Appendix IV info:

Our reviewers need to have information that allows them to perform risk assessments on our biosafety permit applications and when permit holders wish to add new biologicals to their permits.

In Appendix IV include all RG2 agents in use (if listed as stored only, do not have to explain in Appendix IV but must notify biosafety office if plans to use are made). Please remember that the reviewers must have enough information for their risk assessments and they do not have access to previous permit applications.

If there is not enough room for all the required information on the Appendix IV form (or on the amendment form), additional pages can be submitted. If working with any viable human pathogens, then Appendix VII must also be filled out.

Give brief (2-3 sentences) overview or goal of research. What are you trying to show? Test?

For each RG2 agent in use (some RG2 agents may be grouped for example cell lines):

1. Describe any potential hazard associated with organism. Is it pathogenic – what about immune-compromised individuals? What procedures are planned (can give list but no need to explain every step of the procedure). Remember to consider the potential of generating aerosols that may spread infectious agents.
2. Will it be used in vitro, or also in vivo?
3. Will you be working with large volumes?
4. Describe risk mitigation strategy which may include:
   a. Any precautions planned i.e. sharps not allowed.
   b. What physical containment level/engineering controls will be used?
   c. Operational requirements, such as: specialized equipment which might include BSC, centrifuge cups with aerosol resistant lids containing O-rings etc.; appropriate PPE; medical surveillance if applicable.
   d. decontamination and disposal methods.

Additional information required for certain biologicals (in addition to the general questions listed above):

Viral vectors:

If working with viral vectors, please include the following information:

1. The back bone of the viral vector
2. The nature of inserts in the viral vector
3. Targets of viral vector
4. Over view of the work
Human tissues, fluids, primary cells:

1. Source of samples: describe the population that the samples are from and any associated risks. Is the population generally healthy? Was the population screened? Is the population known to have pathogens for example are they all positive for HIV?
2. Have the samples been screened? For what pathogens? If screened include certification of screening with permit application.
3. What volume will be handled? Risk increases with large volumes.
4. Training – have all your people working with human samples taken the Blood borne Pathogen course (EHS 603)?
5. Medical surveillance – include any medical clearance certificates or indicate whether appropriate medical surveillance has been obtained.

Toxins:

Fill out section below K-II labelled toxins, include quantity in mg,

In Appendix IV:

1. Briefly describe what you will use the toxin for.
2. Describe deactivation and disposal procedures.

Crispr/Cas9 systems:

Crispr systems are not currently being tracked in our biosafety permits but due to possible regulatory implications we are asking researchers that are or are planning to use Crispr/Cas9 to send us answers to the following questions:

1. Target genes? - Germ lines or somatic cells?
2. Nature of insert
3. Vector?
4. Is the guide RNA and the Crispr together or separate?
5. Is the system dead ended or continuous?

If you are not yet working with Crispr/Cas9 systems, but decide to during the duration of your permit, please send an email to the Biosafety office informing us of the proposed work and answering the above questions.

Note: re listing of HPTA exempt cell lines in Permit Renewals and Permit Amendments (Section K-II in renewals):

You do not need to list all of modified/derivative cell lines if you have the parent cell line on your permit unless you have introduced something that is regulated by CFIA (as an ex. a potential animal pathogen, zoonotic pathogen or part thereof) or has the potential to alter the pathogenicity of the parent cell line in any way (as an ex. introduced a toxin, alter tropism etc.).
Waste Procedures and Information for Bio Labs

Purpose and Introduction
• Note this document does not cover chemical or radioactive waste (see EPS website for more details)
• These procedures should be incorporated into your lab’s standard operating procedures (SOPs) (general tissue culture, viral vector surgery, etc)
• All personnel must be trained on your lab’s waste procedures and show proficiency in your lab’s waste procedures
  o This training must be documented and kept for 5 years after the personnel have left the lab

Key Contacts and Resources
Environmental Protection Services | EPS
• Website: https://ehs.utoronto.ca/our-services/environmental-protection-services/
• Manager: Rob Provost 416-978-7000 | rob.provost@utoronto.ca
  o Contact the manager if you have any questions on hazardous material disposal/waste
• Environmental Protection Technicians (EPTs): 416-946-3473 | hazwaste.ehs@utoronto.ca
  o Contact the EPTs to set up a pickup service or request chemical/biological waste buckets

Training on Hazardous Waste Management at U of T
• A short, optional, online course (EHS803) is available for U of T personnel
• https://ehs.utoronto.ca/our-services/environmental-protection-services/eps-training-presentations/

Pathogen Safety Data Sheets

Bleach Quick Facts
• The active ingredient in bleach is sodium hypochlorite
• Bleach stocks come in different concentrations of sodium hypochlorite (e.g., 3-12%) 
• Lab members MUST know the concentration in their stock to be able to calculate the final dilution of sodium hypochlorite
• Lab SOPs should state the final dilution of sodium hypochlorite required for disinfection NOT the % of bleach (since bleach stocks are variable)
  o EXAMPLE → If your bleach stock is 6% and you need a 1% sodium hypochlorite solution (dilution often used for spills), then add 100 mL of your 6% bleach stock to 500 mL of liquid
• Diluted bleach breaks down very quickly and must be remade fresh every 24 hours

Safety Precautions
• Bleach is very corrosive
  o If using a sodium hypochlorite solution of 0.5% or higher, then rinse the surface after the required contact time
• Bleach must never be autoclaved → it can cause hazardous chlorine gas to be released!
Waste Disposal Guidelines

Note: the procedures outlined below may need to be modified, for example, if biological waste is also contaminated with chemicals or radioisotopes.

<table>
<thead>
<tr>
<th>Waste Procedures for Bio Labs Version 3.1 Revised September 14th, 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk Group 1</strong> Waste <strong>Liquid</strong></td>
</tr>
<tr>
<td>- Pour bleach stock into the solution so that the final concentration is 1% sodium hypochlorite, let sit for 30 minutes, then pour down the sink</td>
</tr>
<tr>
<td>- You can use a different disinfectant and contact time as long as you validate the efficacy of this procedure (it decontaminates your bioagent)</td>
</tr>
<tr>
<td>- <strong>Or</strong> autoclave at 121°C for at least 20 minutes and once cooled dispose by pouring down sink. Do not autoclave bleach</td>
</tr>
<tr>
<td><strong>Solid</strong></td>
</tr>
<tr>
<td>- Autoclave at 121°C in autoclave bags without the biohazard symbol for at least 20 minutes and then dispose in regular garbage or as directed by EPS</td>
</tr>
<tr>
<td>- Note that bags with the international biohazard symbol can NOT be disposed of in the regular garbage. You must use bags without the symbol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Viral Vectors and Aerosolisable Bioagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>- There could be delayed complications with exposure to these agents so they require more stringent waste procedures</td>
</tr>
<tr>
<td>- All contaminated material must be fully decontaminated <strong>inside the biosafety cabinet (BSC)</strong></td>
</tr>
<tr>
<td>- Includes: pipette tips, tubes, flasks, plates, solutions, etc</td>
</tr>
<tr>
<td>- Use the appropriate disinfectant and contact time for your bioagent</td>
</tr>
<tr>
<td>- E.g., sodium hypochlorite, accelerated hydrogen peroxide, etc</td>
</tr>
<tr>
<td><strong>Solid</strong></td>
</tr>
<tr>
<td>- Contact time with the disinfectant needs to be min 30 minutes</td>
</tr>
<tr>
<td>- Then pour off the disinfectant into the sink</td>
</tr>
<tr>
<td>- The remaining solid waste goes into the yellow biohazard bags/buckets supplied by EPS</td>
</tr>
<tr>
<td><strong>Liquid</strong> → follow the same procedures as liquid Risk Group 2 waste</td>
</tr>
<tr>
<td>- Check the PSDS, product sheet, or consult your HSO if you are unsure which disinfectant to use and the contact time</td>
</tr>
</tbody>
</table>
## DNA Staining Reagents
- Examples: ethidium bromide, Redsafe, Sybr safe, etc
- Includes: running buffers, gels, pipette tips, etc
- Dispose of in green chemical waste buckets supplied by EPS
- Do not dispose of liquid waste down the drain!
  - Local municipal by-laws do not allow for disposal down drains

## Toxins and Human Tissues
- Examples: medical waste, cholera toxin, contaminated supplies, etc
- Some biologicals (medical waste) and some toxins (e.g., cholera toxin) require incineration. Use the designated incineration waste buckets supplied by EPS
- Contact the Manager of EPS for approval of waste methods

## Animal Tissues
- Return to the animal facility for incineration
- Contact your animal facility for instructions and waste containers

## Plant Pathogens and Pests
- Plant pathogens/pests listed by the Canadian Food Inspection Agency (CFIA) and any contaminated material such as plants, soil, pots.
- Must dispose as Risk Group 2 waste
- Contact EPS for instructions and waste containers

## Genetically Modified Organisms (GMOs)
- Examples: genetically modified invertebrates, vertebrates, plants, their products (i.e., germ cells-pollen, spores, etc), etc
- Ensure that the GMOs are no longer viable before disposing of them
- There must be NO release into the environment
- Discuss the waste procedure with your Health and Safety Officer
- A risk assessment must be performed by the investigator

## Non-Native Species
- Examples: non-native invertebrates, vertebrates, plants, their products, etc
- They must NOT be released into the environment.
- Discuss the waste procedure with your Health and Safety Officer
- A risk assessment must be performed by the investigator

## Soil
- All untreated soil that is foreign (from any other country) and from regulated areas in Canada must be sterilized prior to disposal
- Discuss your disposal procedures with EPS

## All Other Waste
- Examples: chemical waste, radioisotopes, mixed waste
- Discuss your waste procedures with EPS