Potentially Hazardous Biological Agents Rules

Rules for use of microorganisms (including bacteria, viruses, viroids, prions, 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, 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 (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 Projects Involving Studies Involving Potentially Hazardous Biological Agents

1) The following types of studies are exempt from prior SRC review and require no additional forms:
   a. Studies involving baker's yeast and brewer's yeast, except when used with rDNA studies.
   b. Studies involving Lactobacillus, *Bacillus thuringinensis,* nitrogen-fixing, oil-eating bacteria, 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 conductive to their 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 mushrooms and amoebozoa (slime mold).

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 similar 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) The use of potentially hazardous microorganisms (including bacteria, viruses, viroids, prions, fungi, and parasites), recombinant DNA (rDNA) technologies or human or animal fresh/frozen tissues, blood, or body fluids, is allowable as follows:
   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. The research must be supervised by a Qualified Scientist. If at an Regulated Research Institution, it must be reviewed and approved by the Institutional Biosafety Committee (IBC) or documentation provided that the institution does not require review.
   e. BSL-3 or -4 research is prohibited.
   f. Laboratory studies culturing known MRSA (Methicillin-resistant *Staphlococcus aureus)*, VRE (Vancomycin-resistant enterococci) and KPC (Klebsiella pneumonia) must be conducted in a BSL-2 laboratory in a Regulated Research Institution with documented IBC Committee review and approval. Students are prohibited from culturing CRE (Carbapenem Resistant Enterobacteriacae).
   g. Studies that genetically engineer bacteria with multiple antibiotic resistance are prohibited.
   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 2 organisms the following disposal methods are acceptable: 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.

l. Any proposed changes in the Research Plan 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:
- Checklist for Adult Sponsor (1), Student Checklist (1A), Research Plan, and Approval Form (1B)
- Regulated Research Institution Form (1C) - when applicable.
- Qualified Scientist (2), when applicable
- Risk Assessment (3), when applicable
- PHBA Risk Assessment Form (6A), when applicable
- Human and Vertebrate Animal Tissue Form (6B) – for all studies involving tissues and body fluids.

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

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 (2-ply) 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 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 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* K12, *S. cerevesiae,* and *B. subtilis* host-vector systems.

2) 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.

3) 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.

4) 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.

5) Propagation of recombinants containing DNA coding for oncogenes or other human, plant or animal toxins (including viruses) is prohibited.

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
   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.
   c. Fresh or frozen meat, meat by-products, pasteurized milk or eggs obtained from food stores, restaurants, or packing houses.
   d. Hair.
   e. 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 recommended.
   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.

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. Documentation of the IACUC approval for the original animal study from which tissues are obtained is required.

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 for studies conducted at a Regulated Research Institution. (See vertebrate animal rules.)

5) Biosafety level 1 tissue studies involve the collection and examination of fresh/frozen tissue and/or body fluids, (not including blood or blood products; see rule 7) from a non-infectious source with little likelihood of microorganisms present. Biosafety level 1 studies must be conducted in a BSL-1 laboratory or higher and must be supervised by a Qualified Scientist or trained Designated Supervisor.

6) Biosafety level 2 tissue 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.

7) All studies involving human or wild animal blood or blood products is considered a BSL-2 and is required to be conducted in a BSL-2 laboratory under the supervision of a Qualified Scientist. 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.

8) Human breast milk of unknown origin, unless certified free of HIV and Hepatitis C and domestic unpasteurized animal milk, are considered BSL-2.

9) Any study involving the collection and examination of body fluids which may contain biological agents belonging to BSL-3 or -4 is prohibited.

10) Studies of human body fluids, where the sample can be identified with a specific person, must have IRB review and approval, and informed consent. Student researchers using their own body fluids are exempt from this requirement unless they are the subject of their own research.

11) Self-sampling of capillary blood for analysis (e.g. glucometer reading) may be conducted in a home setting.

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.

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:

- Assignment of the biological agent to a risk group
  - 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.
  - The study of unknown microorganisms and the use of fresh tissues relies on the expertise of the supervising adult(s).
- Determination of the level of biological containment available to the student researcher to conduct the experimentation. (See "Levels of Biological Containment" for details.)
- Assessment of the experience and expertise of the adult(s) supervising the student.
- 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.

If a study is conducted at a non-regulated site (e.g. school), the biosafety level must be confirmed by the local or affiliated fair SRC. If the research is conducted at a Regulated Research Institution, the biosafety level must be assigned by an Institutional Biosafety Committee (IBC) or equivalent approval body or a letter from an institutional representative certifying that the research does not require review. If no approval body exists at the Regulated Research Institution, a letter or document from the Regulated Research Institution that the research does not require review is required and the local or affiliated fair SRC must review the project and assign a biosafety level. If possible, this review should be before experimentation.

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 tumifaciens, 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 pneumonia, 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 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 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.

Hazardous Chemicals, Activities or Devices Rules

(Includes DEA-controlled substances, prescription drugs, alcohol & tobacco, firearms and explosives, radiation, lasers, etc.)

The following rules apply to research using hazardous chemicals, devices and activities. These include substances and devices that are regulated by local, state, country, or international law, most often with restrictions of their use by minors such as DEA-controlled substances, prescription drugs, alcohol, tobacco, firearms and explosives. Hazardous activities are those that involve a level of risk above and beyond that encountered in the student's everyday life.

These rules are intended to protect the student researcher by ensuring proper supervision and the consideration of all potential risks so that the appropriate safety precautions are taken. Students are required to meet all standards imposed by Intel ISEF, school, local, and/or regional fair(s).

Rules for ALL Projects Involving Hazardous Chemicals, Activities and Devices

1) The use of hazardous chemicals and devices and involvement in hazardous activities require direct supervision by a Designated Supervisor, except those involving DEA-controlled substances, which require supervision by a Qualified Scientist.

2) The student researcher must conduct a risk assessment in collaboration with a Designated Supervisor or Qualified Scientist prior to experimentation. This risk assessment is documented on the Risk Assessment Form 3.

3) Student researchers must acquire and use regulated substances in accordance with all local, state, U.S. federal and country laws. For further information or classification for these laws and regulations, contact the appropriate regulatory agencies.

4) For all chemicals, devices or activities requiring a Federal and/or State Permit, the student/supervisor must obtain the permit prior to the onset of experimentation. A copy of the permit must be available for review by adults supervising the project and the local and affiliated and the ISEF Scientific Review Committee in their review prior to competition.

5) The student researcher must minimize the impact of an experiment on the environment. Examples include using minimal quantities of chemicals that will require subsequent disposal; ensuring that all disposal is done in an environmentally safe manner and in accordance with good laboratory practices.

6) The following forms are required:
   a. Checklist for Adult Sponsor (1), Student Checklist (1A), Research Plan and Approval Form (1B)
   b. Regulated Research Institution Form (1C), when applicable
   c. Qualified Scientist Form (2), when applicable
   d. Risk Assessment Form (3)

Additional Rules for Specific Regulated Substances

There are additional rules for the following regulated substances described below:

A. DEA-Controlled Substances

The U.S. Drug Enforcement Administration (DEA) regulates chemicals that can be diverted from their intended use to make illegal drugs. Other countries may have similar regulatory bodies; students outside of the U.S. must adhere to their own country's drug regulatory agency requirements in addition to U.S. DEA regulations. DEA-controlled substances and their schedule number are at the DEA website under Sources of Information. It is the responsibility of the student to consult this list if there is a possibility that substances used in experimentation could be regulated.

1) All studies using DEA-controlled substances must be supervised by a Qualified Scientist who is licensed by the DEA (or other international regulatory body) for use of the controlled substance.
2) All studies using DEA Schedule 1 substances must have the research protocol approved by DEA before research begins. Schedule 2, 3 and 4 substances do not require protocol approval by DEA.

B. Prescription Drugs

Prescription drugs are drugs regulated by federal or country laws and are available only through a pharmacy to protect against inappropriate or unsafe use. Special precautions must be taken in their use for a science project as follows:

1) Students are prohibited from administering prescription drugs to human participants.
2) A veterinarian must supervise student administration of any prescription drugs to vertebrate animals.

C. Alcohol and Tobacco

The U.S. Alcohol and Tobacco Tax and Trade Bureau (TTB) regulates the production of alcohol and distribution of alcohol and tobacco products. Many such products are restricted by age for purchase, possession and consumption. Students outside of the U.S. must adhere to U.S. regulations and to their local and country laws and regulations.

1) The Designated Supervisor is responsible for the acquisition, usage and appropriate disposal of the alcohol or tobacco used in the study.

2) Production of ethyl alcohol (wine or beer) is allowable in the home under parental supervision and must meet the TTB home production regulations.

3) Fermentation studies in which minute quantities of ethyl alcohol are produced are permitted.

4) Students are allowed to distill alcohol for fuel or other non-consumable products. To do so, the work must be conducted at school and a TTB permit must be obtained by school authorities. Details regarding this process are available from the Alcohol and Tobacco Tax and Trade Bureau (TTB) website.

D. Firearms and Explosives

The U.S. Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF), along with state agencies, regulates the purchase and use of firearms and explosives. A firearm is defined as a small arms weapon from which a projectile is fired by gunpowder. An explosive is any chemical compound, mixture or device, the primary purpose of which is to function by explosion. Explosives include, but are not limited to, dynamite, black powder, pellet powder, detonators, and igniters.

The purchase of a firearm by a minor is generally unlawful. The use of a firearm, without proper state certification, is illegal. Students are required to check the training and certification requirements of individual states and countries.

1) Projects involving firearms and explosives are allowable when conducted with the direct supervision of a Designated Supervisor and when in compliance with all federal, state and local laws.

2) A fully assembled rocket motor, reload kit or propellant modules containing more than 62.5 grams of propellant are subject to the permitting, storage and other requirements of federal explosive laws and regulations.

3) Potato guns and paintball guns are not firearms unless they are intended to be used as weapons. They must be treated as hazardous devices.

Guidance for Risk Assessment

Please find below guidance on conducting risk assessment by category:

A. Hazardous Chemicals

A proper risk assessment of chemicals must include review of the following factors:

Toxicity – the tendency of a chemical to be hazardous to health when inhaled, swallowed, injected or in contact with the skin.
Reactivity — the tendency of a chemical to undergo chemical change.
Flammability — the tendency of a chemical to give off vapors which readily ignite when used under normal working conditions.
Corrosiveness — the tendency of a chemical, upon physical contact, to harm or destroy living tissues or physical equipment.

When assessing risk, the type and amount of exposure to a chemical must be considered. For example, an individual's allergic and genetic disposition may have an influence on the overall effect of the chemical. The student researcher must refer to Material Safety Data Sheets (MSDS) provided by the vendor to ensure that proper safety precautions are taken. Some MSDS sheets (e.g., Flinn) rank the degree of hazard associated with a chemical. This rating may assist students and adult sponsors in determining risk associated with the use of a chemical. Information on pesticides can be obtained from the websites listed in the Sources of Information section of this document.

A risk assessment must include proper disposal methods for the chemicals used in an experiment. The Flinn Catalog (referenced in the Sources of Information section) provides information for the proper disposal of chemicals. If applicable, the student researcher must incorporate in the research plan disposal procedures required by federal and state guidelines.

Environmentally Responsible Chemistry

The mission of environmentally responsible (green) chemistry is to avoid the use or production of hazardous substances during a chemical process. The principles of green chemistry are described on the EPA website in the Sources of Information section. The following principles must be incorporated into the research plan:

- Waste prevention
- Use of the safest possible chemicals and products
- Design of the least possible hazardous chemical syntheses
- Use of renewable materials
- Use of catalysts in order to minimize chemical usage
- Use of solvents and reaction conditions that are as safe as possible
- Maximization of energy efficiency
- Minimization of accident potential

B. Hazardous Devices

The documentation of risk assessment Risk Assessment (Form 3) is required when a student researcher works with potentially hazardous/dangerous equipment and/or other devices, in or outside a laboratory setting that require a moderate to high level of expertise to ensure their safe usage. Some commonly used devices (Bunsen burners, hot plates, saws, drills, etc.) may not require a documented risk assessment, assuming that the student researcher has experience working with the device. Use of other potentially dangerous devices such as high vacuum equipment, heated oil baths, and high temperature ovens must have documentation of a risk assessment.

C. Radiation

A risk assessment must be conducted when a student uses non-ionizing radiation beyond that normally encountered in everyday life. Non-ionizing radiation includes the spectrum of ultraviolet (UV), visible light, infrared (IR), microwave (NW), radiofrequency (RF) and extremely low frequency (ELF). Lasers usually emit visible, ultraviolet or infrared radiation. Lasers are classified into four classes based upon their safety. Manufacturers are required to label Classes II – IV lasers.

Projects involving radionuclides (radioisotopes) and X-rays must involve a careful examination of the risks associated with the study and appropriate safety precautions must be taken. Depending upon the level of exposure, radiation released from these sources can be a health hazard.

A risk assessment must take into account the time of exposure, distance and shielding involved in the study.

1) A study of natural radiation that is no more than than encountered in everyday life is exempt from the following requirements.
2) All studies may not exceed the dose limits set by the Nuclear Regulatory Commission of 0.5 mrem/hr or 100 mrem/year of exposure.
3) If the voltage needed in the study is <10 kvolts, a risk assessment must be conducted. The study may be done at home or school, and SRC preapproval is not required.
4) A study using 10-25 kvolts must have a risk assessment conducted and must be preapproved by the SRC to assess safety. Such a study must be conducted in a metal chamber using a camera only, not direct view through glass. A dosimeter or radiation survey meter is required to measure radiation exposure.
5) All studies using > 25 kvolts must be conducted at an institution with a Licensed Radiation Program and must be preapproved by the Institutions' Radiation Safety Officer or the Committee which oversees the use of ionizing radiation to ensure compliance with state and federal regulations.

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