

❖ Potentially Hazardous Biological Agents ❖

(includes rules involving microorganisms, rDNA, and human and vertebrate animal tissues)

Projects involving **microorganisms** (including bacteria, viruses, viroids, prions, rickettsia, fungi, and parasites), **recombinant DNA (rDNA) technologies** or **human or animal fresh/frozen tissues, blood, or body fluids** may involve working with potentially hazardous biological agents. Students are permitted to do research projects with potentially hazardous biological agents as long as every effort is made to ensure that they work safely and that the projects meet the conditions and rules described below. The following rules were developed to protect students and to help them adhere to federal and international biosafety regulations and guidelines.

When dealing with potentially hazardous biological agents it is the responsibility of the student and all of the adults involved in a research project to conduct and document a **risk assessment, (Form 6A)** to define the potential level of harm, injury or disease to plants, animals and humans that may occur when working with biological agents. The risk assessment determines a final biosafety level which then determines the laboratory facilities, equipment, training, and supervision required for the research project to proceed. See page 23.

All projects involving microorganisms, recombinant DNA technologies and human or animal fresh/frozen tissues, blood or body fluids must adhere to the rules below AND, depending on the study, to the additional rules in Section A, B or C.

Rules for ALL Studies Involving Potentially Hazardous Biological Agents

- 1) The use of potentially hazardous microorganisms (including bacteria, viruses, viroids, prions, rickettsia, fungi, and parasites), recombinant DNA (rDNA) technologies or human or animal fresh/frozen tissues, blood, or body fluids is allowable under the conditions and rules that follow. All of these areas of research may involve potentially hazardous biological agents and require special precautions.
- 2) An appropriate review and approval committee (SRC, IBC, IACUC) must approve all research before experimentation begins. The initial risk assessment determined by the student researcher and adults supervising the project must be confirmed by the SRC.
- 3) Experimentation involving culturing of potentially hazardous biological agents, even BSL-1 organisms, **is prohibited in a home environment**. However, specimens are allowed to be collected at home as long as they are immediately transported to a laboratory with the appropriate level of biosafety containment.
- 4) **Research determined to be biosafety levels 3 or 4 is prohibited for precollege students.**
- 5) **Laboratory studies utilizing MRSA** (Methicillin resistant *Staphylococcus aureus*) **and VRE** (Vancomycin-resistant enterococci) **are prohibited.**
- 6) **Studies intended to genetically engineer bacteria with multiple antibiotic resistance are prohibited.** Extreme caution should be exercised when selecting out antibiotic resistant organisms. Studies using such organisms require at least BSL-2 containment.
- 7) Naturally-occurring plant pathogens may be studied (not cultured) at home, but may not be introduced into a home/garden environment.
- 8) A risk assessment must be conducted by the student and adult supervisors prior to experimentation and a final biosafety level must be determined or confirmed by the SRC. See p. 23.
- 9) Research determined to be at Biosafety Level 1 (BSL-1) may be conducted in a BSL-1 or higher laboratory. The research must be supervised by a trained Designated Supervisor or a Qualified Scientist. The student must be properly trained in standard microbiological practices.
- 10) Research determined to be a Biosafety Level 2 (BSL-2) **MUST** be conducted in a laboratory rated BSL-2 or above (commonly found in a regulated research institution). The research must be reviewed and approved by the Institutional Biosafety Committee (IBC) or a letter obtained from an institutional representative that the research does not require review. The research must be supervised by a Qualified Scientist. The student researcher must receive extensive training, demonstrate competency and be directly supervised while conducting microbiological procedures.
- 11) All potentially hazardous biological agents must be properly disposed of at the end of experimentation in accordance with their biosafety level. Following are acceptable procedures for disposal of cultured materials: Autoclaving at 121 degrees Celsius for 20 minutes, use of 10% sodium hypochlorite, incineration, alkaline hydrolysis, biosafety pick-up and other manufacturer recommendations.
- 12) Studies involving the culturing of human or animal waste, including sewage sludge, must be treated as a BSL-2 study.
- 13) The following types of studies are exempt from prior SRC review:
 - A. No additional forms required:
 - 1) Studies involving baker's yeast and brewer's yeast, except when involved with rDNA studies
 - 2) Studies involving *Lactobacillus*, *Bacillus thuringensis*, nitrogen-fixing, oil-eating bacteria, slime mold and algae-eating bacteria introduced into their natural environment. (Not exempt if cultured in a petri dish environment that could potentially be contaminated).
 - B. Require completed Risk Assessment Form 3:
 - 1) Studies involving protists, archae and similar microorganisms
 - 2) Research using manure for composting or other non-culturing experiments and fuel production.
 - 3) Commercially-available color change coliform water test kits which will remain sealed and will be properly disposed.
- 14) Any proposed changes in the **Research Plan** by the student after initial SRC approval must have subsequent SRC or IBC review and approval before such changes are made and before experimentation resumes.

- 15) The following forms are required:
- Checklist for Adult Sponsor (1), Student Checklist (1A), Research Plan, and Approval Form (1B)**
 - Regulated Research Institution Form (1C)** - when appl.
 - Qualified Scientist (2)**, when applicable
 - Risk Assessment (3)**, when applicable
 - PHBA Risk Assessment Form (6A)**
 - Human and Vertebrate Animal Tissue Form (6B)** – for all studies involving tissues and body fluids.

A. Additional Rules for Projects Involving Unknown Microorganisms

Studies involving unknown microorganisms present a challenge because the presence, concentration and pathogenicity of possible agents are unknown. In science fair projects these studies typically involve the collection and culturing of microorganisms from the environment (e.g. soil, household surfaces, skin, etc.)

- Research with unknown microorganisms can be treated as a BSL-1 study under the following conditions:
 - Organism **is cultured** in a plastic Petri dish (or other standard non-breakable container) **and sealed**. Other acceptable containment include doubled heavy-duty (2-ply) sealed bags.
 - Experiment involves only procedures in which the Petri dish remains sealed throughout the experiment (i.e. counting presence of organisms or colonies).
 - The sealed Petri dish is disposed of in the appropriate manner under the supervision of the Designated Supervisor.
- If a culture container is opened for any purpose, it must be treated as a BSL-2 study and involve BSL-2 laboratory procedures.**

B. Additional Rules for Projects Involving Recombinant DNA (rDNA) Technologies

Studies involving rDNA technologies in which microorganisms have been genetically modified require close review to assess risk level assignment. There are a few rDNA studies that can be safely conducted in a BSL-1 high school laboratory with prior review by a knowledgeable SRC.

- All rDNA technology studies involving BSL-1 organisms and BSL-1 host vector systems may be conducted in a BSL-1 laboratory under the supervision of a Qualified Scientist or trained Designated Supervisor and must be approved by the SRC prior to experimentation. Examples include cloning of DNA in *E. coli K12*, *S. cerevisiae*, and *B. subtilis* host-vector systems.
- Commercially available rDNA kits using BSL-1 organisms may be conducted in a BSL-1 laboratory under the supervision of a Qualified Scientist or trained Designated Supervisor and must be approved by the SRC prior to experimentation.
- A rDNA technology study that involves BSL-1 agents that may convert to BSL-2 agents during the course of experimentation must be conducted entirely in a BSL-2 facility.

- All rDNA technology studies involving BSL-2 organisms and/or BSL-2 host vector systems must be conducted in a regulated research institution and approved by the IBC prior to experimentation.
- Propagation of recombinants containing DNA coding for oncogenes or other human, plant or animal toxins (including viruses) is prohibited.**

C. Additional Rules for Projects Involving Tissues & Body Fluids, including Blood and Blood Products

Studies involving fresh/frozen tissue, blood or body fluids obtained from humans and/or vertebrate may contain microorganisms and have the potential of causing disease. Therefore, a proper risk assessment is required.

- If tissues are obtained from an animal that was sacrificed for a purpose other than the students' project, it may be considered a tissue study. Documentation of the IACUC approval for the original animal study from which tissues are obtained is required.
- If the animal was euthanized solely for the student's project, the study must be considered a vertebrate animal project and adhere to the vertebrate animal rules for studies conducted at a regulated research institution. (See the vertebrate animal rules, pg 17.)
- Biosafety level 1 studies involve the collection and examination of fresh/frozen tissue and/or body fluids, (not including blood or blood products, see rule 5) from a non-infectious source with little likelihood of microorganisms present. Biosafety level 1 studies can be conducted in a BSL-1 laboratory and must be supervised by a Qualified Scientist or trained Designated Supervisor.
- Biosafety level 2 studies involve the collection and examination of fresh/frozen tissues or body fluids that may contain microorganisms belonging to BSL-1 or 2. These studies must be conducted in a regulated research institution in a BSL-2 laboratory under the supervision of a Qualified Scientist.
- All studies involving human or wild animal blood or blood products should be considered a Biosafety level 2 study and must be conducted in a BSL-2 laboratory under the supervision of a Qualified Scientist. All studies involving domestic animal blood may be considered a BSL-1 level study. All blood must be handled in accordance with standards and guidelines set forth in the OSHA, 29CFR, Subpart Z. Any tissue or instruments with the potential of containing bloodborne pathogens (eg. blood, blood products, tissues that release blood when compressed, blood contaminated instruments) must be properly disposed of after experimentation.
- Human breast milk of unknown origin, unless certified free of HIV and Hepatitis C and domestic unpasteurized animal milk are considered BSL-2. Pasteurized domestic animal milk may be considered BSL-1.
- Any study involving the collection and examination of body fluids which may contain biological agents belonging to BSL-3 or 4 is prohibited.
- Studies of human body fluids, where the sample can be identified with a specific person, must have IRB review and informed consent. Students using their own body fluids are exempt from this requirement.

- 9) Studies involving human embryonic human stem cells must be conducted in a registered research institution and reviewed and approved by the ESCRO (Embryonic Stem Cell Research Oversight) Committee.
- 10) The following types of tissue do not need to be treated as potentially hazardous biological agents:
- Plant tissue
 - Established cell and tissue cultures (e.g., obtained from the American Type Culture Collection). The source and catalog

- number of the cultures should be identified in the Research Plan
- Meat or meat by-products obtained from food stores, restaurants, or packing houses
 - Hair
 - Teeth that have been sterilized to kill any blood borne pathogen that may be present. Chemical disinfection or autoclaving at 121 degrees Celsius for 20 minutes is a recommended procedure.
 - Fossilized tissue or archeological specimens
 - Prepared fixed tissue

Risk Assessment

(Use this information to complete PHBA Risk Assessment Form 6A)

Risk assessment defines the potential level of harm, injury or disease to **plants, animals and humans** that may occur when working with biological agents. The end result of a risk assessment is the assignment of a final biosafety level which then determines the laboratory facilities, equipment, training, and supervision required for the research project to proceed.

Risk assessment involves:

- **Assignment of the biological agent to a risk group**
 - Studies involving a known microorganism should begin with an initial assignment of the microorganism to a biosafety level risk group based on information available through a literature search.
 - The study of unknown microorganisms and the use of fresh tissues should rely on the expertise of qualified adults supervising the project.
- **Determination of the level of biological containment** available to the student researcher to conduct the

experimentation. (Please see Levels of Biological Containment below for more details.)

- **Assessment of the experience and expertise of the adult(s)** supervising the student.
- **Assignment of a final biosafety level** for the study based on risk group of biological agent, level of biological containment available and the expertise of the Qualified Scientist or Designated Supervisor who will be supervising the project.

If a study is conducted at a non regulated site (e.g. school), the final biosafety level must be confirmed by the SRC. If the research is conducted at a regulated site, the final biosafety level must be assigned by an Institutional Biosafety Committee (IBC) or equivalent approval body or a letter obtained from an institutional representative that the research does not require review. If no approval body exists at the regulated site, the SRC should review the project and assign a final biosafety level.

Classification of Biological Agents Risk Groups

Biological agents, plant or animal, are classified according to biosafety level risk groups. These classifications presume ordinary circumstances in the research laboratory, or growth of agents in small volumes for diagnostic and experimental purposes.

BSL-1 risk group contains biological agents that pose low risk to personnel and the environment. These agents are highly unlikely to cause disease in healthy laboratory workers, animals or plants. The agents require Biosafety Level 1 containment. Examples of BSL-1 organisms are: *Escherichia coli* strain K12, *Agrobacterium tumefaciens*, *Micrococcus leuteus*, *Neurospora crassa*, *Bacillus subtilis*.

BSL-2 risk group contains biological agents that pose moderate risk to personnel and the environment. If exposure occurs in a laboratory situation, the risk of spread is limited and it rarely would cause infection that would lead to serious disease. Effective treatment and preventive measures are available in the event that an infection occurs. The agents require Biosafety Level 2 containment. Examples of BSL-2 organisms are: *Mycobacterium*, *Streptococcus pneumoniae*, *Salmonella choleraesuis*.

BSL-3 risk group contains biological agents that usually cause serious disease (human, animal or plant) or that can result in serious economic consequences. **PROHIBITED**

BSL-4 risk group contains biological agents that usually produce very serious disease (human, animal or plant) that is often untreatable. **PROHIBITED**

Levels of Biological Containment

There are four levels of biological containment (Biosafety Level 1 - 4). Each level has guidelines for laboratory facilities, safety equipment and laboratory practices and techniques.

BSL-1 containment is normally found in water-testing laboratories, in high schools, and in colleges teaching introductory microbiology classes. Work is done on an open bench or in a fume hood. Standard microbiological practices are used when working in the laboratory. Decontamination can be achieved by treating with chemical disinfectants or by steam autoclaving. Lab coats are required and gloves recommended. The laboratory work is supervised by an individual with general training in microbiology or a related science.

BSL-2 containment is designed to maximize safety when working with agents of moderate risk to humans and the environment. Access to the laboratory is restricted. Biological safety cabinets (Class 2, type A, BSC) must be available. An autoclave should be readily available for decontaminating waste materials. Lab coats, gloves and face protection are required. The laboratory work must be supervised by a competent scientist who understands the risk associated with working with the agents involved.

BSL-3 containment is required for infectious agents that may cause serious or potentially lethal diseases as a result of exposure by inhalation. **PROHIBITED**

BSL-4 containment is required for dangerous/exotic agents that pose high risk of life-threatening disease. **PROHIBITED**

Sources of Information

American Biological Safety Association: ABSA Risk Group
Classification – list of organisms
<http://www.absa.org>

American Type Culture Collection
(703) 365-2700; 1(800) 638-6597 (US, Canada, & PR)
<http://www.atcc.org>

Bergey's Manual of Systematic Bacteriology website –
follow the links for resources and microbial databases for a
collection of international websites of microorganisms and
cell cultures: <http://www.bergeys.org>

Biosafety in Microbiological and Biomedical Laboratories
(BMBL) - 4th Edition. Published by CDC-NIH,
To order: Office of Health and Safety
Centers for Disease Control and Prevention
1600 Clifton Road, NE, Mailstop F05
Atlanta, GA 30333

<http://www.cdc.gov/od/ohs/biosfty/biosfty.htm>

World Health Organization
Laboratory Safety Manual-3rd Edition
<http://www.who.int/csr/bioriskreduction/>

Available online in English, French, Spanish, & Portuguese.
Provides practical guidance on biosafety techniques for use
in laboratories at all levels. Includes risk assessment and
safe use of recombinant DNA technology, and provides
guidelines for the commissioning and certification of
laboratories.

Canada – Agency of Public Health – list of non-pathogenic
organisms
[http://www.phac-aspc.gc.ca/ols-bsl/pathogen/
organism_e.html](http://www.phac-aspc.gc.ca/ols-bsl/pathogen/organism_e.html)

Microorganisms for Education Website – list of organisms
<http://www.science-projects.com/safemicrobes.htm>

NIH Guidelines for Research Involving Recombinant DNA
Molecules. Published by National Institutes of Health.
<http://oba.od.nih.gov/oba/index.html>

OSHA – Occupational Health and Safety Administration
<http://www.osha.gov>

The Mad Scientist Network at Washington University
School of Medicine: <http://www.madsci.org>