# General Use Standard Operating Procedure (SOP)

# Lipopolysaccharide (LPS)

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*Globally Harmonized System Hazard Class: Acute toxicity, Oral (Category 2), H300Examples: Lipopolysaccharide from Escherichia coli, from Salmonella enterica, from Pseudomonas aeruginosa*

**Note**: This SOP is intended to provide general guidance on how to safely work with LPS and only addresses safety issues specific to LPS. Other hazard classes may also apply. Review Safety Data Sheets (SDS) and refer to other general use SOPs relevant to the chemical you are working with. Contact the Principal Investigator/ Laboratory Supervisor or the WSU Chemical Hygiene Officer for questions concerning the applicability of any item listed in this SOP (OEHS: 313-577-1200).

## Hazard Description

Lipopolysaccharide (LPS), also known as endotoxin, is a structural component of the cell wall of gram negative bacteria. It is a substance that is considered a pyrogen (fever-inducing substances when introduced or released into the blood). LPS induces a strong immune response, including inhalation airway inflammation, the progression of asthma, and other forms of airway disease. Humans are the most susceptible species for immune response. LPS stimulation of the inflammation cascade is the cause of sepsis. Some types of LPS are considered reproductive effectors, teratogens, and mutagens. Laboratory exposure risks are primarily from accidental injection, absorption through skin or mucous membranes, inhalation, and ingestion.

## Control of Hazards – General

* If possible, do not work with toxins in solid or powder form. If it is necessary to purchase it in powder or solid form, purchase pre-weighed toxin in the minimum quantity needed to perform work.
* When opening packages of biological toxins shipped to the lab, use a functional and certified chemical fume hood or biosafety cabinet.
* Conduct a hazard assessment to identify proper use and handling techniques, storage, and waste disposal issues specific to the procedures being conducted.
* Conduct literature search and review about LPS, including safe work practices.
* Develop a written laboratory-specific SOP.
* Provide and document lab specific SOP training to personnel working with LPS and any other personnel authorized or required to be in the laboratory during toxin work. This should include but is not limited to reviewing the SDS, training on the health and physical hazards of LPS, symptoms of exposure, appropriate work practices, use of PPE, decontamination, and appropriate disposal.
* Use good microbiological laboratory techniques.
* Personnel should not work with LPS if skin is cut or scratched.
* Do not work alone when working with biological toxins.
* Before starting work, prepare a fresh solution of 10% bleach in water for decontaminating items that come in contact with LPS and for decontaminating work surfaces. Minimum contact time of 30 minutes.
* Designate a specific work area for work with toxins. When toxins are in use, post a warning sign in the area (e.g. WARNING! LPS WORK AREA – TOXIC).
* Verify your experimental set-up and procedure prior to use.
* Line work area with absorbent, plastic-backed bench paper. Change at the end of the procedure or at least daily.
* If supplied in glass ampules, wrap a paper towel around the top of the ampule when snapping the ampule open to protect against cuts. If supplied in septum sealed vials, use a hands-free device to stabilize the vial to avoid an accidental needle stick when resuspending.
* Use in the smallest practical quantities for the work being done.
* Change gloves at least every 2 hours, or immediately after contamination, and wash hands at time of glove change.
* Re-suspend the toxin carefully and slowly, rinsing down the walls of the tube in the process.
* Toxin should be removed from the BSC or fume hood only after the exterior of the closed primary container has been decontaminated with 10% bleach for 30 minutes and placed in a clean secondary container.
* The interior of the BSC or chemical fume hood should be decontaminated periodically, for example, at the end of a series of related experiments or at the end of each day.
* Until thoroughly decontaminated, the fume hood or BSC should be posted to indicate that toxins remain in use, and access should remain restricted.
* Remove and dispose or decontaminate protective clothing and wash hands with soap and water before leaving the work area.

## Engineering/Ventilation Controls

* The preparation of LPS including reconstitution, weighing, and diluting should be performed in a fume hood or biological safety cabinet or glove box. Do not use a laminar flow hood or cabinet (e.g. clean bench) for toxin work.
* In-line HEPA filters are required if vacuum lines are used with LPS.
* If centrifuging materials containing toxin, centrifuge safety cups or sealed rotors must be used and the outside surfaces routinely decontaminated. Open the sealed cups or rotors inside BSC or fume hood.
* Limit the use of glassware (no glass Pasteur pipettes).
* Sharps with engineering controls (i.e. self-sheathing) must be used. Have a sharps container next to the work area for quick and easy disposal.

## Personal Protective Equipment

In addition to proper street clothing (long pants or equivalent that cover legs and ankles, close-toed non-perforated shoes that completely cover the feet), wear the following Personal Protective Equipment (PPE) when performing lab operations/tasks:

* Safety glasses (If splash potential exists, use goggles + face shield instead)
* Lab coat
* Nitrile gloves
* Refer to Section 8 “Exposure controls/personal protection” of SDS or a glove selection guide (e.g. [Ansell Chemical Protection Guide](https://www.ansellguardianpartner.com/chemical/home#hp)) to identify appropriate glove type.

## Special Handling Procedures and Storage Requirements

* Designate toxin storage area in a locked container (freezer, refrigerator, cabinet or other container) in a secure location.
* Store and transport toxin in a minimum of 2 layers of sturdy, leak-proof non-breakable containers (e.g. plastic container with sealing lid as a secondary container).

## Decontamination Procedures

* **LPS is strongly heat resistant and not efficiently inactivated with regular heat sterilization procedures (e.g. standard autoclaving conditions). Chemical inactivation is the preferred method for decontamination.**
* Ensure supplies for inactivation of toxin are readily available at all times.
* Spills must be cleaned immediately by properly protected and trained staff.
* Cover spill with absorbent materials and inactivate by applying **10% bleach solution**, starting at the perimeter and working toward the center. Clean the spill area by applying 10% bleach solution and incubate for 30 minutes, followed by cleaning with soap and water. Collect clean-up materials as hazardous chemical waste.
* For powder spills inside chemical fume hood or biosafety cabinet, cover with absorbent materials that have been wetted with 10% bleach solution, in order to prevent air dispersal. Apply the appropriate chemical inactivating agent starting at the perimeter and working toward the center, allowing prescribed contact time before clean up. Clean the spill area by applying 10% bleach solution and incubate for 30 minutes, followed by cleaning with soap and water. Collect clean-up materials as hazardous waste.
* For powder spills outside of a containment device, personnel should be instructed to leave the laboratory and entrance should be restricted. Contact OEHS for clean-up.

## Waste Disposal

Unused biological toxins, toxin waste and materials contaminated with biological toxins must be collected and disposed of as hazardous chemical waste. Waste may need to be chemically inactivated prior to disposal.

Do not dispose of waste by dumping down a drain or discarding in regular trash containers, unless authorized by OEHS. [Submit requests to OEHS](https://research.wayne.edu/oehs/forms/chem-waste) for waste containers, labels, and waste collection. Also, refer to the [OEHS Hazardous Waste Management web page](http://research.wayne.edu/oehs/hazardous/index.php) and [WSU Chemical Hygiene Plan](http://research.wayne.edu/oehs/pdf/chemical-hygiene-plan.pdf) for more information.

# Spill procedures

## Spills

For hazardous material or releases which have impacted the environment (via the storm drain, soil, or air outside the building) or which cannot be cleaned up by local personnel due to size of spill, hazard level, or hazards are unknown:

* 1. Call WSU Police (313) 577-2222. Available 24 hours a day, 7 days a week.
  2. Evacuate the spill area
  3. Post someone or mark-off the hazardous area with tape and warning signs to keep other people from entering.
  4. Remain in the vicinity until emergency personnel arrive and provide them with information on the chemicals involved.

For additional information regarding spill response procedures, refer to the [WSU Chemical Hygiene Plan](http://research.wayne.edu/oehs/pdf/chemical-hygiene-plan.pdf) and [American Chemical Society (ACS) guide for chemical spill response](https://www.acs.org/content/acs/en/about/governance/committees/chemicalsafety/publications/guide-for-chemical-spill-response.html).

## Small Spills

In the event of a minor spill or release that can be safely cleaned up by local personnel using readily available equipment (e.g., absorbent materials) and appropriate PPE:

* 1. Alert personnel in the immediate area of spill and restrict access.
  2. Review the SDS for the spilled material, or use your knowledge, to assess the hazards and to determine the appropriate level of protection.
     1. **DO NOT** clean up spills requiring respiratory protection. Contact OEHS for help (313-577-1200).
  3. Choose appropriate personal protective equipment (e.g. goggles, face shield, chemical resistant gloves, lab coat or apron).
  4. Protect floor drains, sinks or other potential avenues of environmental release as much as possible. Make a dike around the outside edges of the spill using absorbent materials.
  5. For powder spills inside chemical fume hood or biosafety cabinet, cover with absorbent materials that have been wetted with 10% bleach solution, to prevent air dispersal. Incubate for 30 minutes, then clean the area with soap and water. Collect clean-up materials in a hazardous chemical waste container.
  6. For powder spills outside of a containment device, personnel should be instructed to leave the laboratory and entrance should be restricted. Contact OEHS for clean-up.
  7. For liquid spills: Cover spill with absorbent materials and inactivate by applying 10% bleach solution, starting at the perimeter and working toward the center. Incubate for 30 minutes, then clean the area with soap and water. Collect clean-up materials in a hazardous chemical waste container.
  8. Collect spill cleanup materials using a scoop or other suitable items and place in a plastic bag.
  9. After spilled material is removed, decontaminate surfaces with an appropriate disinfectant. Refer to Safety Data Sheet, product information, or other references to determine appropriate disinfectant.
  10. Place all contaminated materials, including contaminated items such as gloves, in a sealable hazardous waste container.
  11. Label waste container with completed hazardous waste tag (available from OEHS).
  12. Submit online [waste pickup request](https://research.wayne.edu/oehs/hazardous/chemical-waste.php) to OEHS.

# Emergency Procedures

**\*\*If medical attention required, call WSU police (313-577-2222) immediately\*\***

* **Fire Extinguishers** – Refer to section 5 of the SDS for chemical specific firefighting measures. Both ABC dry powder and carbon dioxide extinguishers are appropriate for most fires.
* **Eyewash/Safety Showers** – An ANSI approved eyewash station and safety shower must be accessible and immediately available within 10 seconds travel time for emergency use. Instruct personnel on the locations of eyewashes and safety showers, and how to activate them, prior to an emergency.

1. **Health Threatening Emergencies**
   1. **Fire, explosion, health threatening hazardous material spill or release, compressed gas leak, or valve failure.**
      1. Call WSU Police (313) 577-2222.
      2. Alert people in the vicinity and activate the local alarm systems.
      3. Evacuate the area and go to your Emergency Assembly Point.
      4. Remain nearby to advise emergency responders.
      5. Once personal safety is established, call OEHS at (313) 577-1200.

Note: For compressed gas leaks, shut off gas supply only if this can be done safely, without risk to personnel.

* 1. **Injuries and Exposures:** 
     1. Remove the injured/exposed individual from the area, unless it is unsafe to do so because of the medical condition of the victim or the potential hazard to rescuers.
     2. Call WSU Police (313) 577-2222.
     3. Administer first aid as appropriate.
        1. Eye contact: Promptly flush eyes with copious amounts of water for a prolonged period (at least 15 minutes). Seek medical attention.
        2. Ingestion: Seek medical attention IMMEDIATELY. See first aid section of chemical Safety Data Sheet.
        3. Skin contact: Remove any contaminated clothing. IMMEDIATELY flush contamination from skin using the nearest emergency shower for a minimum of 15 minutes. Seek medical attention.
        4. Inhalation: Get to a source of fresh air. Seek medical attention.
     4. Call (313) 577-1200 to report the exposure to OEHS and complete [Report of Injury](https://risk.wayne.edu/files/rofi.pdf) form.
     5. Bring to the hospital copies of the Safety Data Sheets for all chemicals to which the victim was exposed.

1. **Non-Health Threatening Emergencies**
   1. **Injuries and Exposures**

For injuries and exposures that are not considered serious or a medical emergency, visit:

University Health Center (UHC)

Monday-Friday 7:00 am – 4:30 pm

4201 St. Antoine Blvd, UHC 4K, Detroit, MI 48201

Phone: 313 745-4522

If University Health Center is closed or for serious injuries, visit:

Emergency Department - Detroit Receiving Hospital

4201 St. Antoine St, Detroit, MI 48201

Phone: 313-745-3000

# Minimum Training Requirements

1. **General Training:**

* Online through the [Collaborative Institutional Training Initiative (CITI)](https://about.citiprogram.org/en/homepage/).
  + Laboratory Safety Training (general lab & chemical safety issues)
  + Hazard Communication
* [Fire Safety](https://risk.wayne.edu/fire-safety).

1. **Laboratory Specific Safety Training:**

* [Laboratory-Specific Safety Training checklist](https://research.wayne.edu/oehs/lab-safety/lab-safety-training-checklist.docx)
* Review of SDS for chemicals involved in process/experiment.
* Review of this SOP.
* Review [WSU Hazardous Waste Management](https://research.wayne.edu/oehs/hazardous/chemical-waste) guidelines.
* Other: \_\_\_\_\_\_\_\_\_

# Laboratory Personnel Review

Prior to initiating work, lab personnel using these types of chemicals must complete the table below confirming that they have read and understood the above SOP and the associated hazards.

| **Name** | **Signature** | **Date** |
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