

Decontamination of Equipment and Clothing to Prevent the Spread of White-Nose Syndrome (the causal fungus: *Pseudogymnoascus destructans*) in Canada.

Background:

The fungus that causes White-nose Syndrome in bats, *Pseudogymnoascus destructans* (*P.d.*), grows on bats, in soil and on a range of different materials. It produces spores (conidia), small units of fungus similar to dust in size and characteristics that can cling to surfaces and can thus be transported to distant locations where they may fall to earth or onto other substrates, grow and produce more fungus and more spores.

Bats themselves carry these spores and have spread *P.d.* over large areas of North America.

People also can spread the fungus, simply by visiting or working in an environment where *P.d.* exists and then traveling to another environment suitable to growth of the fungus. Spores on clothing and equipment can be transported from place to place in this manner and could easily introduce *P.d.* to new areas where it will grow and threaten the health of any hibernating bats in the same environment.

Why Decontaminate?

White nose-Syndrome is a very serious disease. It has killed millions of bats in North America since 2006, and some of the affected species, once some of the most numerous mammals on the continent, already now are rare and may become extinct altogether in affected areas. Bats are valuable for many different reasons – ecological, economic and aesthetic.

The single most important thing any person can do to reduce the impact of White-nose Syndrome is to ensure that he or she does not spread the fungus to new areas. The only way to make sure of this is to decontaminate any clothing or equipment that may have come in contact with the fungus and will be transported to locations where the fungus does not now exist, be that 10 km or 1000 km away.

Who Should Decontaminate?

If you enter potential bat habitat in Canadian provinces that are WNS-positive or suspect, it is your duty to decontaminate your clothing and equipment before going anywhere else that bats might live (for the most recent map of the distribution of WNS/*P.d.* in Canada, go to http://www.ccwhc.ca/wns_maps.php). White-nose Syndrome is spreading westward in Canada and the United States, and it is being

spread to new sites within regions where White-nose Syndrome already exists. Anything people can do to slow or stop this spread of White-nose Syndrome should be done, and decontamination of equipment and clothing is something that people can do, whether you are a tourist, a scientist, a government official, a mining engineer or a land-owner.

- People entering potential bat habitat in western Canada may feel distant from the problem of White Nose Syndrome. This is both correct and incorrect. Some special guidelines for western Canada to avoid spreading WNS/*P.d.* have been prepared by a consortium of cavers and biologists in BC and Alberta will be available Fall 2014.

What Can You Do?

In the following pages, you will find guidelines for decontamination of clothing and equipment to prevent the spread of the fungus that causes White-nose Syndrome.

- These are guidelines that provide you with the best information available about how to treat clothing and equipment so that no living fungus (*P.d.*) remains and the clothing and equipment has the least chance of spreading the fungus to new locations when used in these new locations.
- As new information becomes available, these guidelines will be revised to provide the most up-to-date information possible.

Laws and Rules and Personal Responsibilities

On some lands in Canada, there are firm rules and regulations that require full decontamination of all clothing and equipment when people leave sites that may contain the fungus that causes White-nose Syndrome (*P.d.*). Many other sites where *P.d.* may occur are closed and entry by anyone simply is not permitted. In most situations, however, each person must evaluate the potential risks of spreading *P.d.* that are associated with his or her activities, and make a decision on decontamination.

To evaluate the risk your actions may pose for White-nose Syndrome:

- Be informed: read the pages of this website and visit the White-nose Syndrome website of the US Fish and Wildlife Service
- Look at the most recent map of the known distribution of *P.d.* in North America (available on the Canadian and US websites), and locate your planned activities on this map.

- Call your provincial bat biologist and confer about risks associated with spread of White-nose Syndrome.
- Read the decontamination guidelines on this page and consider how you can work these into your activities.

Decontamination Products for Use in Canada

Testing of the effectiveness of various chemicals and processes for decontamination of clothing and equipment against spores of *P.d.* is incomplete at present. Some testing of products sold in the United States has been done and further testing is underway on products available specifically in Canada. The results of these tests will be added to the guidelines below as they become available. Many products are manufactured for use only on non-porous surfaces (hard surfaces) and carry no product guarantees for use on porous surfaces (e.g. clothing). Soon, test results and generic recommendations on effective concentrations of particular decontamination compounds will be available, making the choice of decontamination product easier.

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(Modified from information provided by the U.S. Fish and Wildlife Service “National WNS Decontamination Protocol – Version 06.25.2012” and the Addendum of Canadian Cooperative Wildlife Health Centre Ontario/Nunavut Region “Biosecurity Protocol for Research Visits to Bat Hibernacula in Ontario” by the Mitigation Technical Working Group of the Inter-agency White-Nose Syndrome Committee in Canada)

For the purposes of clarification, the use of the word “decontamination,” or any similar root, in this document entails both the 1) cleaning and 2) treatment to disinfect exposed materials.

I. GOALS OF BIOSECURITY AND DECONTAMINATION:

Prevent the spread of *Pseudogymnoascus destructans* by:

1. **Using protective gear** to prevent contamination of personnel, their clothing and equipment in known or suspected contaminated sites
2. **Preventing transfer of spores** from bat to bat within a site, or from infected sites to non-infected sites
3. **Removing spores** from protective gear and equipment
4. **Inactivating spores** still present on protective gear and equipment

II. PROTECTIVE GEAR

1. Vinyl or rubber rain suit (overalls and coat) of adequate size to be worn over clothes.
2. Disposable Tyvek[®] coveralls, with hood, of adequate size to be worn over rain gear.
3. Caving or similar helmet with light source. Hood of Tyvek[®] suit is worn up over the hair under helmet. Loose hair should be tied back.
4. Cheap rubber boots to be worn on site. Take one pair per person per site. Wear boots with rain suit cuffs outside the top, and Tyvek[®] suit cuffs outside that. Do not tuck garments into boots. Change to clean unused boots the last thing before leaving a site.
5. Nitrile or latex disposable gloves, worn pulled over sleeve cuffs of Tyvek[®] suit.
6. Caving ropes and gear if needed for site.
7. Disposable or washable plastic or other impervious bags for transport of cameras.
8. Disposable impervious plastic garbage or other bags for bagging contaminated gear.

III. DISINFECTANT TREATMENTS AND PRODUCT USE:

The most universally available option for treatment of submersible gear is:

Submersion in Hot Water: Effective at sustained temperatures $\geq 50^{\circ}\text{C}$ (122°F) for 20 minutes

Disinfectant products must be used in accordance with the label:

Ensuring the safety of those who use any of the above products for treatment is of utmost importance. Material safety data sheets (MSDS) developed by product manufacturers provide critical information on the physical properties, reactivity, potential health hazards, storage, disposal, and appropriate first aid procedures for handling or working with substances in a safe manner. Familiarization with MSDS for chemical products prior to use will help to ensure appropriate use of these materials and assist in emergency response.

It is a violation of federal law to use, store, or dispose of a regulated product in any manner not prescribed on the approved product label and associated MSDS. Disinfectant products, or their contaminated rinse water, should be managed and disposed of as per product label directions to avoid contamination of groundwater, drinking water, or non-municipal water features such as streams, rivers, lakes, or other bodies of water. Follow all local, provincial and federal laws. Note: Quaternary ammonium wastewaters should not be drained through septic systems because of the potential for system upset and subsequent leakage into groundwater.

Secondary and non-submersible treatment options include:

1. **Quaternary ammonium products containing at least 0.3% ammonium quaternary compounds.** These quaternary ammonium products must be used at the label dilution for best fungicidal activity. (Note: The Lysol Professional cleaning products listed by the U.S. Fish and Wildlife Service (US FWS) decontamination protocol [Table 1] are not available in Canada, according to the manufacturer Reckitt Benckiser). A selection of products available in Canada containing ammonium quaternary compounds, the agent in the Lysol products, include:
 - A. **ASEPTOL 2000 S.E.C. Repro Inc.**
http://www.secrepro.com/en/sanitary/aseptol_2000.php
 - B. **EP51B Av-mixx Avmor**
<http://www.avmorgreen.com/English/products.php?cat=1>
 - C. **Aqua San Zep Inc.**
http://webfiles.acuitysp.com/MSDS/2410_1_EN1_CDN.P.D.F
 - D. **Vanguard 256 Dustbane Products Ltd.**
http://www.dustbane.ca/msds/english/Vangard%20256_en.P.d.f
 - E. **Dyna Quat Plus Zep Inc.**
http://webfiles.acuitysp.com/psrCanada/psr_q161.P.D.F
 - F. **Clinicide (Bimeda-MTC Animal Health Inc.)**
<http://veto.naccvp.com/product/basic/view/1194013?key=ds2597>
 - G. **Virocid CID Lines**
http://www.belgagri.com/images/store/files/1272_VIROCID_FDS_FR.pdf
2. **Household chlorine bleach solution (e.g. Javex) diluted to 10% by volume (1 part bottled bleach solution, 9 parts water).**

Note: Alcohol based disinfectants, such as wipes and hand wash, are not effective for destroying *P.d.* spores as determined by US FWS.

Table 1: Treatment options for decontamination of non-porous and porous surfaces^{1,2,3}

PRODUCT	Preferred Treatment	Other Treatments				
	Submersion in Hot Water	Clorox® (6% HOCl) Bleach	Lysol® IC Quaternary Disinfectant Cleaner	Professional Lysol® Antibacterial All-purpose Cleaner	Formula 409® Antibacterial All-Purpose Cleaner	Lysol® Disinfecting Wipes
Hard, non-porous surfaces	Yes	Yes	Yes	Yes	Yes	Yes
Non-porous personal protective safety equipment	Yes	No	Yes (headgear, goggles, rubber boots, etc.)	No	No	No
All surfaces, including: porous clothing, fabric, cloth footwear	Yes	Yes (do not use on ropes, harnesses or fabric safety equipment)	No	No	No	No
DILUTION/TREATMENT (as per label)	Effective at sustained temperatures ≥ 50°C (122°F) for 20 minutes	Effective at 1:10 dilution (bleach : water) for 10 minutes	Effective at 1:128 dilution (1 ounce: 1 gallon of water) for 10 minutes	Effective at 1:128 dilution (1 ounce: 1 gallon of water) for 10 minutes	Effective at concentrations specified by label for 10 minutes	Effective at 0.28 % di-methyl benzyl ammonium chloride for 10 minutes

Other effective disinfectant(s) with similar chemical formulas (e.g., a minimum of 0.3% quaternary ammonium compound) or water based applications may exist and are currently being tested

IV. PLAN AHEAD! BEFORE EACH CAVE/MINE VISIT:

1. Determine the *P.d.* /WNS status⁴ of the province/county where your gear was previously used.
2. Determine the *P.d.* /WNS status⁴ of the province/county to be visited.
3. Contact local provincial/federal regulatory or land management agencies to determine additional requirements for site visits.

¹ The use of trade, firm, or corporation names in this protocol is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by provincial and/or federal agencies of any product or service to the exclusion of others identified in the protocol that may also be suitable for the specified use.

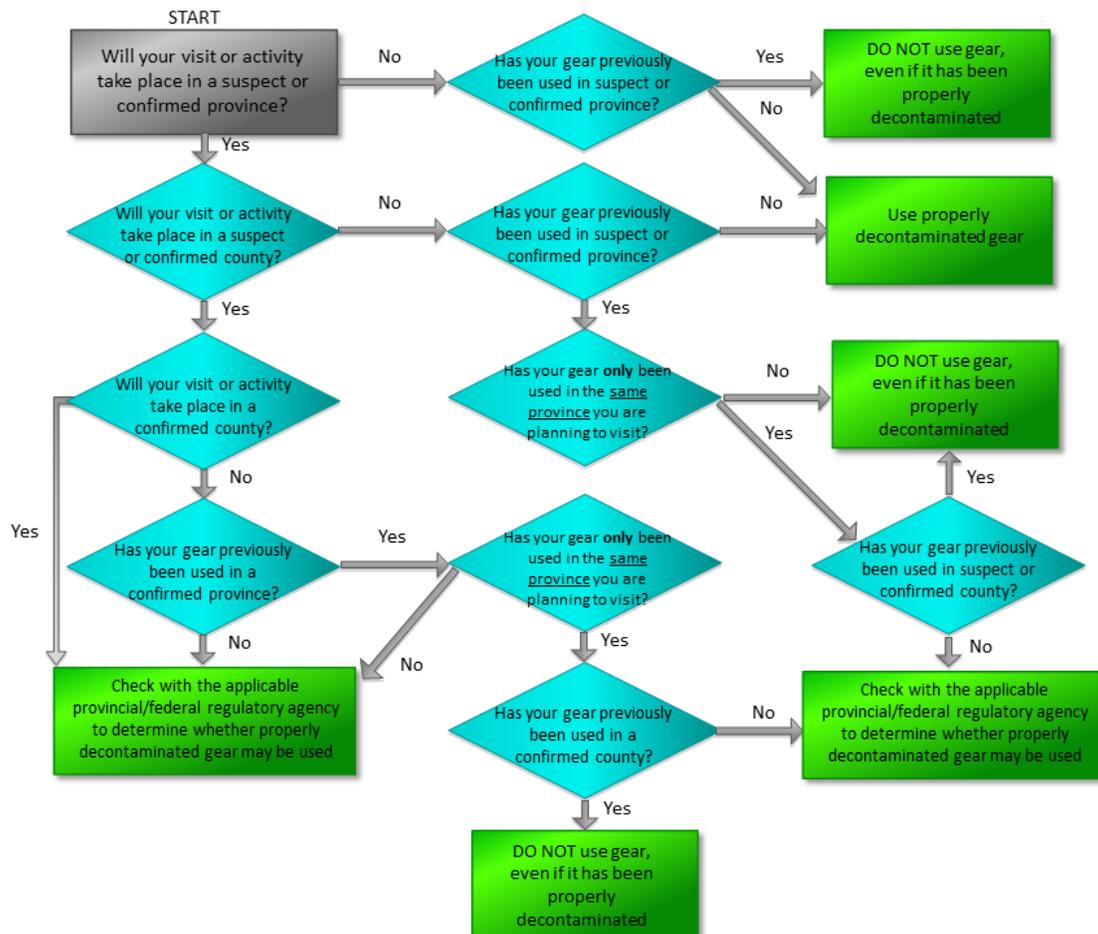
² Product guidelines should be consulted for compatibility of use with one another before using any decontamination product. Also, detergents and quaternary ammonium compounds (i.e., Lysol® IC Quaternary Disinfectant Cleaner) should not be mixed directly with bleach as this will inactivate the bleach and in some cases produce a toxic chlorine gas. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

³ Final determination of suitability for any decontaminant is the sole responsibility of the user. Use of some treatments which utilize such method need to be applied carefully, especially in confined spaces, due to inhalation or contact risks of the product. All users should be aware of these risks

⁴ Visit http://www.ccwhc.ca/wns_maps.php for the most up-to-date WNS status of a county or province/territory

4. Dedicate your gear – If possible, have separate sets of gear for each cave/mine visited, or simply do not enter caves/mines that require such equipment. Many types of rope and webbing have not been thoroughly tested for integrity after decontamination. Reminder: **Safety equipment must never be treated with chemicals, temperature modifications, or manual treatments that have not been approved by the manufacturer.**
5. Choose gear that can be most effectively decontaminated (keeping in mind what items can be submerged in hot water and what cannot).
6. Bring bags – Isolate (quarantine) all gear not decontaminated on site at the cave/mine entrance, in a sealed plastic bag/s or container/s, to be cleaned and disinfected off-site. Wet bags with disinfectant and seal in an additional bag before placing in vehicle to ensure non-contamination of vehicle.
7. Prepare a strategy (i.e., how/where all equipment and waste materials will be contained, stored, treated and/or discarded after returning to your vehicle/base area) for cleaning and treatment of equipment.
8. Clean and treat all gear between each separate cave, mine or other site. If you must visit known *P.d.* contaminated sites or sites likely to be contaminated with *P. d.*, visit these sites after you have visited sites for which the presence of *P.d* or WNS is unknown, to further reduce the risk of carrying *P.d* to new locations.
9. **Remember, under no circumstances should any gear that was used in a WNS-affected province or county be used in a WNS-unaffected province or county.**

Flow Chart to Determine Gear Use or Decontamination



V. AFTER EACH CAVE/MINE VISIT:

ON SITE:

1. Decontamination is most successful when mud and sediment are removed first (Shelley et al. 2013). Do this immediately upon emerging from cave/mine (e.g. with a bristle brush), and before equipment is sealed for transport and further decontamination.
2. All used, disposable gloves, Tyvek® coveralls and paper towels are discarded in a plastic garbage bag which is then sealed and sprayed with disinfectant and returned for disposal. Ideally, these bags are incinerated or buried in a landfill.
3. Isolate (quarantine) all gear not decontaminated on site at the cave/mine entrance, in a sealed plastic bag/s or container/s, to be cleaned and disinfected off-site. Wet bags with disinfectant and seal in an additional bag before placing in vehicle to ensure non-contamination of vehicle. ALWAYS clean and disinfect the outside surfaces of containers and bags prior to putting them in vehicles, labs, or storage areas.

OFF SITE:

1. **Clean** submersible and non-submersible equipment according to approved manufacturer's specifications. Laboratory trials (Shelley et al. 2013) demonstrate that the use of conventional cleansers like Woolite® detergent and Dawn® dish soap aid in the removal of dirt and organic debris prior to treatment with a disinfectant contributes to the overall effectiveness of the disinfection. Once cleaned, rinse gear thoroughly in water. Clean/treat gear used in a suspect or confirmed province prior to transport when traveling back to or through a province **without** known cases of *P.d./WNS*.
2. **Disinfect** submersible or non-submersible equipment with one of the appropriate treatments listed in Section IV.
 - A. **Submersible Equipment** (i.e., clothing, footwear, and/or equipment that can be submerged in liquid):

The preferred treatment for all submersible equipment is complete submersion in water that maintains a temperature of at least 50°C (122°F) for a minimum of 20 minutes.

Some submersible gear (depending on material) may be soaked for a minimum of 10 minutes in the appropriate products listed in Section IV, rinsed thoroughly with water, and air dried overnight.

Note: Although commercially available washing machines with sanitation cycles often sustain desired water temperatures; their efficacy for killing *P.d.* conidia is untested.

- B. **Non-submersible Equipment** (i.e., electronics, and/or gear that may be damaged by liquid submersion):

Clean and disinfect all non-submersible equipment according to the manufacturer's recommendations. Rinse with damp paper towels and allow to air dry, or dry smooth surfaces with paper towels. Equipment that is not approved for disinfection should be dedicated to individual sites.

- i. **Cameras/Electronics**

- Place camera and flash inside a clean plastic bag (e.g. resealable freezer bags), or a plastic underwater camera housing. Carry camera into cave in this bag. Wipe down surface of bag with quaternary ammonium solution before opening and removing camera. Handling camera with clean gloves; use camera; put it back in the bag for transport out of cave. Change gloves to clean ones after re-bagging camera. After emerging from hibernaculum, remove camera from bag on site using clean gloves, and discard into plastic garbage bag containing other contaminated disposables. If camera is enclosed in a re-usable case, remove camera from case on exiting hibernaculum and place case in plastic bag for later decontamination according to manufactory instructions. Wipe camera down with appropriate quaternary ammonium solution as described above and place in another clean case or disposable bag.

3. Reduce the risk of vehicle contamination and transport of *P.d.* to new areas by making sure to:
 - A. Transport gear in clean containers.
 - B. Remove outer clothing/footwear and isolate in a sealed plastic bag or container prior to entering a vehicle. Storage container options vary considerably depending on the type of vehicle; but **always clean and disinfect the outside surfaces of storage containers prior to putting them in the vehicle.**
 - C. Remain outside of the vehicle after exiting a cave/mine or completing field work.
 - D. Change into clean clothing and footwear prior to entering the vehicle.
 - E. Clean dirt and debris from the outside of vehicles (especially wheels/undercarriage).

VI. EQUIPMENT- SPECIFIC RECOMMENDATIONS:

A. Clothing and Footwear

- i. Wear disposable, Tyvek[®] coveralls for each site visit. Do not reuse suits as they are easily ripped when crawling through small spaces in caves/mines. Discard in a sealed, plastic garbage bag and spray with disinfectant and return for disposal.
- ii. Use one pair of clean boots per person per site, preferably rubber boots, which has a surface that is easy to clean. Put on clean boots in vehicle. Before leaving site remove boots and place in garbage bags for later decontamination. Do not wear those boots again at the potentially contaminated site, and put on (different) clean boots in the vehicle.
- iii. Wear gloves at all times inside hibernaculum, and while handling potentially contaminated gear outside. Carry an ample supply of clean gloves of appropriate sizes, in the box if convenient, inside a plastic bag. After handling a bat, remove and discard gloves in a plastic garbage bag. Put on a new uncontaminated pair of gloves before handling the next bat, or equipment such as a camera. **Degloving:** with gloved fingers, grasp exterior of glove near wrist (without touching skin) and pull off, inverting glove so that contaminated exterior ends up on the interior of glove once removed. Remove second glove by slipping fingers inside the wrist and inverting the contaminated external surface as you pull the glove off. **Regloving:** handling the exterior of the clean glove near the wrist, using bare fingers, pull on first glove. Repeat using gloved hand to pull on second glove. Pull gloves up over Tyvek[®] suit at wrists.
- iv. Clean and decontaminate all other clothing via the most appropriate method previously mentioned (e.g. hot water for 20 minutes).

VII. DECONTAMINATING OTHER GEAR, AND FURTHER INFORMATION:

- Please consult the U.S. Fish and Wildlife Service website at:
<http://www.fws.gov/whitenosesyndrome/>
- For more information about White-nose Syndrome in Canada:
http://www.ccwhc.ca/white_nose_syndrome.php

- Other informative websites regarding White-nose Syndrome:
<http://www.whitenosesyndrome.org/>
<http://batcon.org/index.php/what-we-do/white-nose-syndrome.html>