

## Botulinum neurotoxin (BoNT)

*Botulinum neurotoxin (BoNT) is a paralytic neurotoxin produced by certain strains of Clostridium botulinum as well as a few other Clostridial species. It is a large molecular weight molecule (150,000 kDa). Certain types of BoNT are the most potent toxins known, with LD<sub>50</sub>s in the nanogram quantity range. Depending on the type of exposure, development of life-threatening paralytic symptoms can develop within hours or days of exposure. However, symptom development in under an hour could be possible with parenteral exposure to concentrated toxin. Potential exposure routes in a laboratory environment include cuts or punctures through the skin but the BoNTs are known to be toxic through oral and aerosol routes.*

### General Safety considerations:

- Exposure to BoNT can be potentially fatal! Upon any exposure to an amount of BoNT that could potentially lead to rapidly dangerous symptoms, call 911 immediately! After exposure to BoNT, the PI and supervisor MUST notify the UW-Madison Office of Biological Safety (OBS), UW-Madison Select Agent staff, and UW-Madison Occupational Medicine staff. A First Report of Exposure/Release MUST be submitted within 24 hours of an exposure to BoNT.
- There are no known fatal laboratory exposures to BoNT. Although extremely potent, the toxin can be safely handled with minimal risk if all of the handling procedures outlined in the laboratory's Biosafety Protocol are stringently followed.
- Exposure routes: Inhalation, ingestion, and parenteral (through the skin).
- Parenteral (through the skin) is the most dangerous type of laboratory exposure to BoNT. For instance, the oral LD<sub>50</sub> for the most potent subtypes of BoNTs for humans is 5.7 ug/kg, but it only takes about 1/10 of that amount (0.6 ug/kg) for an exposure through the skin (injected).
- Botulinum antitoxin may be available from CDC after a significant exposure.
- In addition to being a neurotoxin, BoNT is classified by OSHA as a highly toxic chemical.
- BoNT is a sodium channel blocker that can prevent the normal function of muscle neurons and lead to subsequent paralysis. Depending on the type of exposure, this paralysis can be localized to specific muscles, or can be systemic (throughout the body), and life-threatening due to paralysis of muscles involved with breathing. The toxin can also lead to irritation of exposed skin and eyes.
- BoNT is generally acquired by laboratories in powdered form. Dry forms of BoNT are **always** handled in a chemical fume hood or ducted certified biological safety cabinet. **Be extremely careful** when handling any amount of BoNT in dry form. Only order as much as you need to use in the immediate future. Resuspend the entire vial at once rather than weighing out aliquots of dry toxin.
- BoNT is sensitive to heat and many chemical methods of inactivation. As a large protein toxin, it is readily inactivated by autoclaving or treatment with 10% bleach solution (0.5% sodium hypochlorite) for at least 30 minutes.

## Special considerations

- BoNT is produced by the *Clostridium botulinum* and certain other strains of toxigenic Clostridia. Under specific conditions, exposure to spores of these BoNT-producing Clostridia in a deep wound could lead to BoNT production and subsequent absorption of the toxin within the anaerobic wound. This is referred to as “wound botulism” and is a significant safety consideration for laboratories handling these strains of bacteria.
- Toxicity varies widely between BoNT subtypes and also with levels of purity. When handling any type of BoNT, it is best to assume that the toxin you are handling is the most potent type.

## Biosafety Protocol

*At UW-Madison, research laboratories that utilize purified BoNT in their research programs must list information about use of the toxin in their Bio-ARROW protocol. The UW-Madison Institutional Biosafety Committee will review all protocols that include use of BoNT, and review will be in the closed session portion of the meeting because of potential biosecurity issues involving its status as a Select Agent Toxin. Following is information about adding BoNT to specific sections of the Bio-ARROW protocol:*

### Select Agents

- The Federal Select Agent Program does not regulate certain Select Agent (SA) toxins if the amount under the control of a PI does not exceed, at any time, the amounts indicated on their website (<https://www.selectagents.gov/permisibletoxinamounts.html>). UW-Madison requires that PIs maintain an accurate inventory of SA toxins and secure the toxins in their laboratory. Inventory reports are submitted semi-annually (every 6 months) to the Office of Biological Safety ([biosafety@fpm.wisc.edu](mailto:biosafety@fpm.wisc.edu)).
- The permissible amount of BoNTs, all types, that an investigator can possess in their laboratories without registering as a Select Agent laboratory, is 1 mg. This amount includes the total of all types of BoNT under the control of that investigator.
- Select Agent Principal Investigator (PI):
  - If the PI of the protocol is a Select Agent registered person, please contact the UW-Madison Select Agent Program for guidance about listing BoNT on the protocol.
  - If the PI of the protocol is not a Select Agent registered person, select “No”.

### Recombinant Materials

- Experiments involving genes coding for fully toxic BoNTs are considered a Major Action under Section III-B of NIH Recombinant DNA Guidelines. These experiments require prior approval by NIH.
- Experiments involving genes coding for smaller portions of BoNTs, such as heavy chain or light chain, aren’t considered a Major Action by NIH. However, these experiments must be reviewed and approved by the UW-Madison IBC.

### Microbes

- Microbes treated with BoNT are handled at BSL2 or (if applicable) BSL3.

### Biological Toxins: Biological Toxins Details

- Select Agent: Yes
- Botulinum Toxin: Yes
- Biological Toxin Select Agent Inventory: Please indicate that toxin is kept in a secured fridge or freezer, a current inventory is maintained, and semi-annual (every 6 months) inventory reports are submitted to the Office of Biological Safety at [biosafety@fpm.wisc.edu](mailto:biosafety@fpm.wisc.edu).
- Amount: Estimated maximum total amount of BoNT you will have in your laboratory.
- Form: Enter all forms handled (dry/lyophilized, aqueous, etc.) and note if the dry/lyophilized form is only handled for reconstituting.
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- LD<sub>50</sub>: 0.6 ug/kg parenteral, 5.7 ug/kg oral.
- Biosafety Level: BSL2
- Storage only: Select “yes” only if all of the BoNT in the laboratory is currently in storage and is not in active use in your research program.

### Cells, Organs, Tissues, or Biological Specimens

- Cells and tissues are treated with BoNT in a biological safety cabinet at BSL2. Requests to handle any amount of BoNT outside of containment must be reviewed by the Office of Biological Safety and approved by the IBC.
- Treated cells or tissues are subsequently handled at BSL2.

### Vertebrate Animals

- ABSL2 housing is adequate for most animal studies involving BoNT. OBS staff will work with labs to determine the required biosafety level for these animals, and whether administration of BoNT to animals must occur in a BSC.
- Note that because BoNT is a very large protein toxin, rodents treated parenterally (IV, IP, subcutaneous, IM) with BoNT will not excrete BoNT in their urine. Thus, bedding for injected rodents should not contain BoNT. However, animals treated orally with BoNT potentially may excrete the toxin in their feces, and thus it might be present in the bedding.

### Containment

- Aerosol Generating Activities: Unless handling of very small amounts of BoNT are approved by the IBC, the toxin must be handled within a Biological Safety Cabinet (BSC).
- Aerosol Generating Activities: Cage changes for animals treated parenterally with BoNT does not require a BSC unless needed for a separate reason (also infected with a pathogen, for instance).

- Aerosol Generating Activities: Cage changes for animals treated orally with BoNT must occur in a BSC.

#### PPE

- At minimum, lab coat, eye protection, and disposable gloves must be worn when handling BoNT or BoNT-treated materials.
- Depending on the research activities being performed with BoNT, a fit-tested respirator (N95, etc.) may also be required.

#### Disinfection and Inactivation - Animal

- Because BoNT is a very large protein toxin, rodents treated parenterally (IV, IP, subcutaneous, IM) with BoNT will not excrete BoNT in their urine. Thus, bedding for injected rodents should not contain BoNT. However, animals treated orally with BoNT potentially may excrete the toxin in their feces, and thus it might be present in the bedding. If bedding may contain BoNT, then bedding should be autoclaved prior to disposal.
- Carcasses of animals injected with BoNT should not need to be autoclaved prior to pick-up for incineration unless they need to be autoclaved for a different reason (also infected with a Risk Group 3 pathogen, for instance.).

#### Disinfection and Inactivation - General: Biotoxins

- BoNT is inactivated by autoclaving or by treatment with 10% aqueous bleach solution for 30 minutes.
- Because of the potential aerosol risk, spills of BoNT must include evacuation of the lab for at least 30 minutes to allow dissipation of aerosols.

#### Spill and Release Procedures:

- Please note that the Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition, recommends the following PPE are worn during a cleanup for a liquid toxin spill: mask, gloves, safety glasses or goggles and laboratory coat.

#### Signage

- A "Toxins in Use" sign must be posted on the laboratory door when BoNT is being handled. The sign can be removed when no toxin is in use.
- Cage cards must specify that animals have been treated with BoNT.

#### Emergency Response

- UW-Medicine Occupational Medicine provides Medical Response plans for biological toxins and infectious agents through a link at <https://ehs.wisc.edu/workplace-safety/occupational-medicine-2/>.
- Emergency Response - General: In the event of exposure to BoNT, immediately wash or flush the affected area with soap and water for 15 minutes. Use an eyewash for 15 minutes after a splash to the eye. After a needlestick, immediately remove gloves and

“bleed out” the wound under running water for 15 minutes. Consult UW-Madison Occupational Medicine or the UW Hospital Emergency Department after any exposure to any amount or type of BoNT. Report exposures as soon as possible to the PI and/or laboratory supervisor. PI or supervisor must submit a First Report of Exposure/Release form as soon as possible, and within 24 hours.

- If concerned about potential rapid effects from exposure to BoNT, call 911.
- Upon adding BoNT to your biosafety protocol, OBS personnel will conduct a risk assessment of the details of the proposed BoNT usage (amount utilized, etc.) and will provide specific handling recommendations, including emergency response.

### Laboratory Training

- Individuals handling BoNT or working in a laboratory where BoNT is being handled must receive training about potential risks from exposure to the toxin as well as safe handling methods as outlined in the laboratory’s biosafety protocol.
- Individuals handling BoNT must receive training about security and inventory requirements for BoNT.
- A separate spill protocol specifically for BoNT may be required.

### Research Description

- Briefly describe what you will be doing with BoNT, including the amount that may be utilized at any one time. Specify the form of BoNT handled (typically aqueous).
- Specify the location(s) and biosafety level(s) for research involving BoNT.
- Staff from the Office of Biological Safety (OBS) will review the potential exposure risks for research activities proposed with BoNT and may require additional PPE or modified handling practices prior to the start of the research.

### **Contacts and Additional Information**

*The sources listed may provide additional information about safe use of BoNT in research laboratories at UW-Madison:*

- Office of Biological Safety (OBS); [biosafety@fpm.wisc.edu](mailto:biosafety@fpm.wisc.edu), 608-263-2037
- Chemical Safety Department; [chemsafety@fpm.wisc.edu](mailto:chemsafety@fpm.wisc.edu), 608-265-5700
- Occupational Medicine; [occmmed@uhs.wisc.edu](mailto:occmmed@uhs.wisc.edu), 608-265-5610
- First Report of Exposure or Release Form; <https://ehs.wisc.edu/first-report-of-biological-exposure-or-release-event/>
- Bio-ARROW KnowledgeBase; <https://kb.wisc.edu/arrow/ibc/page.php?id=43082>
- UW-Madison Select Agent Program; <https://www.selectagents.gov/sat/permissible.htm>; 608-890-4951