

Disinfection Protocol for Bat Field Studies
U.S. Fish and Wildlife Service – Region 3
July 2009

To minimize the potential for transmission of white-nose syndrome (WNS) while handling bats (both between handler and bats and between bats), these procedures shall be implemented. To date, WNS has been discovered in the northeastern U.S. and mid-Atlantic states.¹ The Midwest Region of the U.S. Fish and Wildlife Service (Service) has implemented these protocols in the interest of preventing WNS from spreading any further. In addition, we recommend that these guidelines be used any time people handle wildlife to minimize potential disease-related impacts to wildlife and people. Please note that individual states may have additional permitting requirements above and beyond these general procedures. In addition, these guidelines may be revised upon review of new information.

Any equipment that comes in contact with bats, with individuals handling bats, or the environments where bats occur, has the potential to be a vector for spread of WNS. Examples include mist nets, harp traps, bat bags, wing biopsy punches, weighing tubes, rulers, clothing, and gloves.

Decontamination requirements target the fungus *Geomyces* sp., which, to date, has been the most consistent pathogen recovered from bats exhibiting signs of WNS. Fortunately, many of the disinfectants and techniques tested for efficacy against the fungus are also suitable to kill other bacterial or viral agents should another causative agent of this disease be identified.

CAUTION: Disinfectant efficacy is based on application to hard, nonporous surfaces and the ability to prevent the regrowth of *Geomyces* sp. on artificial culture media. Tests are currently being conducted on porous fiber materials such as ropes and harnesses to determine disinfectant efficacy to kill the fungus on these substrates and their effects on gear integrity. The repeated use of disinfecting agents may compromise the effective use of vertical equipment; therefore, this equipment should be dedicated to one cave or not used at all.

Although a site may be affected with WNS, it should not be assumed that all individual bats within the site are infected or will become infected, and thus, care should be taken not to cross-contaminate specimens by lax handling methods. This is especially true if samples are to be submitted for diagnostic purposes.

Decontaminate all clothing, footwear, and gear prior to departing for a bat netting or cave outing if you did not decontaminate these items after last netting activity or exiting a cave. In affected and adjacent states, you may not take gear into a cave if that gear cannot be thoroughly decontaminated or disposed of (i.e. if harnesses, ropes, or webbing cannot be decontaminated, we advise that you not enter caves or parts of caves requiring use of this gear). In addition, only bring essential equipment used for bat netting and processing to a site; other non-essential items should be left home as they may contribute to spreading the fungus.

¹WNS Affected States: Connecticut, Massachusetts, New York, New Jersey, New Hampshire, Pennsylvania, Vermont, Virginia and West Virginia

PROCEDURES:

Vehicles:

Do not put bats in vehicles. Vehicles used to transport equipment may harbor spores. Do all processing on vehicle hood or on a table away from the vehicle. The tailgate is not preferred since it is likely near netting equipment. A drawstring garbage bag should be placed at each site outside the field vehicle each night so all contaminated bags, gloves, wipes, etc., are contained.

Submersible Gear (i.e. clothing and soft-sided equipment):

- For clothing – Wash all clothing and any appropriate equipment in washing machine using the hottest cycle possible for material and conventional detergents. Laboratory testing has found Woolite[®] fabric wash the best surfactant for clothing. Rinse and air dry. Then follow by soaking with sodium hypochlorite bleach (i.e. household bleach) solution diluted to 1 part bleach to 10 parts water in a tub or plastic container. Soak for 10 minutes. Rinse and air dry.
- For other submersible gear (i.e. bags, gloves, nets, etc.) – Disinfect any equipment that can be submersed in a solution with an appropriate and compatible disinfectant such as sodium hypochlorite bleach (i.e. household bleach) solution diluted to 1 part bleach to 10 parts water in a tub or plastic container or $\geq 3\%$ concentration of quaternary ammonium compounds (i.e. Sparquat 256, Lysol[®] All-purpose Professional Cleaner, or the antibacterial form of Formula 409[®]). Keep submersed for 10 minutes. Rinse and air dry.

Nets:

- Use separate sets between states affected by WNS¹ and unaffected states.
- Under no circumstances should nets that have been used in an affected site be used in an unaffected site. Contact your state wildlife agency (www.fws.gov/offices/statelinks.html) for county by county listings for WNS affected and unaffected sites.

Bats should be kept in breathable holding bags rather than holding cages. To avoid cross-contamination of samples, it is imperative to keep bats separated using holding bags that are kept as clean as possible. Non-disposable holding bags should be used only once per night of field work and should be washed and decontaminated (following procedures above) and dried between nights of use. Disposable paper bags are also a convenient option for holding bats temporarily. Only one bat should be in a given bag, and that bag should not be reused during the field night. White paper bags are best to avoid misplacing bats in the woods.

Disposable latex gloves should be worn over handling gloves and changed in between handling each bat. Disposable gloves should be one size larger than the handling gloves. Smooth leather gloves may be wiped down with a disinfectant (i.e. Purell[®], Lysol[®] disinfecting wipes or alcohol wipes) in between handling bats. If only using leather gloves, each handler should have several sets of gloves to interchange in between handling bats. This allows time to effectively kill the fungus and for the

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disinfectant to completely dry. After each night of netting (or prior to the next night of use), remove heavy soil deposits from surface of bags and gloves, soak in an appropriate disinfectant, then dry completely.

For situations when gloves may hinder field work (i.e. transmitter attachment) and bats come in contact with bare hands, apply hand sanitizer with alcohol (i.e. Purell[®]) after handling each bat. Make sure it dries completely before handling the next bat.

Non-submersible Gear (i.e. hard-sided equipment):

- **For non-submersible gear** (i.e. bat processing equipment, mist net poles, harp trap frames and legs, folding chairs, etc.) – Disinfect any equipment that cannot be submersed by applying an appropriate and compatible disinfectant to the outside surface by using $\geq 3\%$ concentration of quaternary ammonium compounds such as Sparquat 256, Lysol[®] All-purpose Professional Cleaner or the antibacterial form of Formula 409[®], or use sodium hypochlorite bleach (i.e. household bleach) solution diluted to 1 part bleach to 10 parts water. Keep on surface for 10 minutes. Rinse and air dry.
- **For boots** – Boots need to be fully scrubbed and rinsed so that all soil and organic material is removed. The entire rubber and leather boot, including soles and leather uppers, can then be disinfected with an appropriate disinfectant such as $\geq 3\%$ concentration of quaternary ammonium compounds (i.e. Sparquat 256, Lysol[®] All-purpose Professional Cleaner or the antibacterial form of Formula 409[®]) and sodium hypochlorite bleach (i.e. household bleach) solution diluted to 1 part bleach to 10 parts water. Keep on surface for 10 minutes. Rinse and air dry.

Use one of the disinfecting agents listed above to sanitize all equipment that comes into contact with a bat's body, including light boxes, banding pliers, rulers, calipers, scale, etc. Any instrument coming into direct contact with bat skin should be rinsed free of chemical disinfectant using clean water or physiologic (0.9%) saline. Clean items after handling each bat. If using containers to weigh bats, separate containers used to weigh tree bats from cave bats, do not place tree bats in the same container previously used for a cave bat. Containers used to weigh bats (film canisters, baggies, cardboard rolls) should be disinfected in between handling each bat. Paper lunch bags can be used for holding and weighing individual bats, and can be immediately discarded after each use. Plastic baggies can also be used to line weighing containers, and bats can even be held in unsealed plastic bags during forearm measurements, reducing contact with wing rulers or calipers. Discard used bags after each bat. Disinfect gloves or discard disposable gloves after handling each bat.

Harp traps:

- Use separate traps between states affected by WNS¹ and unaffected states. Under no circumstances should traps that have been used in an affected site be used in an unaffected site. Contact [your state wildlife agency](#) for county by county listings for WNS affected and unaffected sites.

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- Each night after use in affected states¹, remove any dirt/debris from wires/lines and bags, and spray on one of the above-listed disinfecting agents. Swab the bag with disinfectant and allow to dry completely (preferably in the sun) prior to the next use. Do not use equipment in an unaffected site following use in affected sites.
- Bats should not be allowed to remain in the catch bag for more than 10 minutes. Checking the catch bag more frequently will reduce the amount of time that bats are in contact with each other. Bats collected should then be put in their own bag until processing is complete. Disposable bags should be discarded after handling each bat and reusable bags should be decontaminated using one of the disinfecting agents listed above. To reduce cross-contamination, the catch bag may also be lined with a sheet of plastic and replaced with new plastic after every hour or wiped down with one of the disinfecting agents above.

Cameras, Computers, and Other Electronic Equipment:

If possible, do not bring electronic equipment to a netting site. If practical, cameras and other similar equipment that must be brought to a site may be wrapped in plastic wrap where only the lens is left unwrapped to allow for photos to be taken. The plastic wrap can then be decontaminated by using Lysol[®] disinfecting wipes and discarded after use. If using plastic wrap is not practical, alcohol wipes or Lysol[®] disinfecting wipes can be applied directly on surfaces.

Wing Biopsies:

If collecting wing biopsies for any approved research studies on Federally threatened or endangered bats, use a new (unused) punch for each bat. For other bats, punches may be reused, but only if they are still sharp enough to make clean punches. If there is evidence of fungal infection on any individual, use new punches. Be sure to completely sterilize recycled punches between bats by dipping the cutting end in alcohol and flaming until it naturally extinguishes, and then allowing them to cool completely. The cutting board must also be disinfected between processing individual bats using one of the agents detailed above. Disposable, stiff cardboard squares (1 per individual) can be used as an alternate surface for biopsy.

Notification of Signs of WNS

As a reminder, the white fungus is only one of the signs of WNS. We do not expect to find bats with fungus on them during the summer or fall, but bats could still be infected during these seasons. Other possible signs of WNS may be damage to wings and tail membranes in the form of lesions, flakiness or dehydrated skin, discolored spots/scarring, multiple holes, or tears to leading edge of membranes. We encourage the use of Reichard's Wing Damage Index (link below) for assessing bats. Please photograph any damage you observe and report it to the nearest U.S. Fish and Wildlife Service Field Office and the state agency that issued your bat handling permit within 24 hours.

http://www.fws.gov/northeast/PDF/Reichard_Scarring%20index%20bat%20wings.pdf

Important Note: These protocols are posted on the U.S. Fish and Wildlife Service Midwest Region website at: <http://www.fws.gov/midwest/Endangered/mammals/BatDisinfectionProtocol.html>. Please visit the site at least once every six weeks to ensure that you are using the most recent protocol in your permitted activities.

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What is known about *Geomyces* sp. viability:

- The fungus survives exposure to mammalian body temperature (38° C/100° F) for at least 3 days, but does not remain viable after 8 days (W. Stone, NYSDEC, pers. communication 4/14/09).
- The fungus survives exposure to temperature (30° C/86° F) for at least 15 days. (W. Stone, NYSDEC, pers. communication 4/14/09).
- Short-term incubation of fungus at higher temperatures reduces the number of conidia present and alters the morphology of the hyphae which may not inhibit growth once returned to colder temperatures (W. Stone, NYSDEC and D. Blehert, USGS NWHC, pers. communication 4/14/09).
- Clothes dryer heat treatment (49° C/ 120° F) alone increases fungal spore germination and does not kill the fungus (H. Barton, NKU, pers. communication 4/22/09).

What kills the *Geomyces* sp. fungus:

Method	Conditions	Kill Time	Source	Cautions*
Disinfectant				
5.25% Chlorine bleach	10% bath solution (1 part bleach: 9 parts water)	10 min	Over the counter	Inactivated by organic material, detergents; corrosive to metals; produces toxic gas if combined with ammonia; skin irritant
Lysol® Professional Antibacterial All Purpose Cleaner	1:128 bath solution (1 oz per 1 gal water)	10 min	Janitorial supply	Corrosive; skin & eye irritant
	1:64 bath solution (2 oz per 1 gal water)	5 min		
Sparquat 256	½ oz per 1 gal water	10 min	www.chemsearch.com	May require license to obtain; requires special disposal methods
Promicidal™	1:128 bath solution (1 oz per 1 gal water)	10 min	www.chemsearch.com	May require license to obtain; requires special disposal methods
Grenadier™	1:64 bath solution (2 oz per 1 gal water)	10 min	www.chemsearch.com	May require license to obtain; requires hazardous waste disposal methods

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	1:32 bath solution (4 oz per 1 gal water)	5 min		
Formula 409 [®]	At least 0.3% concentration	10 min	Over the counter	
Woolite [®]	Refer to product label		Over the counter	
Dawn [®] antibacterial hand soap	Refer to product label		Over the counter	
Purell [®]	Refer to product label		Over the counter	
Lysol [®] disinfecting wipes	Refer to product label		Over the counter	
70%-95% ethanol	Undiluted bath	2 min	Lab supply distributor	Flammable, skin irritant
Temperature				
Dry heat	110° F/ 43° C	12 hr	Oven, incubators	
	165° F/ 74° C	15 min		
	175° F/ 79° C	5 min		
	180° F/ 82° C	5 min		
Sterilization				
Steam autoclave	121 F; 15 psi	15 min	Laboratory or hospital settings	
Gas sterilization	Ethylene oxide	16-18 hr	Only available at hospitals	
Flame sterilization	Alcohol & open flame	15-20 sec		Fire hazard; burn injuries

* Effects of different decontamination methods on the integrity of caving equipment are currently being tested.

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