

Potentially Hazardous Biological Agents (PHBA) Rules

Potentially Hazardous Biological Agents Rules for use of 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.

Research using 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 potentially hazardous biological agents. Students are permitted to do some research projects with potentially hazardous biological agents meeting the conditions and rules described below which were designed to protect students and to ensure adherence 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 on 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 biosafety level which in turn determines if the project can proceed, and if so, the laboratory facilities, equipment, training, and supervision required.

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 with Potentially Hazardous Biological Agents (PHBA)

1. The following types of studies involve BSL-1 organisms and are exempt from prior SRC review and require no additional forms:
 - a. Studies involving baker's yeast and brewer's yeast, except in rDNA studies.
 - b. Studies involving *Lactobacillus*, *Bacillus thuringiensis*, nitrogen-fixing, oil-eating, and algae-eating bacteria introduced into their natural environment. (Not exempt if cultured in a petri dish environment.)
 - c. Studies involving water or soil not concentrated in media conducive to their microbial growth (please review all rules below to ensure that there are not more specific rules that may apply).
 - d. Studies of mold growth on food items if the experiment is terminated at the first evidence of mold.
 - e. Studies of slime molds and edible mushrooms.
 - f. Studies involving *E. coli* k-12 which are done at school and are not recombinant DNA studies.
2. The following types of studies are exempt from prior SRC review, but require a Risk Assessment Form 3:
 - a. Studies involving protists, archaea and known non-pathogenic microorganisms.
 - b. Research using manure for composting, fuel production, or other non-culturing experiments.
 - c. Commercially-available color change coliform water test kits. These kits must remain sealed and must be properly disposed.
 - d. Studies involving decomposition of vertebrate organisms (such as in forensic projects).
 - e. Studies with microbial fuel cells.
3. Prior review and approval is required for 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:
 - a. An affiliated fair SRC, an IBC or an 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, IBC or IACUC.
 - b. Experimentation involving the culturing of potentially hazardous biological agents, even BSL-1 organisms, is prohibited in a home environment. However, specimens may be collected at home as long as they are immediately transported to a laboratory with the BSL containment determined by the affiliated fair SRC.
 - c. Research determined to be at Biosafety Level 1 (BSL-1) must 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.
 - d. Research determined to be a Biosafety Level 2 (BSL-2) must be conducted in a laboratory rated BSL-2 or above (commonly limited to a Regulated Research Institution). The research must be reviewed and approved by the Institutional Biosafety Committee (IBC) if the Regulated Research Institution requires the review. The research must be supervised by a Qualified Scientist. For a high school BSL-2 laboratory, the SRC must review and approve.
 - e. Students are prohibited from designing or participating in an experiment associated with the following types of PHBA studies:
 - BSL-3 or BSL-4 Research
 - Culturing CRE (Carbapenem Resistant Enterobacteriaceae)
 - f. Insertion of antibiotic resistance markers for the clonal selection of bioengineered organisms is permitted. Students may not genetically engineer organisms with multiple drug resistance traits for the intended purpose of investigation of the pathology or treatment of antibiotic-resistant infections. Insertion of antibiotic-resistance traits or selection of organisms expressing traits that may affect the ability to provide effective treatment of infections acquired by humans, animals, or plants is strictly prohibited.
 - g. Laboratory studies culturing known MRSA (Methicillin-resistant *Staphylococcus aureus*), VRE (Vancomycin-resistant enterococci) and KPC (*Klebsiella pneumoniae*) must have a written justification for usage and be conducted at a Regulated Research Institution with a minimum BSL-2 laboratory with documented IBC Committee review and approval.
 - h. Extreme caution must be exercised when selecting and sub-culturing antibiotic-resistant organisms. Studies using such organisms require at least BSL-2 containment.
 - i. Naturally-occurring plant pathogens may be studied (not cultured) at home, but may not be introduced into a home/garden environment.
 - j. The culturing of human or animal waste, including sewage sludge, is considered a BSL-2 study.

- k. All potentially hazardous biological agents must be properly disposed at the end of experimentation in accordance with their biosafety level. For BSL 1 or BSL 2 organisms: Autoclave at 121 degrees Celsius for 20 minutes, use of a 10% bleach solution (1:10 dilution of domestic bleach), incineration, alkaline hydrolysis, biosafety pick-up and other manufacturer recommendations are acceptable.
 - l. Any proposed changes in the Research Plan/Project Summary by the student after initial local or affiliated fair SRC approval must undergo subsequent SRC or IBC review and approval before such changes are made and before experimentation resumes.
4. The following forms are required:
 - a. Checklist for Adult Sponsor (1), Student Checklist (1A), Research Plan/Project Summary, and Approval Form (1B)
 - b. Regulated Research Institution Form (1C) - when applicable
 - c. Qualified Scientist (2), when applicable
 - d. Risk Assessment (3), when applicable
 - e. PHBA Risk Assessment Form (6A), when applicable
 - f. 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.)

1. Research with unknown microorganisms can be treated as a BSL-1 study under the following conditions:
 - a. Organism is cultured in a plastic petri dish (or other standard non-breakable container) and sealed. Other acceptable containment includes two heavy-duty sealed bags.
 - b. Experiment involves only procedures in which the petri dish remains sealed throughout the experiment (e.g., counting presence of organisms or colonies).
 - c. The sealed petri dish is disposed of via autoclaving or disinfection under the supervision of the Designated Supervisor.
2. If a culture container with unknown microorganisms is opened for any purpose, (except for disinfection for disposal), 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, plants and/or animals have been genetically modified require close review to assess the risk level assignment. Some rDNA studies can be safely conducted in a BSL-1 high school laboratory with prior review by a knowledgeable SRC:

1. All rDNA technology studies involving BSL-1 organisms and BSL-1 host vector systems, including commercially available kits, must be conducted in a BSL-1 laboratory under the supervision of a Qualified Scientist or Designated Supervisor and must be approved by the SRC prior to experimentation. Examples include

cloning of DNA in *E. coli* K-12, *S. cerevisiae*, and *B. subtilis* host-vector systems.

2. An rDNA technology study using BSL-1 agents that may convert to BSL-2 agents during the course of experimentation must be conducted entirely in a BSL-2 facility.
3. 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.
4. Propagation of recombinants containing DNA coding for human, plant or animal toxins (including viruses) is prohibited.
5. All genome editing studies that include alteration of germline cells, insertion of gene drives, use of rapid trait development systems (RTDS[®]), etc., should be categorized as a BSL-2 study and must be conducted at an RRI and approved by the IBC from the institution. Qualified scientists are expected to ensure that student research protocols address appropriate intrinsic and extrinsic containment precautions.
6. Introduction or disposal of non-native, genetically-altered, and/or invasive species (e.g. insects, plants, invertebrates, vertebrates), pathogens, toxic chemicals or foreign substances into the environment is prohibited. Students and adult sponsors should reference their local, state and national regulations and quarantine lists.

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

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

1. The following types of tissue do not need to be treated as potentially hazardous biological agents:
 - a. Plant tissue (except those known to be toxic or hazardous)
 - b. Plant and non-primate established cell lines and tissue culture collections (e.g., obtained from the American Type Culture Collection). The source and/or catalog number of the cultures must be identified in the Research Plan/Project Summary.
 - c. Fresh or frozen meat, meat by-products, pasteurized milk or eggs obtained from food stores, restaurants, or packing houses
 - d. Hair, hooves, nails and feathers
 - e. Teeth that have been sterilized to kill any blood-borne pathogen that may be present.
 - f. Fossilized tissue or archeological specimens.
 - g. Prepared fixed tissue
2. Research involving human and/or non-human primate established cell lines and tissue culture collections (e.g., obtained from the American Type Culture Collection) must be considered a BSL-1 or BSL-2 level organism as indicated by source information and treated accordingly. The source and/or catalog number of the cultures must be identified in the Research Plan/Project Summary.
3. If tissues are obtained from an animal that was euthanized for a purpose other than the student's project, it may be considered a tissue study. Use of tissues obtained from

research conducted at a Regulated Research Institution requires a copy of the IACUC certification with the name of the research institution, the title of the study, the IACUC approval number and date of IACUC approval. Use of tissues obtained from agricultural/aquacultural studies require prior SRC approval.

4. If the animal was euthanized solely for the student's project, the study must be considered a vertebrate animal project and is subject to the vertebrate animal rules. (See vertebrate animal rules.)
5. The collection and examination of fresh/frozen tissue and/or body fluids, (not including blood or blood products; see rule 8) from a non-infectious source with little likelihood of microorganisms present must be considered Biosafety level 1 studies and must be conducted in a BSL-1 laboratory or higher and must be supervised by a Qualified Scientist or trained Designated Supervisor.
6. The collection and examination of fresh/frozen tissues or body fluids or meat, meat by-products, pasteurized milk or eggs NOT obtained from food stores, restaurants, or packing houses may contain microorganisms. Because of the increased risk from unknown potentially hazardous agents, these studies must be considered biosafety level 2 studies conducted in a BSL-2 laboratory under the supervision of a Qualified Scientist.
7. Human breast milk of unknown origin, unless certified free of HIV and Hepatitis C, and domestic unpasteurized animal milk are considered BSL-2.
8. All studies involving human or wild animal blood or blood products should be considered at a minimum a Biosafety level 2 study and must be conducted in a BSL-2 laboratory under the supervision of a Qualified Scientist. Known BSL-3 or BSL-4 blood is prohibited. 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 blood-borne pathogens (e.g. blood, blood products, tissues that release blood when compressed, blood contaminated instruments) must be properly disposed after experimentation.
9. Studies of human body fluids, where the sample can be identified with a specific person, must have IRB review and approval, and informed consent.
10. Any study involving the collection and examination of body fluids may contain biological agents belonging to BSL-3 or BSL-4 is prohibited.
11. A project involving a student researcher using their own body fluids (if not cultured)
 - a. can be considered a BSL-1 study
 - b. may be conducted in a home setting
 - c. must have IRB review if the body fluid is serving as a measure of an effect of an experimental procedure on the student researcher (e.g. Student manipulates diet and takes a blood or urine sample). An example of a project not needing IRB review would be collecting urine to serve as a deer repellent.
 - d. must receive prior SRC review and approval prior to

experimentation.

12. Studies involving 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.

Sources of Information are available as a separate section at the end of the document.

Potentially Hazardous Biological Agents 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 biosafety level which then determines the laboratory facilities, equipment, training, and supervision required.

Risk assessment involves:

1. Assignment of the biological agent to a risk group
2. Studies involving a known microorganism must begin with an initial assignment of the microorganism to a biosafety level risk group based on information available through a literature search.
3. The study of unknown microorganisms and the use of fresh tissues relies on the expertise of the supervising adult(s).
4. Determination of the level of biological containment available to the student researcher to conduct the experimentation. (See "Levels of Biological Containment" for details.)
5. Assessment of the experience and expertise of the adult(s) supervising the student.
6. Assignment of a 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
7. Documentation of review and approval of study prior to experimentation:
 - a. If a study is conducted at a non-regulated site (e.g. school), the SRC reviews the Research Plan/Project Summary.
 - b. If the study was conducted at a Regulated Research Institution, and was approved by the appropriate institutional board (e.g. IBC, IACUC), the SRC reviews the institutional forms provided and documents SRC approval (Form(6A)).
 - c. If a PHBA study was conducted at a Regulated Research Institution but the institution does not require review for this type of study. The SRC must review the study and document approval on Form 6A that the student received appropriate training and the project complies with Intel ISEF rules.

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: *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. Projects in the BSL-3 group are prohibited.

BSL-4 risk group contains biological agents that usually produce very serious disease (human, animal or plant) that is often untreatable. Projects in the BSL-4 group are 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 an appropriate biosafety 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 and gloves are required. 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 and gloves are required; eye protection and face shields must also be worn as needed. The laboratory work must be supervised by a 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. Projects in the BSL-3 group are prohibited.

BSL-4 containment is required for dangerous/exotic agents that pose high risk of life-threatening disease. Projects in the BSL-4 group are prohibited.