to handle bats with their bare hands when conducting certain field activities (e.g., transmitter attachment, etc.). For this reason, standard personal hygiene practices, using Dawn® antibacterial dish soap has proved very effective against the growth of *Geomyces* species. As always, researchers should make sure hands dry completely before handling the next bat. Since handling of bats with bare hands is extremely hazardous to humans and bats through transmission of various diseases, all researchers are strongly encouraged to consider more protective measures (e.g., wearing disposable exam gloves over smooth leather gloves).

All equipment (i.e. light boxes, banding pliers, rulers, calipers, scale, etc) that comes into direct contact or has been used in the handling of bats should be sanitized and soaked for a minimum of 10 minutes in the appropriate decontamination products listed in Section II. Any instrument coming into direct contact with bat skin must be rinsed free of chemical product using clean water or physiologic (0.9%) saline. If using containers to weigh bats, separate containers used to weigh tree bats (e.g., *Lasiurus cinereus*, *Lasiurus borealis*, *Lasionycteris noctivagans*, and other similar species) from cave-hibernating bats, and never place tree bats in the same container previously used for a cave-hibernating bat. Non-disposable containers used to weigh bats (film canisters, baggies, cardboard rolls) should never be reused on the same night and must be decontaminated and properly dried in between uses. Paper lunch bags are permitted for holding and weighing individual bats. Plastic baggies are permitted for individual use when lining weigh containers. Furthermore, bats can be held in unsealed plastic bags during forearm measurements, reducing contact with wing rulers or calipers. All disposable equipment (i.e., paper lunch bags, plastic bags, etc.) and gloves should be properly discarded after holding or handling each bat.

B. Non-submersible Gear (i.e. equipment that will be damaged by submersion):

The following recommendations for the various types of non-submersible gear each pose their own significant challenges to ensure decontamination efficacy. Therefore, such gear, with the exception of vehicles, that has been or is suspected of being exposed to *G.d.* are only permitted for reuse at other contaminated caves. The following decontamination protocols should continue to be followed for items used in contaminated caves. The general protocol for decontamination of non-submersible gear involves the cleaning of gear thoroughly with soap (i.e., Dawn® antibacterial dish soap) and water, where appropriate, and then the decontamination of all equipment by applying one of the recommended chemical products (understanding some products are tougher on surfaces than others) in Section II to the outside surface for a minimum of 10 minutes, then rinse and air dry.

1. Harp traps:

While the following measures do help to minimize the risk of WNS transmission via harp trapping, fall swarming represents a heightened risk and a particular challenge for decontamination. Furthermore, many experts are concerned that it is not possible to develop protocols to sufficiently address the risk of transmission during this time. The potential for transmission in the collecting bag of the harp trap is of particular concern. For that reason, additional restrictions, similar to those in Region 3 and 4 of the USFWS, are highly recommended during this season. Researchers are encouraged to seek alternative techniques, such as emergence counts or acoustic surveys, in lieu of harp trapping. However, it is realized that use of harp traps may be critically necessary for study of white-nose syndrome during this season, in which case such research may need evaluation on a case by case basis.

If harp traps are used then such research must use separate traps between confirmed WNS-affected and unaffected states. Furthermore, regardless of location or other circumstances, traps used at an affected cave are not permitted for use at an

unaffected cave (even within the same state or region). Contact your state wildlife agency for updated information regarding specific WNS affected caves by visiting the following webpage <http://www.fws.gov/offices/statelinks.html>. In both WNS-affected and unaffected states or regions, all traps are required to be cleaned nightly after use to remove any dirt/debris that could inhibit the effectiveness of decontamination on the wires/lines and bags (it is highly recommended that the bag be removed to reduce risk of transmission). Upon cleaning, all surfaces shall be sprayed with one of the decontamination products listed in Section II. The harp trap bag must be sanitized and soaked for a minimum of 10 minutes using one of the decontamination products listed in Section II, rinsed thoroughly, and allowed to dry completely (preferably in the sun) prior to the next use.

We recognize that some of the recommended decontamination procedures may not be practical when using harp traps where regular bat to bat contact occurs. Therefore, we recommend checking the catch bag more frequently in order to reduce the amount of time that bats are in contact with each other and the bag. Bats should not be allowed to remain in the catch bag for more than 10 minutes. Bats collected should then be put in their own holding bag until processing is complete. To reduce cross-contamination, the catch bag is required to be lined with a sheet of plastic and replaced with new plastic periodically or wiped down using one of the decontamination products in Section II. Disposable gloves must be worn over handling gloves and swapped out between handling of each bat, or frequently decontaminated according to previously mentioned protocol for using smooth leather gloves.

2. Cameras, Computers, and Other Electronic Equipment:

If possible, do not bring electronic equipment into a cave. If practical, cameras and other similar equipment that must be brought in a cave should be placed in plastic casing (i.e., underwater camera housing) or wrapped in plastic wrap where only the lens is left unwrapped to allow for photos to be taken. The plastic wrap shall then be decontaminated by using Lysol® Disinfecting Wipes and properly discarded after use. If using plastic wrap is not practical, respective wipes can be applied directly on camera surfaces or plastic casing (Caution: This could damage the body of the camera on repeated treatments).

3. Wing Biopsies:

Given the high risk of transmission inherent with wing punch biopsies, as well as concerns regarding the sharpness of re-used punches, all applicable research on bat species requires the use of a new (unused) sterile punch for each bat. In such cases where researchers/biologists have the ability to sterilize used punches by means of autoclaving, punches may be used a total of 3 times. This will insure they are sharp enough to make clean punches. However, if a biopsy punch is used on a bat with evidence of fungal infection, then researchers/biologist must discard the used punch (not reusable even if sterilized) after use on that individual. Furthermore, no punch should ever be reused on the same day.

The cutting board must be disinfected between processing individual bats using an appropriate decontamination product listed in Section II. Disposable, stiff cardboard squares (1 per individual) can be used as an alternate support for the biopsy. It may be necessary to have multiple people to assist with wing biopsies to avoid contaminating equipment during this process; one for holding the bat down, one for note taking and one for taking the biopsies.

4. Vehicles:

Do not work on live bats in vehicles. Vehicles used to transport equipment may harbor spores. It is important to keep vehicles as clean as possible by storing gear in clean, decontaminated containers, using an appropriate decontamination product listed in Section II. Do all processing on vehicle hood or on a table away from the vehicle; as the tailgate is not an allowable surface (i.e., nearest to potentially contaminated equipment). A drawstring garbage bag should be placed at each cave, outside the field vehicle each night so to properly contain all contaminated bags, gloves, wipes, and other items. Dead bats should be placed in a sealed plastic container and placed inside a second bag or container handled only with clean gloves. This outer packaging layer is considered clean and uncontaminated and safe to transport inside the vehicle (preferably contained within a clean cooler).

IV. SIGNS OF WNS:

First and foremost, *G.d.* may be present with NO apparent signs at all, so always take the necessary and appropriate precautions in decontaminating equipment, clothing, and associated items. If signs are present, realize that the white fungus is only one of the many signs. Other possible WNS signs may include damage to wings and tail membranes in the form of lesions, flakiness or dehydrated skin, discolored spots/scarring, multiple holes, or tears to the leading edge of

membranes. Such conditions should be put in context of the season, but have been quantified by the Reichard's Wing Damage Index (http://www.fws.gov/northeast/PDF/Reichard_Scarring%20index%20bat%20wings.pdf). Any researcher or field biologist should photograph any damage you observe and report it to the nearest U.S. Fish and Wildlife Service Field Office and/or local state agency that issued your bat handling permit within 24 hours.

V. CLOSING REMARKS:

Please, understand the effectiveness of this protocol depends solely on the adherence and successful implementation by each researcher/biologist. **ALL researchers and biologists must FIRST consider the risk of transmission and decontaminate, to the extent necessary, their clothing, boots, and equipment prior to entering and/or upon exiting the cave environment, both within and between States.** Furthermore, no decontamination procedure can ever be 100% effective, so it is essential that only the most critical research or surveys deemed necessary for WNS occur, especially those that involve handling bats or entering cave environments.

Important Note: The most updated information on known WNS affected states (mapped version) and protocols are posted on the U.S. Fish and Wildlife Service White Nose Syndrome website at: <http://www.fws.gov/whitenosesyndrome/>. We recommend that you visit the website periodically to ensure that you are using the most recent protocol in your permitted activities.

VI. LITERATURE CITED

- Hicks, A. J.C. Okoniewski, S. R. Darling, D.N. Redell, R.B. Smith, R.I. VonLinden, K.E. Langwig, T. Ingersol, J. Flewelling, and C.U. Meteyer. 2010b. Investigations in to the Environmental Transmission of WNS to hibernating *Myotis lucifigus*. Page 12 *In* Abstracts of Presented Papers and Posters for 2010 White-nose Syndrome Symposium, Pittsburgh, Pennsylvania, May 25-27, 2010. Available at: <http://www.fws.gov/WhiteNoseSyndrome/pdf/AbstractsofPresentedPapersandPostersFor.pdf> (Accessed on August 23, 2010).
- Okoniewski, J.C., J. Haines, A.C. Hicks, K.E. Langwig, R.I. VonLinden, and C.A. Dobony. 2010. Detection of the Conidia of *Geomyces destructans* in Northeast Hibernacula, at Maternal Colonies, and on Gear – Some Findings Based on Microscopy and Culture. Pages 17-18 *In* Abstracts of Presented Papers and Posters for 2010 White-nose Syndrome Symposium, Pittsburgh, Pennsylvania, May 25-27, 2010. Available at: <http://www.fws.gov/WhiteNoseSyndrome/pdf/AbstractsofPresentedPapersandPostersFor.pdf> (Accessed on August 23, 2010).