Lipopolysaccharide (LPS)

Lipopolysaccharide (LPS), also known as endotoxin, is a structural component of the cell wall of gram-negative bacteria. It is a large protein ranging from 50-100 kDaltons in size.

General Safety considerations:

- LPS can cause a strong immune response, particularly in the respiratory tract after aerosol exposure. This immune response may lead to inflammation, the progression of asthma, and may worsen other forms of airway disease.
- Exposure routes: Respiratory and parenteral (through the skin)
- LPS can cause inflammation of the respiratory tract in susceptible individuals after aerosol exposure. Because most individuals handling LPS don't know if they are susceptible to such an inflammatory response, avoid aerosolization of the toxin to minimize the likelihood of exposure to themselves or others.
- LPS is generally acquired in powdered form. Dry forms of LPS are always handled in a chemical fume hood or ducted certified biological safety cabinet
- LPS is considered a pyrogen, which is substance that causes fever when introduced or released into the blood. Thus, exposures to LPS through the skin are also a concern in addition to aerosol exposures. Use caution when handling LPS with sharps and other materials that may cut the skin such as glass. Disposable gloves should always be worn when handling LPS.

Special considerations

- Although the LD_{50} for LPS is relatively high, it's important to recognize that exposure to significantly lower amounts can cause lung inflammation in susceptible persons.

Biosafety Protocol

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

<u>Select Agents</u>

- LPS is not a Select Agent Toxin.

<u>Microbes</u>

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

<u>Biological Toxins: Biological Toxins Details</u>

- Select Agent: No

- Botulinum Toxin: No
- Biological Toxin Select Agent Inventory: Not applicable (or N/A)
- Amount: Estimated maximum amount 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: Approximately 8 mg/kg (IV, mouse)
- LPS is not a Select Agent Toxin.
- Biosafety Level: BSL2
- Storage only: Select "yes" only if all of the LPS 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 LPS in a biological safety cabinet at BSL2. Requests to handle any amount of LPS 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 LPS. OBS staff will work with labs to determine the required biosafety level for these animals and whether administration of LPSto animals must occur in a BSC
- Note that because LPS is a very large protein toxin, rodents treated parenterally (IV, IP, subcutaneous, IM) with LPS should not excrete LPS in their urine. Thus, bedding for injected rodents should not contain LPS. However, animals treated orally with LPS 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 LPS 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 LPS 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 LPS must occur in a BSC.

PPE

- At minimum, lab coat, eye protection, and disposable gloves must be worn when handling LPS or LPS-treated materials.
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Disinfection and Inactivation - Animal

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

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

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 LPS, 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 LPS. 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.

Laboratory Training

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

Research Description

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