BOG TURTLE HEALTH BULLETIN
For the Northern Population Range
(Revised October 26, 2018)

Over the past several years, the U.S. Fish and Wildlife Service (Service) has received multiple reports of dead and apparently diseased bog turtles from Delaware, Maryland, Massachusetts, New Jersey, New York, and Pennsylvania. Most notably, in 2014, 14 bog turtles were found dead at one site in early May in Pennsylvania and 4 were found dead at one site in New Jersey between mid-April and mid-June. In August 2009 and May 2013, the Service issued an Advisory Bulletin for all surveyors, researchers, and agency biologists to follow should dead, dying, or injured bog turtles be found. Since that time, this Bog Turtle Health Bulletin has provided updated information and guidance to states for collecting and processing animals should any dead or sick turtles be found at bog turtle sites, including other turtle species, and for decontamination of clothing and equipment when working at bog turtle sites.

Pursuant to the “Emergency Provisions” of Section 6 Cooperative Agreements, the Service, with the state wildlife agencies in the northern range of the bog turtle, permits that "Any employee or agent of the Division who is designated by that Agency for such purposes, may, when acting in the course of his official duties, take federally listed Endangered and Threatened fish or wildlife species without a permit if such action is necessary to: (1) aid a sick, injured, or orphaned specimen; or (2) dispose of a dead specimen; or (3) salvage a dead specimen which may be useful for scientific study. . . ." Guidelines for taking such actions, data forms for documentation (Attachment A), and decontamination protocols (Attachment B) to reduce the risk of disease spread are provided below to support agencies and their agents for addressing these emergency situations.

Reports and collections of sick and dead animals since 2009 have included a variety of clinical signs and results. In addition to empty (no soft tissue remaining) shells, some dead bog turtles have been found entirely intact, with no obvious cause of death. Others have been incomplete specimens due to predation, scavenging, and/or decay. On several live bog turtles, a grayish or whitish substance and/or discoloration has been documented on the skin of the head, neck, and limbs, as well as on the claws. In some cases, these appear as skin lesions, scute sloughing, and/or loss of claws, toes, or limbs.

At this time, no causative agent(s) of many observed morbidities or mortalities has been identified. The vast majority of test results are inconclusive due to predation or decay, but some results have shown a variety of potential causative factors including injury, infection, pneumonia, and carcinoma. Positive detections of pathogens such as *Herpesviruses*, *Mycoplasma*, and *Ranavirus* have been documented in bog turtles in all northern states, but to date, there is no correlation with death of individuals. Despite this, the Service takes this matter seriously due to the ever increasing threat of disease and fungal pathogens on wildlife and is seeking the voluntary cooperation of bog turtle surveyors and researchers in implementing the following guidance regarding (1) fresh-dead, (2) carcasses and shells, and (3) sick or injured turtles.
1. *Fresh-dead bog turtle specimens*¹ (including road-killed turtles) should be collected and sent immediately to one of two locations: the **Wildlife Health Center at the Wildlife Conservation Society** (WCS)/Bronx Zoo in Bronx, NY, for those states actively engaged in health assessment studies with WCS (DE, MA, NJ, NY, PA) OR the **USGS National Wildlife Health Center** (NWHC) in Madison, WI. Also, ship fresh-dead *turtles of any species* from known bog turtle sites (and within 1 km of known bog turtle sites) according to the same protocol.

Complete the attached **Turtle Data Sheet** (Attachment A, or a similar data sheet that records the same information) and, IF sending to the NWHC, the **USGS Wildlife Mortality Reporting and Diagnostic Services Request Worksheet**², as well. When the specimen is shipped, please also provide an email notification and attach copies of completed forms to:

1. The Service’s New York Field Office (Attn.: Noelle Rayman-Metcalf, Northern Population Bog Turtle Recovery Coordinator);
2. The Service’s point-of-contact for your state³; and
3. The appropriate state agency point-of-contact³.

**Instructions for sending turtles to the Wildlife Health Center at the WCS/Bronx Zoo:**

a. Wearing disposable gloves, place the remains into a plastic bag or leak-proof container. Place that container into another one. If sending multiple specimens, use separate gloves and submit specimens in separate containers.

b. Label each container with the ID of the animal, person collecting, and contact information for that person. Attach the Turtle Specimen Form.

c. Place the container into a cooler with cold packs (not ice packs) as soon as possible or into a refrigerator (not a freezer). Keep the samples cool.

d. Contact a veterinarian or pathologist at the Wildlife Health Center at the WCS/Bronx Zoo by calling 718-220-7100 (daytime) or 718-220-5095 (nighttime) for further instructions.

e. The body should be transported **within 24 hours** to the Wildlife Health Center at the WCS/Bronx Zoo or sent via FedEx or UPS overnight to:

¹ This refers to fresh-dead turtles whose cause of death is unknown (*e.g.*, do not ship road-killed turtles). Partially scavenged, fresh-dead turtles should be sent in for analysis, because one cannot confidently conclude that predation was the actual cause of death (*i.e.*, the cause of death is unknown).


³ Contact information for Service and state agency biologists is on pages 6 and 7 of this bulletin.
Please write in conspicuous lettering: **For Immediate Delivery; keep cold (not frozen).**

Prior to transporting an animal or body or sending samples via overnight service, contact a veterinarian or pathologist at 718-220-7100 (daytime) or 718-220-5095 (nighttime) and also email kconley@wcs.org, leidlin@wcs.org, or sbartlett@wcs.org.

**Instructions for sending to the USGS NWHC in Madison, WI:**

a. Greater detail regarding collection and shipment of specimens is found in the link under letter “b.” In general, however, you must wear disposable rubber, vinyl, or nitrile gloves, place each animal in its own plastic bag, close, and seal the bag. Cover zipper bag closure with strapping or duct tape after sealing the zipper. Twist non-zipper bags closed, fold over on itself, and secure with package strapping or duct tape.

b. The NWHC prefers to receive fresh, chilled specimens if they can be sent within 24-36 hours of collection or death, as freezing/thawing impedes isolation of some pathogens and causes tissue damage. As a general guideline, if you cannot call or ship within 24-36 hours, immediately freeze the animal(s) and keep frozen during shipment.


d. See Attachment B for Collection, Preservation, Packaging, and Shipping of Amphibians for Diagnostic Examinations instructions.

e. **Please Note:** Fresh-dead specimens should be shipped for 1-day (overnight) delivery on a Monday, Tuesday, or Wednesday to ensure they will arrive during the same work week at the NWHC. If specimens are fresh and need to be shipped on Thursday or Friday, prior arrangements must be made with the NWHC (Field Epidemiologist on-duty: 608-270-2480). Email/fax shipment tracking number to NWHC-epi@usgs.gov.
Unless otherwise specified, the bog turtle remains will be shipped back to the submitter, or to
the agency requested by the submitter.

2. **Carcasses and shells** (*i.e.*, the ones that are **NOT** fresh-dead and have no tissue remaining)
should be collected, labeled, and **shipped to your state wildlife agency**, in accordance with
State Scientific Collector’s Permit conditions. Complete the **Turtle Data Sheet** and provide
copies to:

   a. The Service’s New York Field Office (Noelle Rayman-Metcalf, Northern
      Population Bog Turtle Recovery Coordinator);

   b. The Service point-of-contact for your state³; and

   c. The appropriate state agency point-of-contact³.

3. **Live bog turtles** (and turtles of other species) found during routine field surveys and/or
research activities at (or < 1 km of) bog turtle sites should be carefully examined. Document
any abnormalities⁴ on bog turtles via close-up photographs and complete the attached **Turtle
Data Sheet**. Submit the data sheet and photographs to:

   a. The Service’s New York Field Office (Noelle Rayman-Metcalf, Northern
      Population Bog Turtle Recovery Coordinator);

   b. The Service’s point-of-contact for your state³; and

   c. The appropriate state agency point-of-contact³.

**NOTE:** If there is a telemetry study occurring at the site where the affected bog turtle was
found, we recommend placing a transmitter on the affected turtle(s) and (1) photo-
documenting the affected area(s) of the body every 1 to 4 weeks, and (2) collecting relevant
health data (*e.g.*, body weight⁵, notes regarding any nasal or eye discharges⁶). Similar
information should be collected and reported on other turtle species that appear to be
diseased at known bog turtle sites. Please note site conditions (*e.g.*, whether the site is flooded
and provides limited turtle basking sites).

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⁴ In this case, “abnormalities” refers to those that appear to be related to disease or infection (*e.g.*, swollen feet or
digits, skin discoloration or blisters, nasal or eye discharges). Also note instances of missing toes, whether they are
healed over or not or any other observation worth reporting.

⁵ Weighing animals at least once a week is now recommended. A precipitous decline in weight can indicate
dehydration and lack of eating. A slow decline in weight would suggest a chronic disease process/infection or lack
of eating.

⁶ Gently put pressure with your index finger on the top of the head and with your thumb under the chin to expel
any pus or discharge that may be in the soft palette, evaluate eyelids for puffiness or discharge, and for
abnormalities.
At this time, we are **NOT** recommending that live, affected bog turtles be removed from their wetland habitat unless the state is engaged with a health assessment study with the WCS **OR** has another approved state and federal agency health emergency plan.

If an obviously ill or injured bog turtle is observed in the field, an assessment should be made as to whether the condition of the animal warrants transfer of the animal out of the field for diagnostics and treatment or whether it should remain in the field. If the animal has a radio transmitter, it should remain in the field after taking detailed notes describing the injury/illness (with photographs). State and Service leads should be contacted for consultation with WCS (or other approved veterinarian/organization) regarding follow-up actions. If the animal cannot be tracked, Service and/or state contacts must be reached while in the field for consultation and approval before the animal may be removed from the site.

If the condition warrants, treatment and approvals have been secured, arrangements should be made to transport the animal in an appropriate carrier with water and vegetation from the site of collection to the Wildlife Health Center at the WCS/Bronx Zoo. Veterinarians are available at all times by telephoning 718-220-7100 (daytime) and 718-220-5095 (nighttime). If it is determined not to move the animal, attempts should be made to contact a WCS veterinarian at the same phone numbers to arrange for samples to be collected from the animal. This could be a valuable step in determining whether the population is at risk for an infectious disease.

### 4. Other Considerations

- **Do NOT** euthanize any bog turtles.

- **Do NOT** collect tissue samples from live bog turtles without specific Service or state wildlife agency authorization.

- **Illness or mortality observed among amphibians occurring at bog turtle sites should also be investigated.** Live, sick (preferably), or fresh-dead amphibian specimens should be collected and packaged for diagnostic evaluation by the USGS NWHC following the NWHC’s Collection, Preservation, Packaging, and Shipping of Amphibians for Diagnostic Examinations instructions (Attachment B), please contact the NWHC Field Epidemiology Team – contact info on page 3).

- To reduce the risk of spreading the agent(s) responsible for the observed mortality and disease, see Attachment C: the *Northeast Partners for Amphibian and Reptile Conservation Disinfection of Field Equipment to Minimize Risk of Spread of Chytridiomycosis and Ranavirus* (February 2014). The Service recommends the use of this protocol.

**IN SUMMARY:** Field biologists play a vital role in the early detection of incidents of wildlife mortality and disease. The Service extends its sincere thanks for your cooperation in implementing this guidance.
U.S. Fish and Wildlife Service Contacts

**Bog Turtle Recovery Coordinator**

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Attachment A

Turtle Specimen Data Sheet
Turtle Specimen Data Sheet
(For use in reporting sick or dead turtles)

Observation Date _______ Time _______ Biologist ____________________________
State ____ Co ____ Site ID Name: ________________________________

Turtle Species: Bog / Spotted / Wood / Painted / Snapping / Box / Musk / Redbelly / Map
Condition: (circle one) Sick - injured/ Fresh-dead /Carcass whole-partial / Shell only
Turtle notch code: Marked / Unmarked Notch code __________
PIT Tag No. __________________________ New today? Y / N
Sex: Male / Female / U Age: Annuli ___ Still growing / Worn
Reproductive Condition: NA Gravid Not Gravid Not Sure
Location of turtle in Microhabitat (circle all that apply):
 On tussock / In Rivulet / In deep water / In short vegetation (<1’) / In tall vegetation (>1’)
 Buried in mud / In upland / Other (describe): ________________________________

Behavior (circle all that apply):
 Basking / Feeding / Stationary / Swimming / Mating / Escaping / Nesting / With another turtles
 Other (describe): ________________________________
Location of turtle in Study Area/Property: Describe and attach map with location and GPS coordinates if possible:______________________________
Canopy: ____ Open ____ Part open (%) ____ Closed
Habitat Type (Circle): Marsh / Wet Meadow / Flood plain / Forest / Other: __________
Surroundings (circle all that apply): Ag field / Fallow field / Development / Forest / Road / Other ______
Measurements: Straight-line Carapace Length: _______ mm (Maximum length)
 Straight-line Plastron Length: _______ mm (Maximum length)
Mass: _______ g (to nearest 0.5 g)
Tail Complete? Y N Limbs: All limbs present and complete: Y N
Carapace/plastron: Diagram and describe below any abnormal scutellation, gnaw marks, old injuries, ectoparasites, swelling, discoloration, open wounds, remaining tissue, etc. Draw feet/legs if abnormal.
Description: ________________________________________________________________
Other signs of disease in sick animals (eye/nose/palette discharge, skin discoloration or blisters).
Description: ________________________________________________________________

Follow-up action
Specimen Sent to/by ____________________________ Date: _______
Contacted: State __________________________ FO __________________________ RO __________________________

Additional notes:
Attachment B

National Wildlife Health Center’s Instructions for the Collection, Preservation, Packaging, and Shipping of Amphibians for Diagnostic Examinations
COLLECTION, PRESERVATION, PACKAGING & SHIPPING OF
Amphibians
FOR DIAGNOSTIC EXAMINATIONS

I. PURPOSE: This Standard Operating Procedure (SOP) provides guidelines for selecting, collecting, preserving, packaging and shipping amphibians related to ongoing mortality to optimize diagnostic value.

II. SCOPE: This SOP applies to normal-appearing live, sick and dead amphibians up to mudpuppy size.

III. EQUIPMENT & SUPPLIES
A. Hard-sided plastic cooler
B. Ice packs (not wet ice)
C. Plastic bags (heavy mil), zip-top bags, and/or plastic food-container boxes of varying sizes. Screw cap plastic jars (for fixative samples)
D. NWHC Mortality Reporting and Diagnostic Services Request Worksheet: www.nwhc.usgs.gov/services
E. Labels for containers
F. Dissecting scissors or scalpels
G. 75% ethanol (or greater concentration) – Best is “denatured ethyl alcohol” available with painting supplies or from laboratory suppliers. Isopropyl alcohol will work in a pinch, but is not ideal. –Molecular grade is even better. Or 10% formalin
H. Packing foam, crumpled newspaper, bubble-wrap, etc.
I. Tape: nylon-reinforced tape or wide clear wrapping tape

J. Sharpie pens (fine point and regular) and pencils

IV. BACKGROUND

Amphibians and aquatic animals decompose rapidly after death, which means amphibian carcasses nearly always will have large numbers of decomposition bacteria and fungi throughout their bodies. This rapid decomposition (autolysis) makes determining cause of death difficult. Carrying supplies in your vehicle and using collection and submission techniques outlined in this protocol will help maximize utility of specimens for diagnostic purposes.

Many amphibian die-offs are fleeting. This means the casualties must be collected as soon as they are found. Returning to the casualty site the next day to collect sick amphibians and carcasses is not optimal because of the highly efficient activity of scavengers during the night and rapid autolysis of carcasses. If any other sick/dead amphibians, mollusks, fish, reptiles, birds or mammals are present at the site, record an estimate of species and numbers. Collect other vertebrate species if they are observed sick or dead. Contact the NWHC to discuss whether to attempt to collect and submit any other species (NWHC generally does not accept fish or invertebrates).

V. COLLECTION & PACKAGING METHODS

Each individual should be individually bagged. Individual bags can be placed into a larger bag for each collection site by date. Label them accordingly.

Amphibian specimen can be collected and submitted live to NWHC for diagnostics. This is usually the best method to preserve diagnostic suitability, but the collection and packaging should be done by personnel with general knowledge of herptiles and experience handling live amphibians. Depending on your agency/institution, you may need Animal Care and Use Committee approval or special permits for collection of live animals and should always work in consultation with your agencies wildlife health or veterinary staff.

**please pre-clear a shipment with live amphibians with a NWG epidemiologist BEFORE sending** *Detailed protocols are in section C, but you are responsible for proper approvals.*
A. Collection of live & Sick or apparently healthy amphibians that can be collected and euthanized

Tadpoles, larvae, neonates, and adults: If possible collect, euthanize, and chill the carcasses (do not freeze) and ship within 24 hours. If prompt shipping isn’t feasible, then follow (B) for dead amphibians. Please note that depending on your agency/institution, you may need Animal Care and Use Committee approval for euthanasia and should always work in consultation with your agencies wildlife health or veterinary staff. Below are recommended humane methods, but you are responsible for proper approvals.

To Euthanize

1) If you have MS222 (Tricaine methanesulfonate) available, immerse the amphibian into MS222 at 250-300mg/L concentration. Make sure the MS222 is neutral buffered to pH 7-7.5. This should be done in individual plastic bags and then leave the carcass in the MS222 and chill them. Do not freeze.
   a) An alternative to MS-222 is to use a 20% benzocaine gel such as Orajel. Apply a strip along the ventral skin of the animal and package as above. American Veterinary Medical Association suggests ~2-3 cm strip for African clawed frogs. Use ~5-10cm strip for a large adult mudpuppy to ensure rapid anesthetic action and death. Use only products that are “gels.” Liquid formulations often have high concentrations of alcohol, which is an irritant.

2) Keep carcasses in individual plastic bags with a small volume of whatever euthanasia agent you use, keep on blue ice packs, and ship to the NWHC as soon as possible.

3) See packaging “Freshly dead specimen” under section D. for more details of how to package

B. Dead Amphibians

Examine the carcasses carefully in the field to determine the quality of postmortem condition for testing.

Freshly dead specimens. Freshly dead specimens show no signs of scavenging, no decomposition, no flies laying eggs, no odor, no bloating, eyes that are not sunken, and skin that appears normal with minimal physical damage, skin sloughing or slime/watermold. If there are any fresh carcasses, collect, chill, and ship immediately (details in section D). If they cannot be shipped immediately, follow guidance of “Not freshly dead specimens” below.

Not freshly dead specimens. Carcasses that are not clearly very freshly dead (this will be most cases), but not yet scavenged or decomposing should be preserved with 1/2 of the individuals frozen and 1/2 fixed in preservative.
1) **Fixing**. About half the dead amphibians should be immediately placed into 10% buffered neutral formalin or 70-75% ethanol for histologic examinations. When possible, the freshest carcasses (those with the least amount of decomposition) should be selected for fixation. Prior to immersing the carcass in the fixative, slit open the body cavity along the ventral midline to assure the fixative gets inside the body cavity to reach internal organs. Clean and disinfect scissors or scalpel between individuals by wiping clean and immersing in alcohol for 1 minute, then air drying. Allow specimens to soak in fixative 3-4 days before shipping. For the first 3-4 days of fixation, the volume of fixative to volume of carcasses should be 10:1. Add more fixative if volume drops owing to evaporation. After 3-4 days of fixation, the carcasses may be transferred to a minimal amount of fresh fixative that prevents drying of the specimen and shipped. Allowing the carcasses to fully fix, allows us to avoid cumbersome chemical declarations for shipping.

**Note for large amphibians (greater than 3cm wide)- to efficiently fix be sure to make the incision so that the body cavity is open and the liquid can reach the internal organs. Place the carcass in a gallon zip-top bag and then add the fixative to fully submerge the carcass. Seal the bag, removing most of the air (do this and store in a well-ventilated area), and place on a tray or in a bucket in case of accidental spills. After 3-4 days, carefully pour off liquid, reseal, and the carcass is ready to ship. A small amount of the fixative should remain to keep the specimen from drying out, but there should not be so much that the liquid sloshed in the bottom of the bag.

![Incision: allow fixative to enter the body cavity](image)

2) **Freezing**. About half the carcasses should be promptly frozen. Preferred freezing temperature is -40 degrees C, but any freezing temperature is preferable to a chilled carcass. *Do NOT freeze amphibians in water.* Frozen carcasses can be used for virus cultures, toxicological examinations, and molecular (DNA) tests. If freezing in a commercial freezer with a freeze-thaw cycle, surround with ice packs to keep frozen during the thaw cycle.

Decomposed carcasses. Clearly decomposed carcasses may have some diagnostic usefulness for molecular testing and toxicological analyses. If fresher carcasses are not available, very decomposed carcasses with fluffy
growths of fungus on the skin; maggots in the mouth, vent and body cavity; or those that consist of just skin and bones, should be frozen and saved. Do not send these unless directed with prior NWHC consultation.

C. Packaging and Sending Live and Sick Amphibians

****Pre-clear a shipment with live amphibians with the NWHC BEFORE sending****

1. Eggs. Place eggs in heavy mil plastic bag or plastic container. Equal volumes of air and water should be present in the bag or container to assure adequate oxygen exchange. Do NOT fill bags or containers completely with water. If possible, place plastic bags in a solid container for support and to avoid crushing specimens or puncture of the bag.

2. Tadpoles, Larvae & Neotenes. Same as for eggs. For small amphibians (<2 grams each), multiple live animals may be placed in one container, but avoid mixing species. For larger aquatic larvae and neotenes, one animal per bag or container is recommended. It is important to assure enough air is present in each container; containers that have a large surface area of water to air are preferred; hence, flat food storage-type plastic boxes with lids (available at nearly any grocery store) are preferred to tall narrow plastic bottles.

3. Adult amphibians (terrestrial amphibians). Plastic boxes or bottles with wide lids may be used for mailing. Sick amphibians should be mailed in separate containers. Two or more live adult amphibians of the same species may be placed in one container, but avoid crowding. Note: if an infectious disease is the cause of the casualties, the disease may be transmitted between amphibians in the container if more than one animal is placed in each container. Wet unbleached (brown) paper towels or wet local vegetation should be added to the container to prevent dehydration of the animal; do not use sponges, because many contain chemicals that are toxic to amphibians. Three or more small holes should be made in the lid of each container. Plastic bags are not recommended for terrestrial amphibians. If plastic bags are the only alternative, be sure to allow plenty of air in the bag to avoid suffocation during shipping.

D. Packaging specimens

Each individual should be individually bagged and labeled. Individual specimen bags can then be placed into a larger bag for each collection site by date.
1) Chilled (Found freshly dead or euthanized and never frozen). Place in cooler with plenty of ice packs and wrap the specimen bags in paper towel or something else to insulate slightly and keep from freezing against ice packs.

2) Fixed (preserved). Once specimens have fixed in a large volume of formalin or ethanol for 3-4 days, the preserved samples should be mailed in a minimal amount of preservative that prevents drying. Do not mail large volumes of liquid fixative. It is prohibited by law. Wrap fixed carcasses in paper towel or other absorbent material that is moistened with the fixative inside the primary bag. Double bag the specimen. Include a few ice packs.

3) Frozen. Frozen specimens should be mailed with plenty of ice packs. If possible, ship frozen carcasses in separate coolers than fixed or chilled carcasses. If frozen and fresh dead (or fixed specimens) must be mailed in the same cooler, separate the shipping container into two compartments with styrofoam panels or other insulating material and place most of the ice packs at one end of the container next to the frozen samples.

E. Labels and data

Place labels with each carcass. They can be placed inside the primary plastic bag (within their own plastic bag) or taped to that bag. Paper labels written in pencil are preferred, especially if there is ethanol in any containers. Most ink will dissolve in ethanol or become streaked during freezing and thawing. Each label should have the following information:

- species
- date collected
- location (state/county/town)
- found dead or euthanized and method
- preservation method (chilled, frozen, fixative solution)

Also fill out a NWHC diagnostic request form (attached) with the contents of the cooler, submitter contact, and any other observations about the mortality event.

E. Mailing

2. **Ice.** Ice packs ("blue ice") minimize leaking during shipment and keep the fedex or UPS staff happy.

4. **Packing the shipping container.** Prepare specimen, as described above. Place unfrozen specimen into plastic containers or add other bracing material (crumpled newspaper or packing peanuts) to keep ice packs from crushing the specimen. Include a copy of the diagnostic specimen submission form (placed inside a plastic bag so it does not get wet) taped to inside of lid or on top of all packing material in the cooler.

5. **Shipping.** Overnight courier service should be used (Fedex or UPS is best). Select the ~10am delivery time. Ship Monday-Wednesday, unless there is a freshly dead specimen, then shipping Thursday is acceptable. Do not ship for weekend or holiday delivery. Securely tape the cooler or box and mail to:

   USGS National Wildlife Health Center  
   Necropsy Loading Dock  
   6006 Schroeder Road  
   Madison WI  53711

6. **Notice of Shipping.** You must notify NWHC prior to shipping by email (nwhc-epi@usgs.gov) that includes the overnight tracking number and an electronic copy or scan of the specimen submission form. Alternatively call an NWHC epidemiologist to provide the expected delivery date, tracking # and what is coming (608-270-2480). Use these contacts for shipping notification and to ask any questions about observations of sick or dead wildlife, as well as for help with packaging and shipping.
Attachment C

Northeast Partners for Amphibian and Reptile Conservation’s
Instructions for the Disinfection of Field Equipment to Minimize
Risk of Spread of Chytridiomycosis and Ranavirus
DISINFECTION OF FIELD EQUIPMENT TO MINIMIZE RISK OF SPREAD OF CHYTRIDIOMYCOSIS AND RANAVIRUS

IMPORTANCE OF DISINFECTION
The spread of pathogens is a major threat to amphibians and reptiles worldwide. This is particularly true for Ranavirus (RV) and Batrachochytrium dendrobatidis (Bd) responsible for chytridiomycosis. Humans can transmit diseases from one place to another and from one organism to another in a short amount of time and over distances the organisms cannot traverse. With the increasing spread of pathogens and reports of die-offs among amphibians and select reptiles worldwide, it is imperative that field biologists, researchers, hobbyists, and anyone interested in recreational herpetology-related field activities employ basic disinfecting procedures to prevent the spread of pathogens.

BEFORE LEAVING THE FIELD
Although other chemicals are effective (see table), NEPARC recommends a 3% bleach solution to inactivate Bd and most RV’s. Concentrated bleach is inexpensive and readily available. However, diluted bleach solutions lose their potency if exposed to air, sunlight, or organic material, and should be discarded after 5 days if exposed. To ensure maximum efficacy, prepare only as much solution as you will need for the sampling event.

Suggested equipment:
- Brushes for scrubbing and/or removing mud and vegetation from equipment.
- Hand sanitizers and antiseptic alcohol wipes
- Handheld bottles and/or pump sprayers for applying bleach and water. Bring clean rinse water.
- Gloves for handling animals. These should be disinfected or discarded between animals.
- Plastic bags of different sizes: examining animals in bag minimizes contact.
- Prepare additional sets of equipment if sampling at multiple locations.
- Trash bags.

INSTRUCTIONS FOR LARGE EQUIPMENT
Brush off mud, wash with biodegradable soap. Disinfect with bleach and rinse all exterior surfaces of boats, canoes, vehicles or trailers and their tires that may have come in contact with potentially affected water (e.g. stream or wetland).

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## Disinfection Options for Ranavirus (RV) and Batrachochytrium dendrobatidis (Bd)

Although these chemicals were not developed specifically for RV or Bd, these recommendations represent the minimum concentration and contact time demonstrated as effective.

<table>
<thead>
<tr>
<th></th>
<th>Clorox Bleach&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nolvasan&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Virkon S&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active Ingredient (AI)</strong></td>
<td>Sodium hypochlorite</td>
<td>Chlorhexidine</td>
<td>Potassium peroxymonosulfate</td>
<td>Ethyl alcohol</td>
</tr>
<tr>
<td><strong>Concentration of AI</strong></td>
<td>6.0%</td>
<td>2.0%</td>
<td>20.4%</td>
<td>70.0%</td>
</tr>
<tr>
<td><strong>Relative cost</strong></td>
<td>$4.99/gal</td>
<td>$65.95/gal</td>
<td>$76.50/10 lb or $1.60/gal</td>
<td>$23.45/L or $68.83/gal</td>
</tr>
<tr>
<td><strong>Min. Contact Time RV&lt;sup&gt;b&lt;/sup&gt;/Bd&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td>1 min / 30 sec</td>
<td>1 min / not determined</td>
<td>1 min / 20 sec</td>
<td>1 min&lt;sup&gt;11&lt;/sup&gt; / 20 sec</td>
</tr>
<tr>
<td><strong>Min. Concentration RV&lt;sup&gt;b&lt;/sup&gt;/Bd&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td>3.0% / 1.0%</td>
<td>0.75% / not determined</td>
<td>1.0% / 1.0%</td>
<td>70% / 70%</td>
</tr>
<tr>
<td><strong>Effective dilution ratio for both RV and Bd</strong></td>
<td>1:32 dilution (bleach:water) for 3% solution using 6% concentration of household bleach.</td>
<td>1:127 (Nolvasan&lt;sup&gt;a&lt;/sup&gt;- water) for 0.75% solution (RV only)</td>
<td>1 scoop (1.3 oz) or 1 tablet per gal of water</td>
<td>Effective when applied undiluted (70%)</td>
</tr>
</tbody>
</table>

### Toxicity to Humans
- Vapor may cause severe irritation or damage to eyes and skin.
- Harmful if swallowed.
- May be fatal if inhaled.
- Avoid breathing spray mist.
- Causes irreversible eye damage.
- Harmful if swallowed.
- Harmful if swallowed.
- Hazardous if swallowed.
- Irritating to respiratory system and skin.
- May cause serious eye damage.
- May be fatal if swallowed or inhaled.
- Can damage liver, kidneys and nervous system by repeated or prolonged exposure.
- May be absorbed through skin.
- Repeated or prolonged contact can cause eye irritation or dermatitis<sup>12</sup>.

### Toxicity to Amphibians
- Fatal at high concentrations.
- Safe for short durations<sup>13</sup>.
- Non-toxic<sup>14</sup>.
- May destroy mucus and wax resulting in dehydration and microbial infection<sup>15</sup>.

### Effects on Equipment
- Corrodes metals.
- Will fade colors and break down cloth fibers.
- None reported.
- Safe on fabric.
- May cause pitting on galvanized or soft metal if not rinsed with water.
- May damage rubber and plastics.
- May cause deterioration of glues<sup>13</sup>.

### Special Instructions:
- Remove debris from equipment prior to treatment.<sup>15</sup>
- Wear safety glasses and gloves when handling chemicals.
- Water pH can affect chemicals; all information in this table assumes the use of tap or municipal water.
- Keep out of lakes, streams, or ponds; stand at least 50 m from any natural water source.
- Do not clean equipment or dispose of waste solutions at field sites.
- For disposal, follow local, state, and federal guidelines.

**Bleach**: Inactivated by organic material. Inactivated by sunlight. If in an opaque container, diluted bleach will last 1 month<sup>16</sup>. If exposed to sunlight or air, it will only last 5 days.

**Nolvasan**: Can be inactivated by organic material.<sup>12</sup>
- Store at room temperature in sealed container.<sup>14</sup>
- Dilute concentrate with water of pH 5-7.<sup>18</sup>
- Remains stable for 1 week if dilute with tap water, and for up to 6 weeks if diluted with deionized water.<sup>17</sup>
- Use concentrate within 36 months.<sup>17</sup>
- Toxic to fish.<sup>18</sup>

**Virkon S**: Store at room temperature.<sup>18</sup>
- Keep solution away from extreme cold or heat.
- Shelf life for tablets is 2 years and for powder is 3 years.
- Remains stable for 1 week if diluted with tap water.

**Ethanol**: Highly flammable. Use and store in a well ventilated area. Evaporation may diminish effective concentration.<sup>12</sup><sup>18</sup>

<sup>11</sup>NEPARC Publication 2014-01 page 2 of 4
CITATIONS FOR DISINFECTION OF FIELD EQUIPMENT TO MINIMIZE RISK OF SPREAD OF CHYTRIDIOMYCOSIS AND RANAVIRUS

1. This information has been compiled in part from Miller, D. L., and M. J. Gray. 2009. Southeastern Partners in Amphibian and Reptile Conservation, Disease, Pathogens and Parasites Task Team, Information Sheet #10.


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CITATIONS FOR DISINFECTION OF FIELD EQUIPMENT TO MINIMIZE RISK OF SPREAD OF CHYTRIDIOMYCOSIS AND RANAVIRUS (CONTINUED)

