UNIVERSITY OF TORONTO

ENVIRONMENTAL HEALTH & SAFETY

In-Lab Procedures for Biological Waste Handling

Introduction

- **Purpose:** This document describes the approved institutional procedures for collection and handling of waste containing biological material generated in a laboratory or research facility.
- **Scope:** This document does not cover chemical or radiological waste. For guidance on disposal of these materials, please refer to the Environmental Protection Services (EPS) website.
- All laboratories working with biological agents must incorporate these procedures into their standard operating procedures (SOPs) describing work with biological agents.
- **Training Requirements:** Every lab/facility member must be trained on the applicable waste collection and handling procedures and demonstrate proficiency. Training records must be documented and retained for a minimum of five years after the individual's tenure in the laboratory or facility ends.

Key Contacts and Resources

EPS handles hazardous waste collection and management at U of T.

- EPS website
- Additional information on biological waste procedures
- **EPS Manager:** Gordon Petre, 416-978-7000, gord.petre@utoronto.ca
  - Contact for guidance on proper hazardous material disposal procedures.
- **Environmental Protection Technicians (EPTs):** 416-946-3473, eps.hazdisposal@utoronto.ca
  - Contact to schedule hazardous waste pickup, or to request hazardous waste collection containers or chemical waste labels.
- **Online Training** (optional) on hazardous waste management at U of T: EHS803

Important Notes

- **Proper use of Bleach:** Page 4 outlines information on the proper use of bleach. All lab members must be familiar with this information and follow correct dilution procedures.
- **Sharps Waste:** Page 5 outlines sharps waste procedures.
- **Mixed Waste:** The procedures outlined below will need to be modified if biological waste is mixed with chemicals or radioisotopes. Contact the EPS Manager for guidance on handling of mixed waste.
- **Autoclaves used for Waste**: These autoclaves must undergo initial validation and subsequently adhere to routine performance verification testing schedules. Consult your Health and Safety Officer for guidance regarding performance and verification testing.
- Some Faculties/Departments may not have autoclaves suitable for decontamination of risk group 1 (RG1) waste. In this case, contact your Health and Safety Officer for guidance.

Waste Procedures

Risk Group 1 Biological Agents

Liquid

- **Option 1:** Add the appropriate amount of concentrated sodium hypochlorite ("bleach" stock) to the RG1 liquid biological waste to achieve a final sodium hypochlorite concentration of 1% in the total solution. After a contact time of at least 30 minutes, dispose of the treated waste down the drain with a copious amount of water.
- **Option 2:** Autoclave RG1 liquid biological waste at 121°C for at least 20 minutes in a suitable vessel. Allow the liquid to cool, then dispose of the waste down the drain.
- Other decontamination methods of RG1 liquid waste may be used only if validation data is provided and the method of decontamination has been approved on your biosafety permit.

Solid

- Autoclave RG1 solid waste at 121°C in autoclave bags **without** the biohazard symbol for at least 20 minutes. (Bags without the biohazard symbol must be used because bags that bear the international biohazard symbol cannot be disposed of in the regular waste stream even after autoclaving.) Allow waste to cool, then dispose of in regular waste stream.

Risk Group 2 (RG2) Biological Agents

(excluding viral vector particles, agents transmitted via the aerosol route, prions and misfolded proteins)

Liquid

- Add the appropriate amount of concentrated sodium hypochlorite ("bleach" stock) to the RG2 liquid biological waste so that the final concentration of sodium hypochlorite is 1% in the total solution. After a contact time of at least 30 minutes, dispose of down the drain with copious amount of water.
- Other decontamination methods of RG2 liquid waste may be used only if validation data is provided and the method of decontamination has been approved on your biosafety permit.

Solid

- Dispose of RG2 solid waste in the lined yellow biohazardous waste pails provided by EPS.
- If working in the biological safety cabinet (BSC), solid waste should be collected in a small bag within the BSC to minimize disruption of the air curtain by moving arms in and out. When work is complete, the bag should be tied shut, removed from the BSC and disposed of in the lined yellow biohazardous waste pails provided by EPS.

Viral Vector Particles and Other Biological Agents Transmitted by the Aerosol Route

Liquid

- Collect liquid waste in a vessel with volume markings **inside the BSC**.
- When work is completed, add the correct amount of concentrated sodium hypochlorite to bring the final concentration to 1% sodium hypochlorite **inside the BSC.** (If using a different disinfectant, concentration or contact time, this must be approved on your biosafety permit.)
- Close the vessel and allow the liquid waste to sit for a contact time of at least 30 minutes **inside the BSC.** Leave the BSC running during the contact time.
- After the contact time is complete, disinfect the vessel's exterior with 70 % ethanol and dispose of the decontaminated liquid it down the drain with copious amount of water.

Solid – Non-Absorbent

- To disinfect the non-absorbent solid waste (*e.g.,* pipette tips, serological pipettes, plates, dishes, *etc.),* fill a vessel with freshly prepared 1% sodium hypochlorite (or other disinfectant approved on your biosafety permit) and place it in the BSC. The opening of the vessel must be wide enough so that all solid waste can easily be deposited into the solution and the vessel must be large enough to accommodate all non-absorbent solid waste and the disinfectant liquid.
- While working in the BSC, submerge non-absorbent solid waste directly in the disinfectant. For items that have an inner barrel (*e.g.,* pipette tips, serological pipettes), draw up disinfectant into the tip/pipette prior to submerging it so that both the outside and the inside of the tip/pipette undergo decontamination.
- After the last item has been deposited in the disinfectant, close the vessel and disinfect the exterior with 70 % ethanol. Allow the solid waste to sit in the disinfectant **inside the BSC** for a contact time of at least 30 minutes. Leave the BSC running during the contact time.
- After the contact time is complete pour the disinfectant off down the drain with copious amount of water and dispose of the solid waste in the lined yellow biohazardous waste pails provided by EPS.

Solid – Absorbent

- Dispose of absorbent solid waste such as serological pipette wrappers and paper towels in a small waste bag inside the BSC. When the bag is 75% full, remove as much air as possible from the bag while within the BSC prior to tying it closed and disinfect the exterior using 70 % ethanol **inside the BSC**. Dispose of in the lined yellow biohazardous waste pails provided by EPS.

Sharps

- The intentional use of sharps or items that could be accidentally broken to yield sharps (*e.g.,* glass) should be avoided, if possible, when working with viral vectors and other biological agents transmitted by the aerosol route.
- While working in the BSC, draw the disinfectant fully into the needle and syringe, then remove the plunger before submerging both the needle/syringe and the plunger in a separate vessel dedicated to this sharp waste decontamination.
- After the last needle/syringe has been deposited in the disinfectant, close the vessel and disinfect the exterior with 70 % ethanol. Allow the solid waste to sit in the disinfectant inside the BSC for a contact time of at least 30 minutes. Leave the BSC running during the contact time.
- After the contact time is complete, carefully place the needles/syringes in a sharps waste container using forceps, pour the remaining disinfectant in the now empty vessel down the drain with an ample amount of water. Never pick up a sharp with your fingers.

Prions

Liquid

- Add the appropriate amount of concentrated sodium hydroxide to the prion liquid waste so that the final concentration of the sodium hydroxide is 2% in the total solution. After a contact time of 60 minutes, dispose of as chemical waste. Ensure waste containers are labelled to indicate only the disinfectant.

Solid

- Dispose of in red biowaste pails provided by EPS.

Misfolded Proteins that Cause Disease

(*e.g.,* alpha-synuclein, tau protein, amyloid beta)

Liquid

- Add the appropriate amount of a 10% solution of sodium dodecyl sulphate (SDS) to the misfolded protein liquid waste to bring the final concentration to 1% SDS. After a contact time of 60 minutes, dispose of as chemical waste. Ensure waste containers are labelled to indicate only the disinfectant.

Solid

- Discard in red biowaste pails provided by EPS.

Biological Toxins

- Disposal procedures for biological toxin waste vary depending on the type of toxin.
- Waste procedures for a specific toxin should be outlined on your approved biosafety permit.
- Consult the EPS Manager for toxin waste procedures.

Cytotoxic Waste

(*e.g.,* chemotherapeutic drugs)

Liquid

- Small quantities (<5 ml): Discard in red incineration waste pails provided by EPS.
- Large quantities (≥5 ml): Consult the EPS Manager for cytotoxic waste procedures.

Solid

- Discard in red biowaste pails provided by EPS.

Anatomical Waste

- Human tissue must be returned to the facility from which it was obtained.
- Animal carcasses and tissue may be returned to the animal facility for incineration off-site or collected in red biowaste waste pails provided by EPS. Contact your animal facility for carcass bags and procedures.
- For material stored in fixative solution, samples must be separated from the fixative and the fixative disposed of separately as chemical waste.
- Contact the EPS Manager if you have additional questions.

Genetically Modified Organisms (GMOs) and Non-Native Species
(*e.g.,* genetically modified invertebrates, plants or plant products such as germ cells, pollen and spores; non-native invertebrates, plants or plant products)

- Ensure they are rendered non-viable before disposing.
- They must **not** be released into the environment.
- Consult your Health and Safety Officer for waste procedures.

Plant Pathogens and Pests

For Canadian Food Inspection Agency (CFIA) Regulated Plant Pests and any material contaminated with them (*e.g.,* plants, soil, pots, *etc.),* follow risk group 2 biological agent waste procedures outlined above.

Soil and Soil-Related Matter
(*e.g.,* logs/lumber, containers, stone)

- All soil imported from outside Canada and from regulated areas within Canada must be sterilized prior to disposal.
- Refer to the CFIA directive D-95-26 for approved sterilization and disposal methods.

DNA Staining Reagents
(*e.g.,* ethidium bromide, RedSafe, SYBR Safe)

Liquid (*e.g.,* running buffer contaminated with stains)
- Dispose of as liquid chemical waste.
- Do **not** dispose of down drain.

Solid (*e.g.,* gels, pipette tips, *etc.*)
- Dispose in green chemical waste pails provided by EPS.

Information on Bleach

- The term “bleach” is the name of a household cleaner, not a solution of defined chemical composition. Bleach may contain anywhere from 2% to 12% sodium hypochlorite (NaOCl) and may also contain varying amounts of sodium hydroxide (NaOH).
- Sodium hypochlorite is the active ingredient responsible for inactivation of biological material.
- The concentration of sodium hypochlorite in the brand of bleach you have purchased is normally printed on the bottle and if not, then refer to the Safety Data Sheet (SDS). Note that commercial products often list a range of concentrations. For example, Lavo Pro 6 lists its sodium hypochlorite concentration as 5%-8%. When calculating how much bleach to add to your liquid biological waste, use the **lowest concentration** guaranteed on the SDS (5% in the case of Lavo Pro 6).
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- Bleach stocks have an expiry date, normally printed on the bottle. Ensure that the expiration dates are checked regularly. Expired stocks must not be used for decontamination of biological agents and should be discarded as a liquid chemical waste.
- When exposed to light, sodium hypochlorite decomposes.

$$2\ NaOCl(aq) \xrightarrow{light} 2\ NaCl(aq) + O_2(g)$$

When concentrated sodium hypochlorite (bleach stock) is decanted from its light-protected bottle and diluted in water, it is exposed to light and begins to decompose. If you are using diluted sodium hypochlorite as a disinfectant, it is only effective for **24 hours**.

- Commercially available bleach is exempt from WHMIS regulations and often does not show WHMIS pictograms on the bottle's label or indicate the amount of sodium hypochlorite (or sodium hydroxide) on the label.
- If concentrated sodium hypochlorite (bleach stock) is decanted from its original bottle into a secondary container (*e.g.,* for dilution purposes), a WHMIS workplace label must be affixed to the secondary container. The WHMIS workplace label should indicate the final concentration of sodium hypochlorite (not the concentration of "bleach"):

1% Sodium Hypochlorite

Avoid inhalation of vapour or mist.
Avoid contact with skin and eyes.

Refer to Safety Data Sheet

- Bleach is very corrosive. If using a sodium hypochlorite solution of 0.5% or higher, rinse the surface with water after the required contact time.
- Bleach must never be autoclaved as it can cause hazardous chlorine gas to be released.
- When disposing of material treated with bleach down the drain, ensure that you flush with ample water.

Sharps Waste

(*e.g.,* needles, needles with syringes, scalpels, blades, glass transfer pipettes, glass capillary tubes)

- Dispose of all sharps in an approved CSA Z316.6-14-compliant sharps container.
- Uncontaminated sharps and those lightly contaminated with biological agents, toxins, cytotoxic agents and/or hazardous chemicals, may be placed in the same container.
- All liquid must be drained from the needles prior to disposal in sharps containers.
- Sharps contaminated with biological agents transmitted via the aerosol route must be decontaminated inside the BSC prior to being disposal.
- Do not over-fill the container. Close the lid when content reach the fill line.
- EPS will pick up filled sharps containers.
- For further details refer to the Sharps Waste Procedures page.
- Online EHS course: EHS117: Working Safely with Sharps