

## Staphylococcal Enterotoxin B (SEB)

*Staphylococcal Enterotoxin B (SEB) is an exotoxin produced by Staphylococcus aureus. There are five subtypes of staphylococcal enterotoxins, A-E, but SEB is the one most commonly used in biological research. SEB is a large protein with a molecular weight of 26,000.*

### General Safety considerations:

- Exposure to SEB can lead to a variety of debilitating symptoms, including severe gastroenteritis, within hours of exposure to a high enough dose. The toxin can also cause toxic shock syndrome.
- After exposure to SEB, 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 SEB.
- There are no known fatal laboratory exposures to SEB. Although 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).
- As with most biological toxins, parenteral (through the skin) is the most dangerous type of laboratory exposure to SEB.
- SEB is a superantigen that activates T-cells and leads to a major inflammatory response.
- Symptoms after exposure to SEB can include respiratory illness (cough, difficulty breathing, fluid in lungs) and/or rapid heart rate, headache, GI symptoms (nausea, vomiting), fever, and muscle pain.
- SEB is generally acquired by laboratories in powdered form. Dry forms of SEB are **always** handled in a chemical fume hood or ducted certified biological safety cabinet. **Be extremely careful** when handling any amount of SEB 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.
- SEB 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

- Although the LD<sub>50</sub> for SEB is fairly low (as low as 0.3 ug/kg), it's important to recognize that exposure to significantly lower amounts can cause debilitating symptoms.
- Microbiology labs handling cultures of Staphylococcal aureus, even if not intentionally purifying staphylococcal enterotoxins, need to use caution to avoid exposures to any SE present in the cultures.

### Biosafety Protocol

*At UW-Madison, research laboratories that utilize purified SEB in their research programs must list information about use of the toxin in their Bio-ARROW protocol. Following is information about adding SEB 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/permisstabletoxinamounts.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 Staphylococcal enterotoxins, all types, that an investigator can possess in their laboratories without registering as a Select Agent laboratory, is 100 mg.
- 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 SEB on the protocol.
  - If the PI of the protocol is not a Select Agent registered person, select “No”.
- 

### Microbes

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

### Biological Toxins: Biological Toxins Details

- Select Agent: Yes
- Botulinum Toxin: No
- 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 SEB 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.
- LD<sub>50</sub>: 0.3 ug/kg oral, 5.7 ug/kg inhalation.
- Biosafety Level: BSL2
- Storage only: Select “yes” only if all of the SEB 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 administered SEB in containment in a biological safety cabinet at BSL2. Requests to handle SEB 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 SEB. OBS staff will work with labs to determine the required biosafety level for these animals, and whether administration of SEB to animals must occur in a BSC.

### Containment

- Aerosol Generating Activities: Unless handling of very small amounts of SEB 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 SEB 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 SEB must occur in a BSC.

### PPE

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

### Disinfection and Inactivation - Animal

- Because SEB is a large protein toxin, rodents treated parenterally (IV, IP, subcutaneous, IM) with SEB should not excrete the toxin in their urine. Thus, bedding for injected rodents should not contain SEB. However, animals treated orally with SEB potentially may excrete the toxin in their feces, and thus it might be present in the bedding. If bedding may contain SEB, then bedding should be autoclaved prior to disposal.
- Carcasses of animals injected with SEB 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

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

### 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 SEB, 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 SEB. 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.

### Laboratory Training

- Individuals handling SEB or working in a laboratory where SEB 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 SEB must receive training about security and inventory requirements for SEB.
- A separate spill protocol specifically for SEB may be required.

### Research Description

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