Biosafety Practices

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1. Introduction

1.1 Biosafety in science fair projects

These requirements apply to all projects in the CWSF, and regional competitions, which use **microorganisms** (e.g. germs) and/or **toxins**. If your experiment involves any of the following microorganisms and/or toxins, your team must follow the appropriate biosafety practices outlined below and in the CWSF Biosafety policy:

a. **Bacteria** are single-celled, prokaryotic organisms without any internal organelles, such as a nucleus. They are classified according to their morphology (i.e. appearance), Gram-stain reactivity (which is determined by the presence or lack of an outer membrane), and nutritional requirements.[1] Bacteria are very diverse. Some species can harm humans, animals and/or the environment but most have beneficial uses in a variety of applications, for example the production of food (e.g. yogurt, cheese), antibiotics and vaccines, providing protection from harmful infections, and aiding in digestion in the gut.
b. **Viruses** are the smallest microorganisms, consisting of genetic information (DNA or RNA) encased in a protein shell. They are obligate parasites, which rely on a host cell to replicate. Some viruses can cause harm, while others help defend against harmful infections in humans and plants and are used in scientific research to deliver DNA into bacteria (via bacteriophages, a type of virus that infects bacteria) and to study cancer.[1]
c. **Fungi** are single-celled or multicellular, eukaryotic microorganisms with a rigid wall and organelles.[2] They can be microscopic or visible to the naked eye. Fungi more commonly cause harmful infections in plants than humans.[2] They decompose organic matter, are commonly found in soil, fix nitrogen for plants, and are used for the production of food (e.g. bread) and antibiotics.
d. **Protists** include a collection of single-celled organisms that scientists have had difficulty classifying: protozoa; algae; and slime moulds.[3] Protists generally live in moist or aquatic habitats. **Protozoa** are single-celled, free-living, motile eukaryotic microorganisms that use cellular extensions (e.g. flagella, cilia, or pseudopodia) to move around. They sometimes associate into complex colonies but are very diverse in structure and form.[4] Protozoa regulate bacteria and produce nutrients (e.g. nitrogen, phosphorous) for plants in soil and water but can live as infectious parasites in humans.[5]**Algae** are a type of photosynthetic protists and they generate chemical energy from sunlight. They also produce oxygen and serve as a food source to support aquatic life. Human use algae as a food source as well as to produce pharmaceutical products.[6]
e. Microbial **toxins** are poisonous chemicals produced by microorganisms that can cause adverse health effects and damage to humans or animals following exposure. Toxins can be dangerous and have an impact on human health at very low doses.

Microorganisms can be found everywhere. Does your experiment involve the use of an environmental sample that you took from a common surface (e.g. toilet seat, computer keyboard), food (e.g. m microbial fuel cell? If yes, then your project involves the use of microorganisms and you must follow the appropriate biosafety practices outlined in this document and the CWSF Biosafety policy.

2. Biosafety Requirements for Your Project

Students and their mentor(s) are expected to work together to ensure experiments are performed in a safe setting with the appropriate biosafety practices and containment, according to the level of risk of the microorganisms and/or toxins. This document outlines what steps you will need to take, to make sure that your project meets the CWSF biosafety requirements.

2.1 Identify your microorganisms and/or toxins

First, you (with the help of your mentor) must identify the specific microorganisms and/or toxins which will be used in your project. This information will enable you to determine the risk level/risk group, as well as the appropriate biosafety practices and containment to use throughout your project.

Information you should gather on your microorganism that will help with identification, includes its common name and scientific name, its classification (e.g. the kingdom, genus, and species), and the variant (for example, *E. coli* K12 non-pathogenic strain vs. *E. coli* O157:H7 pathogenic strain). Some of this information should be provided by either the laboratory manager, online retailer, or the person or supplier providing the microorganism or toxin for your experiment.

Be careful when purchasing materials online! You cannot buy Risk Group 2 microorganisms unless you have a licence from the Public Health Agency of Canada (PHAC).

Note: Environmental samples may contain a variety of unknown microorganisms and it is not necessary to identify them.

If you are having trouble identifying your microorganism or toxin, ask your mentor or teacher for help.

2.2 Determine the Risk Group

Now that you?ve identified your microorganisms and/or toxins, the next step is to determine the Risk Group of the microorganisms and whether the toxins are listed in Schedule 1 of the *Human Pathogens and Toxins Act* (HPTA). A list of common Risk Group 1 and Risk Group 2 microorganisms is provided below in Table 1. The use of Risk Group 3 and 4 microorganisms and toxins in science fair projects is prohibited.

Environmental samples may contain different microorganisms that have the potential to be either Risk Group 1 or Risk Group 2 and are subject to special biosafety considerations. If you are using an environmental sample which contains unidentified microorganisms, treat it as a Risk Group 1 sample if it will not be cultured or if it is cultured but remains unopened in a secure container. Treat it as a Risk Group 2 sample if it will be cultured and opened at any point during the experiment.

Table 1. Examples of Risk Group 1 and 2 microorganisms

Risk Group 1 ? Do not cause disease in healthy adult humans	
Agrobacterium spp.	Found in plants, vegetation
Bacillus licheniformis	Found in soil, bird feathers
Bacillus subtilis	Found in soil, gastrointestinal tract of ruminants and humans
Bacteriophages	Found in soil, water, plants
Escherichia coli (*E. coli*) DH5 alpha, K-12	Part of normal human flora, found in the laboratory
Geobacter spp.	Found in soil, aquatic environments
Lactobacillus spp.	Found in fermented food products, part of normal human flora
Micrococcus spp.	Found in the environment, especially in water, dust, and soil
Peptococcus spp.	Part of normal human flora
Saccharomyces cerevisiae	Found on fruits, used to make food
Shewanella spp.	Found in marine, aquatic environments
Staphylococcus epidermis	Part of normal human flora
Risk Group 2 ? Can cause disease in humans, but the disease is treatable or preventable	
Aspergillus spp.	Found in decomposing organic matter, soil, food, water
Bacillus cereus	Found in food, feces, soil
Candida albicans	Part of normal human flora

Citrobacter spp.	Found in feces, soil, water, food
Enterococcus faecalis	Found in feces, soil
Escherichia coli (*E. coli*) O157:H7	Found in food, soil, feces
Listeria monocytogenes	Found in food, soil, water, decomposing organic matter
Proteus spp.	Found in soil, water, feces
Pseudomonas aeruginosa	Found in soil, water, part of normal human/animal flora
Salmonella spp.	Found in food, animals, feces
Serretia spp.	Found in food, soil, animals
Staphylococcus aureus	Found in food, part of normal human flora

A more comprehensive list of Risk Groups can be found on the PHAC Pathogen Safety Data Sheets and Risk Assessment [1] site and the ePATHogen Risk Group Database [2]. Other useful resources are also available.[7-9]

Remember you are not permitted to use Risk Group 3 or Risk Group 4 microorganisms for microbiology experiments at the CWSF or regional competitions.

For help determining the Risk Group of your microorganism or toxin, you can contact:

- Your mentor or school
- The CWSF National Ethics & Safety Committee (NESC) [3]

2.3 Determine the appropriate biosafety practices and containment

It is important for you to use proper biosafety practices and containment during your experiment to ensure your own safety and that of the people around you, the community and the environment. This involves working with the appropriate physical safeguards and safety practices.

After identifying the level of risk associated with the microorganisms and/or toxins used in your project, you must determine the appropriate biosafety practices and containment required.

- If there are multiple microorganisms and/or toxins with different Risk Groups used in the same experiment, the work must be performed following the requirements for the highest Risk Group.

2.3.1 Biosafety requirements for experiments involving Risk Group 1 microorganisms or unlisted toxins

When working with known Risk Group 1 microorganisms or toxins not listed in Schedule 1 of the HPTA, handling practices outlined in the Containment Level 1: Physical Design and Operational Practices [4] Guideline are recommended. Although it is not necessary to conduct the work in a laboratory, it is recommended when possible. Other helpful resources are also available.[10-12] The following are examples of proper biosafety practices that should be employed throughout the experiment:

a. Minimally, gloves should be worn at all times when handling any type of biological materials, including unknown environmental and biological samples, microorganisms, and toxins.
b. Hands should be washed with soap and water for 15-20 seconds immediately after removing gloves and before leaving the work area.
c. Long hair should be tied back.
d. Never apply cosmetics, eat or drink, insert or remove contact lenses while performing an experiment.
e. Never pipette by mouth.
f. Work surfaces and equipment should be cleaned and disinfected before and after the handling of biological materials (with, for example, 70% v/v ethanol, 70% rubbing alcohol, bleach diluted 1 part bleach to 9 parts water for 10 minutes[BC3]).
g. Properly dispose of waste material. Sterilize waste either by autoclaving or using freshly prepared 10% bleach (a contact time of 30 minutes is recommended[BC4]) before disposing of in the garbage.

Although it is not necessary to conduct the work in a laboratory, it is recommended when possible.

2.3.2 Biosafety requirements for experiments involving Risk Group 2 microorganisms or listed toxins

If working with Risk Group 2 microorganisms or toxins listed in Schedule 1 of the HPTA, students must work under supervision at all times in a Containment Level 2 laboratory holding a valid licence from the PHAC [5]. For more information on the physical safeguards and biosafety practices required in a Containment Level 2 facility, consult the Canadian Biosafety Standard [6] (also

available as an app [7]) and Canadian Biosafety Handbook [8] or talk with the laboratory manager.

2.3.3 Biosafety requirements for experiments involving environmental or biological samples with unknown microorganisms

Science fair competition projects involving environmental and biological samples could contain a variety of microbes, ranging from harmless, non-pathogenic microorganisms (Risk Group 1) to dangerous pathogenic microorganisms (mainly Risk Group 2).

a. If working with an environmental (e.g. soil, food) or biological sample (e.g. saliva) and the microorganisms are not cultured, work can be performed according to the biosafety requirements for working with Risk Group 1 microorganisms.
b. If microorganisms are to be cultured from an environmental or biological sample, either in liquid or on solid media (e.g. broth or agar plates), students may follow the biosafety requirements for working with Risk Group 1 microorganisms with appropriate supervision if the cultured samples remain unopened (i.e. covers remain on petri dishes or flasks during the entire experiment). However, working in a Containment Level 2 laboratory is recommended, when possible.
c. If microorganisms are to be cultured from an environmental or biological sample and the cultured samples will be opened at any point during the experiment, work must be performed in a Containment Level 2 laboratory holding a valid licence from the PHAC. The student should be supervised at all times.

Did you know... **When cultures are gown with limited air supply, there is a potential for extremely dangerous anaerobic pathogens to grow. To avoid this, tape petri dish covers with only 2-4 pieces of tape to allow air exchange, instead of taping all the way around the rim.** [1]

If you have any questions or concerns about your project, you can contact the CWSF National Ethics & Safety Committee (NESC) [3].

3. Additional Resources

1. Canadian Biosafety Guideline: Biosafety in the Classroom (2018). The Public Health Agency of Canada.
2. Barnett, D., Science ASSIST Team, Australian Science Teachers Association (2017). Guidelines for best practice for microbiology in Australian schools. Science Education Technicians Association. Science ASSIST Project.
3. Hebert, P. D. N., Draggan, S. Protozoa. Encyclopedia of Earth. Biodiversity Institute of Ontario. May 21, 2012. http://eol.org/info/456 [9]
4. Laybourn-Parry, J. E. M., Diaz, J. M. (2018). Protozoan. Encyclopedia Britannica. February 23, 2018. https://www.britannica.com/science/protozoan [10]
5. Diaz, J. M., Laybourn-Parry, J. E. M. (2018). Protozoan: Ecological and industrial importance of protozoans. Encyclopedia Britannica. February 23, 2018. https://www.britannica.com/science/protozoan/Ecological-and-industrial-importance-of-protozoans [11]
6. Anderson, R. A., Lewin, R. A. (2018). Algae. Encyclopedia Britannica. May 10, 2018. https://www.britannica.com/science/algae [12]
7. ABSA Risk Group Database [13] (ABSA International)
8. DSMZ Catalogue of Microorganisms [14] (German Collection of Microorganisms and Cell Cultures)
9. NIH Guidelines ? Appendix B [15] (National Institutes of Health)
10. The Microorganisms Safety Guide [16]
11. Good Microbiological Practices ? Biosafety in the Laboratory [17]

Biosafety Posters (from PHAC) [18]

Links
[1] https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html
[2] http://health.canada.ca/en/epathogen
[3] https://www.youthscience.ca/contact
[4] https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/containment-level-1-physical-design-operational-practices.html#a5
[5] https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/licensing-program.html
[6] https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html#a2.2
[7] https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/cbs-biosafety-app.html
[8] https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition.html
[9] http://eol.org/info/456

[10] https://www.britannica.com/science/protozoan
[11] https://www.britannica.com/science/protozoan/Ecological-and-industrial-importance-of-protozoans
[12] https://www.britannica.com/science/algae
[13] https://my.absa.org/Riskgroups
[14] https://www.dsmz.de/catalogues/catalogue-microorganisms.html
[15] https://osp.od.nih.gov/biotechnology/nih-guidelines/
[16] https://www.sciencebuddies.org/science-fair-projects/references/microorganisms-safety
[17] http://youthscience.ca/sites/default/files/04_biosafety_in_the_laboratory.pdf
[18] https://youthscience.ca/biosafety-posters