

Cholera Toxin (CTX)

Cholera toxin (CTX) is a protein toxin produced by Vibrio cholera. CTX is a large molecular weight (85,000) protein consisting of five subunits.

General Safety considerations:

- There have not been any known fatal lab exposures to cholera toxin. 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: Typically oral.
- After exposure to CTX, the PI and supervisor MUST notify the UW-Madison Office of Biological Safety (OBS) and UW-Madison Occupational Medicine staff. A First Report of Exposure/Release MUST be submitted within 24 hours of any exposure to CTX.
- CTX causes severe diarrhea, which typically leads to dehydration.
- Cholera toxin is generally acquired in powdered form. Dry forms of cholera toxin are **always** handled in a chemical fume hood or ducted certified biological safety cabinet. **Be extremely careful** when handling any amount of cholera toxin 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.
- As a large protein, CTX 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.

Biosafety Protocol

At UW-Madison, research laboratories that utilize purified CTX in their research programs must list information about use of the toxin in their Bio-ARROW protocol. Following is information about adding Cholera Toxin to specific sections of the Bio-ARROW protocol:

Select Agents

- Cholera toxin is not a Select Agent Toxin.

Microbes

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

Biological Toxins: Biological Toxins Details

- Select Agent: No
- Botulinum Toxin: No
- Biological Toxin Select Agent Inventory: Not applicable.
- Amount: Estimated maximum total amount of CTX 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.
- LD50: 10 ug/kg (IV, rodent)
- Biosafety Level: BSL2
- Storage only: Select “yes” only if all of the CTX 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 Cholera Toxin in a biological safety cabinet at BSL2. Requests to handle any amount of Cholera Toxin 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 CTX. OBS staff will work with labs to determine the required biosafety level for these animals and whether administration of CTX to animals must occur in a BSC
- Note that because CTX is a very large protein toxin, rodents treated parenterally (IV, IP, subcutaneous, IM) with CTX should not excrete CTX in their urine. Thus, bedding for injected rodents should not contain CTX. However, animals treated orally with CTX 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 CTX outside of containment is 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 cholera toxin 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 CTX must occur in a BSC.

PPE

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

Disinfection and Inactivation - Animal

- Because cholera toxin is a large protein toxin, rodents treated parenterally (IV, IP, subcutaneous, IM) with cholera toxin should not excrete the toxin in their urine. Thus, bedding for injected rodents should not contain cholera toxin. However, animals treated

orally with cholera toxin potentially may excrete the toxin in their feces, and thus it might be present in the bedding. If bedding may contain cholera toxin, then bedding should be autoclaved prior to disposal.

- Carcasses of animals injected with cholera toxin 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

- CTX is inactivated by steam autoclaving or by treatment with 10% aqueous bleach solution for 30 minutes.
- Because of the potential aerosol risk, spills of CTX 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 is posted on the laboratory door when tetrodotoxin is being handled. The sign can be removed when no toxin is in use.
- Cage cards must specify that animals have been treated with CTX.

Emergency Response

- UW-Madison 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 CTX, 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 ASAP after any exposure to any amount or type of CTX. Report exposures as soon as possible to the PI and/or laboratory supervisor. Submit a First Report of Exposure/Release form as soon as possible, and within 24 hours.
- Note that during relevant emergency response for labs conducting research with the B subunit of CTX (CTX-B), it is important to specify to everyone involved (medical care providers, etc.) that the toxin involved is the less toxic B subunit and not highly toxic cholera toxin.

Laboratory Training

- Individuals handling CTX or working in a laboratory where CTX is being handled need to receive training about potential risks from exposure to the toxin as well as safe handling of the toxin as outlined in the biosafety protocol.
- A separate spill protocol specifically for PTX may be required.

Research Description

- Briefly describe what you will be doing with CTX, including the amount that may be utilized at any one time. Specify the form of CTX handled (typically aqueous).
- Specify the location(s) and biosafety level(s) for research involving PTX.
- Staff from the Office of Biological Safety (OBS) will review the potential exposure risks for research activities proposed with PTX 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 cholera toxin in research laboratories at UW-Madison:

- Office of Biological Safety (OBS); biosafety@fpm.wisc.edu, 608-263-2037
- Chemical Safety Department; chemsafety@fpm.wisc.edu, 608-265-5700
- Occupational Medicine; 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=43188>