

**NRT Quick Reference Guide:
Botulinum Toxin (causes botulism)**

For reference, please see "Key References Cited/Used in National Response Team(NRT) Quick Reference Guides (QRGs) for Biotoxins 2011 Revision." QRGs are intended for Federal On-Scene Coordinators (OSCs) and Remedial Program Managers (RPMs).

Agent Characteristics	<p>Agent Classification: Biotoxin Type: Neurotoxic protein produced by <i>Clostridium botulinum</i> and related bacteria Description: Botulinum neurotoxin (BoNT) is a term that refers to 7 distinct types of neurotoxins (A through G), produced by different bacterial strains. These neurotoxins interfere with transmission of nerve impulses to muscles, leading to paralysis. Most BoNT cause botulism in humans, although some primarily affect animals. BoNT type A is the most thoroughly studied, and is the basis of this QRG. BoNT has been weaponized as an inhaled aerosol using dispersion devices for liquid solutions, but BoNT can also exist in forms ranging from crude microbiological cultures to isolated powders which may be white or colorless crystals. Intentional (bioterrorism) release may lead primarily to ingestional or inhalational exposure. Although BoNT is sometimes referred to a biological weapon, it is a protein and is not a living organism! Suitable bacteria must be present for further BoNT production.</p>	
	<p>Biosafety Level: 2-3 (dependent upon form and quantity) CDC Category: A HHS Select Agent: Yes Duration of Illness: 2-8 weeks (paralysis), months/years for severe cases Person-to-Person Transmission: No. However, lab workers have developed symptoms after performing autopsies on exposed animals Treatment: Antitoxin accompanied by supportive care</p>	<p>Molecular Weight: ~150,000 g/mol (purified toxin, varies with toxin type) Persistence/Stability: Dependent on release scenarios. For example, while easily denatured by environmental factors (e.g. heat, desiccation), substantial inactivation of BoNT may take 2 days after aerosolization. Inactivation of BoNT and <i>C. botulinum</i> (if present) by oxidants is related to dose and contact time, so can take minutes to hours. Inactivation by boiling takes a few minutes. Note that normal inactivation processes may be defeated, so active BoNT should be assumed unless otherwise known.</p>
Release Scenarios	<p>CAUTION: REAEROSOLIZATION IS A CONCERN FOR ALL RELEASE SCENARIOS. Aerosol release of BoNT into the environment or adulteration of water, food, and beverages are considered most likely. Air/Aerosolization: Efficacy of aerosolized BoNT is related to atmospheric conditions, the particle size of the aerosol, and purity. Aerosol refers primarily to mists. Reaerosolization via mists can occur when using water for fire fighting. Soil/Surfaces: BoNT may present a surface hazard, particularly to moist surfaces. Water: BoNT is a possible water threat. Deactivation by chlorine can occur but is dependent on chlorine dose, contact time, temperature, pH, and BoNT concentration, so deactivation must not be assumed. Reaction with monochloramine is much slower, and coagulation has limited effectiveness.</p>	
Health Effects	Onset	6 hours - 10 days (typically 12-36 hours) after ingestion. 24 hours - several days after inhalation.
	Signs/Symptoms per Exposure Route	<p>General: Botulism is characterized by paralysis and other neurological symptoms. It does not cause fever. Patients with botulism typically present with difficulty seeing, speaking, and/or swallowing. Botulism may mimic stroke and other paralyzing medical conditions. Symptoms are similar across exposure routes, but onset times and effect levels vary. Inhalation: No natural cases of inhalational botulism have been reported. Weaponization activities were focused on inhalational exposure. Skin: BoNT cannot be absorbed through intact skin; exposure via skin is usually associated with trauma, surgery, or IV drug use. Botulism can naturally occur when <i>C. botulinum</i> enters a deep wound, multiplies, and produces toxin. Ingestion: Botulism can occur naturally or as a result of bioterrorism when a person ingests pre-formed BoNT or <i>C. botulinum</i> bacteria.</p>
Effect Level	<p>Lethal Dose: Based on non-human primate studies, the lethal dose for a 70 kg (154 lb) human is estimated as 0.09-0.15 µg (injection), 0.70-0.90 µg (inhalational), and 70 µg (ingestion). Lethality: For naturally occurring cases, 50-60% die without treatment. Lethality with treatment is 5-10%.</p>	
Personnel Safety	Concerns	Check with the Health & Safety Officer regarding PPE, Medical Surveillance, & Health & Safety Plan (HASP). Level of PPE may vary depending upon the incident & site specific circumstances. The PPE Levels listed are general suggestions only & are appropriate only for BoNT; they may not provide protection for some decon & other chemicals that workers may be exposed to during response/recovery operations. For decon of workers, use warm soapy water, taking care to avoid abrading the skin.
	Medical	<p>Baseline: Annual physical & respiratory function exams. Treatments Available: Antitoxin is available from CDC and/or local health department, but must be administered soon after symptoms are exhibited. Different antitoxins are available depending on BoNT type. Accompany antitoxin with supportive care.</p>
	First Aid	<p>During Incident: Conduct medical monitoring; use PPE as designated by the HASP; record the PPE levels used; monitor for signs/symptoms as listed under Health Effects &, if necessary, ensure medical attention is obtained as soon as possible. Post Incident: Monitor for signs/symptoms &, if necessary, ensure medical attention is provided as soon as possible.</p>
	PPE	<p>Emergency Response to a Suspected BoNT Incident: Possible PPE Levels for emergency responders based on scenario risks from highest level of protection to least: 1) Pressure-demand Self Contained Breathing Apparatus (SCBA) with Level A protective suit, when: a) Event is uncontrolled, b) type(s) of airborne agent(s) is unknown, c) The dissemination method is unknown, d) Dissemination via an aerosol-generating device is still occurring, e) Dissemination via an aerosol-generating device has stopped, but there is no information on the duration of dissemination, or what the exposure concentration may be. 2) Pressure-demand SCBA with Level B protective suit, when: a) The suspected BoNT aerosol is no longer being released, b) Other conditions may present a splash hazard. 3) Full-facepiece respirator with P100 filter or PAPR with HEPA filters, when an aerosol-generating device was not used to create high airborne concentration. 4) Disposable hooded coveralls, gloves, & foot coverings, when dissemination was by a letter, package, or other material that can be bagged, contained, etc. Other Workers: PPE recommendations for workers other than emergency responders must be developed in the HASP for the specific scenario. PPE recommendations will vary by job type (e.g., cleanup, decon, etc.), type of exposure (e.g., airborne or surface/liquid/soil hazard), & any other site hazards (e.g., chemical, physical, etc.).</p>
Field Detection	<p>Various immunoassay field detection tests are commercially available, but are not generally kept in inventory for EPA responders. Performance data is available for some of these field tests, & should be carefully reviewed to ensure applicability to site-specific conditions to avoid tragic misinterpretation of results. Many of these tests have been verified under laboratory conditions, & applicability to field use cannot be assumed.</p>	
Sampling	<p>Concerns: BEFORE OBTAINING SAMPLES: Identify sample transportation requirements; Contact EPA/HQ-EOC (202-564-3850) for Environmental Response Laboratory Network (ERLN) laboratories able to analyze these types of samples; Clearly identify & coordinate with the lab since most labs cannot analyze all types of media (e.g., wipes, swabs, & HEPA vacuum samples); Coordinate with the sample disposal facility for acceptance criteria (i.e., sample decon requirements); Coordinate with investigative units (EPA-CID & FBI) to ensure sample chain-of-custody is maintained between the groups. Note: Detection/analytical equipment & sampling techniques will be highly site-specific & depend on: 1) the characteristics of the agent; 2) the types of contaminated surfaces (e.g., porous v. nonporous); 3) the phases/purposes of sampling (initial ID v. post-decon sampling); 4) the way in which samples are handled so as not to adversely affect BoNT activity; few labs currently have capability to determine BoNT activity, particularly for large numbers of samples & in all types of media; 5) transportation regulations 6) the acceptance criteria of the analytical lab & 7) the sample decon requirements for the waste disposal facilities to be used. See LABORATORY ANALYSIS, below.</p>	

	<p>CAUTION: ONLY MANUFACTURER CERTIFIED HEPA VACUUM EQUIPMENT SHOULD BE USED. A site-specific sampling plan should be reviewed & approved by appropriate Subject Matter Experts &/or through ICS channels. Sampling Location Plans: If release was limited to a single point (e.g. letter or container), start with an area thought to be free of contamination & work in concentric circles towards the initial point of contamination. Be concerned about other contaminated areas due to foot traffic/ventilation systems (elevator buttons, mail, corners of hallways, baseboards, light switches, door knobs, etc). Based on site characteristics & laboratory capacity, sampling plan may be judgmental, probabilistic, or a combination thereof.</p> <p>Consult EPA/HQ-EOC at 202-564-3850 for ERLN laboratory contact information for personnel who can explain/describe the sampling procedure most compatible with their current analytical procedure. Note: While BoNT can be detected long after the protein has been inactivated & might be of forensic interest, the presence of inactive BoNT says little about the potential human health risk in the days following a release. Air: Collect air samples with gel filter or liquid impinger. Refer to the manufacturer's aseptic sampling methods, flow rates, & sampling times. Ensure that the appropriate pump is used for the selected sampling method. Due to possible denaturation on drying, wet collection techniques Water: Since BoNT can persist in water; any potable water source should be sampled. If the potable water is chlorinated, the chlorine needs to be neutralized immediately with a sodium thiosulfate or other neutralizer at the concentration specified by the analytical laboratory being used prior to shipment. As chlorine levels can vary substantially throughout a drinking water system, it is not always appropriate to assume that a sample is chlorinated based solely on a description of the water treatment processes in use. Soil: For the localized areas where soil deposition of BoNT is suspected to have occurred (i.e., aerosol or liquid droplets), a surface soil sample from a depth of less than 1 inch (2.54 cm) should be obtained from non-vegetated area. Surfaces: 1) Wipe & Swab Sampling (for non-porous surfaces): Sterile macrofoam swabs moistened with solution recommended by the ERLN laboratory. Do NOT use dry wipes or swabs. 2) HEPA Vacuum Sampling (for both porous & non-porous surfaces): collect samples in a HEPA sock designed to fit into an inlet nozzle of a HEPA vacuum cleaner. Good for screening & determining the extent & location of contamination in large areas.</p> <p>Sample Packaging & Shipping: The packaging & shipping of samples are subject to strict regulations established by DOT, CDC, USPS, OSHA, & IATA. Contact the sample-receiving laboratory to determine if they have additional packaging, shipping or labeling requirements. Samples should be packaged in an air-tight container & kept at temperatures of 40-50°F (4-10°C). Ensure samples are not placed directly on the ice used for cooling the shipping container.</p>
Laboratory Analysis	<p>CAUTION: Many labs may not be able to perform analysis on all matrices (e.g., wipes & soil). The ERLN will use uniform, compatible sample prep & analytical methods. (See http://www.epa.gov/sam). NOTE: The selected laboratory may use a tiered approach. If so, the initial analysis may only determine if select/particular components of BoNT are present in the sample (e.g., indicating presence or absence). It may take additional time (up to weeks depending on the laboratory) to determine if BoNT is active & still able to cause adverse effects.</p> <p>Laboratory Information: Contact EPA/HQ-EOC (202-564-3850) for laboratories able to analyze BoNT samples.</p>
Decontamination/Cleanup	<p>CAUTION: ONLY MANUFACTURER CERTIFIED HEPA VACUUM EQUIPMENT SHOULD BE USED. Decon Planning: Site-specific decon/cleanup plan should be developed & approved by all necessary organizations/SMEs via ICS channels. Responders should develop a plan that takes into account: 1) Nature of contamination including purity, physical properties, how it entered the facility, etc.; 2) Extent of contamination, including the amount & possible pathways that have spread the agent. It is advisable to isolate the contaminated area; & 3) Objectives of decon, including decon of critical items for re-use & the treatment, removal, or packaging of other items for disposal. Note: Crisis exemptions from EPA's Office of Pesticide Programs might be necessary depending on decontaminating products used, particularly if disinfection of <i>C. botulinum</i> is intended and the product is not labeled for this use. CAUTION: DECON SOLUTIONS SHOULD NOT BE DEPLOYED AS A SPRAY WHENEVER POSSIBLE. Decon Methods: As BoNT is a protein, information from inactivation of other protein toxins (e.g. ricin) may be useful when considering decon options. Include decon methods for bacteria if <i>C. botulinum</i> may be present. Decon decisions will be site & situation specific but due to re-aerosolization concerns, under NO circumstances should a non-HEPA vacuum cleaner or a broom be used. EPA's National Decon Team (800-329-1841) can provide specific decontamination parameters & requirements for using readily available commercial items such as household bleach. For large areas, low-tech cleanup methods most likely won't be used – rather, widespread fumigation would be the most expedient & cost effective method selected. However, site specific fumigant application method is required. For small areas of contamination, discreet area decon methods would typically proceed as follows: allow aerosols to settle & wear protective clothing; gently cover any contaminated areas with paper towel(s) (overlapping each other if necessary) & apply decon starting at the perimeter & wet towards the center of the contaminated area. Ensure sufficient contact time (e.g., 30 minutes) is provided & ensure the paper towel is kept "sopping" wet during this time. Remove the paper towel(s) then wipe up the residual dampness/drops of decon solution until surface is dry. Reapply decon solution to the bare surface & wipe up again with more paper towel(s) then let surface air dry. All contaminated decon materials (e.g., paper towels, etc.) should be labeled & discarded as hazardous waste. Methods used on surfaces: 1) Source reduction steps, including HEPA vacuuming; 2) Liquid decon solutions such as pH-amended bleach (mixture of 1 part household bleach (5.25% to 6.0%) to 1 part white vinegar to 8 parts water is recommended). This product affects surfaces differently in terms of corrosiveness, staining, & residue. The product will be most efficient a) at higher temperatures (i.e., >70°F or 21°C), b) when plain bleach (e.g., no added fragrance) is used to make the pH-amended bleach solution, c) when pH is < 7, d) when presence of other surface contaminants is minimal, & e) when surfaces remain wet with amended bleach solution for at least 30 minutes. pH-amended bleach can be deployed as a liquid. Note: Store-bought bleach does degrade with time – check the expiration date. For hard surfaces including floors (with attention to base boards & molding), walls, & horizontal surfaces of furniture & equipment, a 30-minute contact time is recommended. Smaller items should be removed & treated with decon solution. Soft surfaces can be treated with decon solutions & then removed (e.g. carpeting cut up & double bagged). Thermal Inactivation. By analogy to ricin, BoNT might be inactivated by 24 hours at 80% relative humidity & 200°F (93°C). It is possible that results could be achieved with higher temperatures for shorter periods of time. Because BoNT can denature dessication, dry heat may be more effective. It is important to evenly space all containers undergoing treatment for an even distribution of heat. <i>In water</i>, BoNT is deactivated after 30 min at 80°C or a few minutes of boiling. Fumigation: Can decontaminate facilities in which there is evidence of high levels of contamination, re-aerosolization, or if decontamination of limited access areas is required (e.g. HVAC systems). Fumigant: Chlorine dioxide at 500 ppm with a contact time of 20 minutes, relative humidity of 80%, and temperature of 25°C has been shown to effectively decon ricin on various building materials, so may be effective for BoNT. Verification of Decon: Site and situation specific. Please contact ERT (732-321-6660) and/or NDT (800-329-1841) for further assistance.</p>
Waste Disposal	<p>CAUTION: Hazardous waste transportation & disposal are regulated federally; however more stringent regulations may exist under state authority. These regulations differ from state-to-state. Detailed state regulations can be found at www.envcap.org.</p> <p>Waste Disposal Planning: Waste generated from assessment & cleanup activities should be autoclaved, chemically disinfected, or fumigated & then tested to be sure the agent(s) were inactivated. Waste disposal for agent-contaminated wastes generated from the decontamination & disposal activities will be problematic. Landfills willing to take these wastes may be limited & incineration may be prohibitively expensive or impractical. All waste disposal options should be investigated as early into the response process as possible. Transportation of the agent contaminated wastes from the site to the landfill or incinerator may be problematic as well. First, agreements must be reached between the waste sender & acceptor BEFORE transport, followed by timely public notification of the transport & disposal phases. Transportation of hazardous waste may cross several states and localities, which may exceed federal regulations. Requirements for transporting hazardous materials, & procedure for exemption, are specified in http://www.fmcsa.dot.gov/safety-security/hazmat/complyhmregs.htm#hp. BoNT is shipped as Hazard Zone A. The U.S. EPA has developed a web-based Incident Waste Management Planning & Response Tool which contains guidance related to waste transportation, contact information for potential treatment, disposal facilities & state regulatory offices, packaging guidance to minimize risk to workers, & guidance to minimize the potential for contaminating the treatment or disposal facility. Access to EPA's web-based disposal tool requires pre-registration (http://www2.ergweb.com/bdrtool/login.asp).</p>