2023

BIOLOGY LABORATORIES

Biology teachers and their students face a wide range of potential hazards. In addition to chemical reagents, there are the hazards associated with the handling of organisms, classroom activities on the school grounds and outdoor study areas, and the containment of biological specimens. Effective control of such hazards involves both the recognition of each hazard and the development of control procedures.

Complete the Waste Disposal Form for removal of all waste (chemicals and broken glass) at the end of each semester. The Science/STEM Coordinator (Dr. Chaundra Creekmur; creekch@boe.richmond.k12.ga.us) will facilitate removal.

BIO 1: Required Materials for the High School Biology Lab

1. Broken Glass Container
2. Sharps Disposal Box
3. Biohazard Bags
4. Household Bleach
5. Fire Extinguisher
6. Spill Kit
7. First Aid Kit
8. MSDS Notebook
9. Chemical Waste Disposal Containers

BIO 2: Eye Protection

BIO 2.1 What is your obligation?

Teachers owe their students a duty of care. A teacher must reasonably address all foreseeable dangers inherent in any laboratory experiment or demonstration that will be performed in the science laboratory or classroom. A teacher must also instruct and ensure that students demonstrate the proper use of protective equipment.

An important obligation of science teachers is to provide students with appropriate eye protection. **Provision and Maintenance of PPE - 29 CFR §1910.132(d) Personal**

Protective Equipment, General Requirements Standard requires a hazard assessment to determine PPE needs and teachers must be trained in use and care of goggles.

BIO 2.2 What circumstances require eye protection?

Eye protection is a must in any hazardous laboratory activity or demonstration in science. As a responsible teacher, you must select eyewear that provides you and your students with the most appropriate protection for the hazards of your science activities. Effective eye protection must include adequate instruction on the hazards of the particular activity and of the precautions to be followed to reduce the risk of injury. It must also include instructions and modeling of the protective equipment.

Protection of the eyes is essential in any laboratory activity. Eye protection is required (but not limited to):

1. When chemicals, glassware, or a heating source is being used
2. When working with solid materials or equipment under stress, pressure, or force that might cause fragmentation or flying particles
3. When an activity generates projectiles, or uses elastic materials under stress (e.g., springs, wires, rubber, glass), or causes collisions
4. When dust or fumes are present (Eye protection reduces the dust or fumes reaching the eye.)
5. When using preserved specimens

BIO 2.3 Choosing the best eye protection

Only safety goggles provide the level of protection needed for your laboratory activities when dealing with hazardous liquids or solids. A safety goggle fits the face surrounding the eyes; it should have a soft pliable flange, which seals around the eyes snugly to protect the eyes. In addition, safety goggles, with side shields or without side shields, provide adequate protection for laboratory activities involving use of solids such as meter sticks, projectiles,

etc. Safety goggles should also be the standard for eye protection when chemicals, glassware, a heating source, or preserved specimens are being used.

BIO 2.4 Disinfecting Goggles

1. When using the safety goggle cabinet, the ultraviolet light timer should be set for a minimum of ten (10) minutes.. Sanitation of goggles is accomplished best by usage of a UV cabinet. Treatment with UV light will destroy the goggles over several years.
2. Hot soapy water and thorough drying between uses of shared goggles is also recommended by the ACS.
3. Chemical Disinfection: After student use, wash the goggles in soapy water followed by a ten (10) minute rinse in five percent bleach solution (10:1 ratio - 10 parts water to 1 part bleach). The goggles should be allowed to air dry.

BIO 2.5 What is the current recommendation for wearing contact lenses?

1. The American Chemical Society Committee on Chemical Safety states that contact lenses can be worn in the laboratory provided that approved eye protection is worn as required of others in the laboratory.
2. The National Institute for Occupational Health and Safety (NIOSH) recommends that workers be permitted to wear contact lenses when handling hazardous chemicals provided adequate face and eye protection is worn.
3. The Council of State Science Supervisors states that contact lenses can be worn provided "specially marked, non-vented safety goggles are available to contact lens wearers".
4. The Occupational Safety and Health Administration (OSHA) believes that contact lenses do not pose additional hazards to the wearer and has determined that additional regulation addressing the use of contact lenses is unnecessary.

a. The agency wants to make it clear, however, that contact lenses are not eye protection devices. If eye hazards are present, appropriate eye protection must be worn instead of, or in conjunction with, contact lenses."
b. Regulations (Preamble to Final Rules) Personal Protective Equipment for General Industry (Amended Final Rule, April 1994) Section 3- III Summary and Explanation of the Final Rule 1910.133, p. 16343.

BIO 3: Glassware

BIO 3.1 Injuries from Glassware

Glassware is the number one source of injury in the laboratory setting. More students are cut by damaged glassware and burned by heated glassware that are harmed by any other object or circumstance in the lab. To ensure the safety of students in the middle school laboratory, substitute plastic lab ware for glassware where possible. New plastics like polycarbonate (Lexan®) have been successfully used for laboratory containers. While not useful for heating, the plastic is clear and extremely hard and can be used for almost all water-soluble compounds. Beakers, flasks, graduated cylinders, and thermometers now are available in plastic. Check with your science supply company.

BIO 3.2 General Cautions

BIO 3.2.1 Broken Glass

1. Use glassware that is without defect and has smooth edges.
2. One of the most important ways to prevent glassware related injuries is to check the pieces for chips or cracks. Any damaged glassware should be disposed of in the appropriate container.
3. Glassware should have no cracks, chips, or scratches. In particular, be wary of "star cracks" that can form on the bottom of beakers and flasks. Any glassware with such cracks should be properly disposed of immediately.
4. All glass tubing should be fire-polished.

BIO 3.2.2. "Frozen" Glass

Be careful with glassware that is "frozen." Only teachers, wearing goggles and gloves, should try to release the "frozen" glassware. If this fails, discard the glassware. Some common cases of "frozen" glassware are:

1. nested beakers that have been jammed together.
2. stoppers that cannot be removed from bottles.
3. stopcocks that cannot be moved.

BIO 3.2.3 Hot Glass

1. Use only Kimax® or Pyrex® brand glassware when heating substances. Common glass can break or shatter, causing serious injuries in the lab.
2. Use care when working with hot glass. Hot glass looks exactly the same as room temperature glass.
3. Do not leave hot glassware unattended, and allow ample time for the glass to cool before touching.
4. Check the temperature of the glassware by placing your hand near, but not touching, the potentially hot glass.
5. Have hot pads, thick gloves, or beaker tongs available for grasping hot glassware.
6. Never set hot glassware on cold surfaces or in any way change its temperature suddenly. Even a Pyrex® or Kimax® beaker will break if cold water is poured into a hot beaker.

BIO 3.2.4 Glass Tubing

1. Make sure that the tubing is without chips or cracks.
2. Use the appropriate diameter tubing for the task.
3. Make sure the ends of the tubing are fire polished.
4. When breaking tubing:
    a. Use gloves or towels to protect hands when breaking glass tubing. Use goggles to protect the eyes.
    b. Scratch the glass once with a file or score. Wrap the glass in a towel.
    c. Place the thumbs together opposite the scratch. Pull and bend in one quick motion.
    d. Fire polish the broken ends: hold the glass so that the sharp end is in the top of the flame of a gas burner.
    e. Rotate the tube so all sides are heated evenly, causing the sharp edges to melt and become smooth.
    f. Place the glass on insulating material to cool.

BIO.3.2.5 Bending.

Bending glass tubing is often necessary. Follow these procedures:

1. Place a wing-top attachment on a gas burner and heat the area of the glass to be bent while holding it with one hand on each end, rotating to ensure even heating.
2. When the glass is soft and pliable, remove it from the flame and quickly bend to the desired shape.
3. Place on insulating material until cool.

BIO.3.3 Types and Appropriate Use of Glassware

To prevent glassware related injuries always use the correct type of glass for the task you are doing. For example, a graduated cylinder should be used to measure the volume of a liquid, not as a container in which to run chemical reactions. Likewise, a watch glass should not be used to mix chemical compounds, but as a cover over a heated reaction vessel.

BIO 3.3.1 Proper Use

Each type of glassware has its proper use and should be used only for its intended purpose.

1. **For measuring volume**:

pipets	burets	graduated cylinders
dropper pipets		volumetric flasks

2. **For storing solids and liquids:**

bottles	vials

3. **For containing reactive chemicals during experiments:**

beakers	flasks	test tubes
crucibles	watch glasses	test plates

4. **For transferring liquids and gases**:

   glass tubing funnels pipets

5. **For measuring temperature:**

   digital thermometers alcohol thermometers

BIO.3.5 Cleaning

1. Clean immediately after use. The longer glassware sits, the harder it is to clean.
2. Use laboratory-grade detergents or liquid dishwashing detergent such as Dawn® for cleaning glassware.
3. When using brushes, make sure to use the appropriate size brush; make sure the metal part of the brush does not scratch the glass.
   Rinse glassware with deionized water.
4. Allow glassware to air dry on paper towels, drying pads, or drying racks.

BIO.3.6 Disposal

1. Defective glassware should be disposed of correctly.
2. Glassware should be disposed of in a separate container from normal trash. Such container should be clearly labeled **BROKEN GLASSWARE ONLY**.
3. When handling broken glassware, wear gloves or use a dustpan and broom. Do not pick up broken glass with bare hands.

BIO 4: Microscope Handling

1. **DO NOT ALLOW STUDENTS WITH ACTIVE EYE INFECTIONS TO USE MICROSCOPES!**
2. Provide students with alcohol wipes to clean lenses before or after use.
3. Microscopes must be carried upright, with one hand supporting the arm of the microscope and the other hand supporting the base. Nothing else should be carried at the same time.
4. Microscope must be positioned safely on the table, NOT near the edge.
5. After plugging the microscope into the electrical outlet, the cord should be draped carefully up onto the table and never allowed to dangle dangerously to the floor.
6. The coarse adjustment must NEVER be used to focus a specimen when the 40x or oil immersion lens is in place.
7. When finished with the microscope, the cord should be carefully wrapped around the microscope before returning it to the cabinet.
8. All prepared microscope glass slides are to be returned to their appropriate slide trays; wet mount preparations are to be disposed of properly.
9. Malfunctioning microscopes should be reported to the department chairperson/laboratory safety manager.

BIO 5: Dissections

The use of preserved animal specimens in instruction should be carefully planned to provide learning that cannot otherwise be achieved. Dissection activities should enable students to develop a greater respect for life. **ALL** such activities, particularly those involving the use of vertebrates should be undertaken by students only when they are prepared and have the maturity to appreciate fully the significance of the instructional activity.

BIO 5.1 General Considerations

1. Most biological supply houses sell specimens that are preserved in methanol or other low toxicity preservatives. When ordering new specimens for dissection, be aware that specimens preserved in formaldehyde are not permitted for use in the State of Georgia.
    a. Some schools may have older specimens which are still stored in formaldehyde or formalin. Formaldehyde and formalin are listed as carcinogens by the EPA and are strong irritants. Good room ventilation is required when working with these specimens.
    b. Any specimen held in a formalin solution should be soaked in a water bath in a fume hood for 24 hours and then thoroughly rinsed under running water for several minutes before use.
    c. The soak solution should be placed into a hazardous waste container and appropriately labeled with the name of the contents, the amount of solution, and the date generated.
    d. Facilities and Maintenance should be contacted for pick-up and disposal. Document the date of contact and the date of pick-up.
2. While not required, it is suggested that dissections should be performed **only** by those students who have obtained a permission note signed by a parent.
3. **Work surfaces should be decontaminated once per class and after any spill of materials. A 1:10 household bleach and water solution may be used for disinfection.**

BIO 5.2 Student Instruction

1. **Students should be instructed in the safe use of dissection instruments.**
    a. Scalpels and dissecting instruments should be sterilized before and after experiments.
    b. Pointed dissection probes, scalpels, razor blades, scissors, and microtome knives must be used with great care, and placed in a safe position when not in use.
    c. Scalpels and other sharp instruments are only to be used to make cuts in the specimen, never as a probe or a pointer.
    d. Leave scalpel blades in the original package when pushing the scalpel onto the blade.
    e. Use tweezers, forceps, or a hemostat to remove the blade, always pushing the blade away from the body.
2. Avoid holding the specimen in the hand during dissection. A waxed pan or similar device should be used for holding the specimen in place.
3. When cutting with a scalpel or other sharp instrument, forceps may be used to help hold the specimen. **NEVER** use fingers to hold a part of the specimen while cutting.
4. Hands should be thoroughly washed after dissection activities.

BIO 5.3 Preserved Specimens

1. When specimens are being removed from the preservative solution, rubber gloves should be worn or forceps or tongs should be used, depending on the size of the specimen. Use chemical splash goggles to protect against splashes and fumes.
2. **Preserved specimens should be thoroughly washed (including the abdominal cavities of large specimens) before being handled by the students.**
3. Preservative fumes may be irritating to the eyes, nose, and throat. Adequate ventilation should be provided whenever preservative fumes are present.
4. When larger specimens are being dissected, the part of the specimen that is not being dissected should be kept enclosed in the plastic bag.
5. When dissecting smaller specimens, seal the bag or container after removing the specimen, so as to confine the preservative in the specimen bag.
6. Specimens are to be clearly labeled and stored in designated containers or cabinets when not in use.

BIO 5.4 Disposal

1. **Body parts or scraps of the specimen should NOT to be disposed of in the sink.**
    a. **Body parts and tissue specimen should be placed into resealable plastic bags then placed into red biohazard bags. The bags should be labeled with the contents and the date the waste was generated.**
    b. **Facilities and Maintenance should be contacted for pick up and disposal. Document the date pick-up was requested and the date it occurred.**
2. Containers designated for the disposal of sharps (scalpel blades, razor blades, needles; dissection pins, etc.) and containers designated for broken glass must be present in each laboratory. Never dispose of any sharp object in the regular trash containers.
3. All bio hazardous disposable glass items (i.e., slides, cover slips, Pasteur pipets, etc.) must be disposed of properly in the **Biohazard Sharps Container, NOT** the regular trash or waste bags.
4. Dispose of dissecting pins or other sharp objects in **Biohazard Sharps Container, NOT** in the Waste Container, waste bags, or regular trash.

BIO 5.5 Cleaning Dissecting Pans

1. All solid debris should be removed from the tray.
2. Dissecting pans should be washed with soapy water.
3. Dissecting pans should be sterilized with a 5:1 ratio of water and hypochlorite solution.
4. Allow the solution to sit in the pan for a minimum of 10 minutes, then rinse thoroughly and allow to air dry.

BIO 4.6 Cleaning Dissecting Tools

1. Scalpels, probes, and other related tools should be carefully wiped with a paper towel to remove solid debris.
    a. Place the paper towel into the waste bag containing discarded body parts and tissues.
2. Tools may be placed in a 5:1 ratio of water and hypochlorite solution or a similar alcohol solution to clean and sterilize them.

3. Tools should be allowed to sit in the solution for a minimum of 90 minutes; leaving them

in the solution overnight is preferable.

4. If necessary, dissecting tools may be autoclaved.

BIO 6: Experiments with Bacteria and Fungi

This section pertains primarily to the use of viruses, bacteria, and other microscopic organisms. The handling of these pathogens is treated in 29CFR 1910.1030. This publication covers definitions, exposure control, specific procedures and protocols to comply with the regulations, precautions for specific pathogens, signs, labels, training, and record keeping. Proper laboratory technique is the basis for all cautions in this section.

Essential equipment for working with microorganisms includes:

1. Sterilization equipment (autoclave, heat sterilizer, or pressure cooker) for media preparation, sterilization of glassware and equipment, and decontamination of disposable material
2. Sterile transfer equipment (micropipettes with disposable tips or sterile pipets) for safe transfer of microorganisms
3. Adequate work space and equipment to prepare media
4. Proper storage facilities, including refrigeration and incubation equipment
5. Supplies for cleaning up and disinfecting work areas
6. Pipets Due to the nature of microorganisms, the use of disposable pipets, pipet tips, dishes and culture plates, etc. is recommended. If you use nondisposable glassware, take care to properly decontaminate it.
7. Special trash containers for all cultures for proper sterilization and disposal
8. Petri dishes for use with noninfectious materials

BIO 6.1 General Considerations

1. Disinfect work surfaces at least once a day (or after each class), and after any spill of active cultures.
2. NEVER pipette by mouth. Pipetting bulbs or pumps should always be used.
3. Wash hands with antibacterial soap when entering and prior to leaving the laboratory, and any time viable cultures are handled.
4. Wear eye protection, aprons, and gloves.

5. Cultures should be kept to the minimum size and number required to do the job.

BIO 6.2 Incubation Temperature

The incubation temperature should be restricted to an upper limit of 30°C to reduce the danger of isolating pathogens adapted to human body temperature.

BIO 6.3 Culturing from the Environment

MICROORGANISMS MAY NOT BE CULTURED FROM THE ENVIRONMENT IN ANY RCSS LAB UNLESS PRIOR PERMISSION IS OBTAINED FROM DAWN PHILLIPS, ED.S.

BIO 6.4 Culturing Commercial Organisms

1. **PATHOGENIC BACTERIA MAY NOT BE CULTURED IN ANY RCSS LAB.**
2. Only pure cultures of nonpathogenic microorganisms should be used in experiments.
3. Petri dishes passed around the classroom for inspection of cultures should be bound together with transparent tape.
4. Any petri dish that contains fungus should be taped shut.

BIO 6.5 Special Concerns in the Study of Fungi and Molds

1. Only commercially prepared fungal and mold cultures are allowed in RCSS labs.
2. **MOLDS AND FUNGAL SPORES MAY NOT BE CULTURED FROM THE ENVIRONMENT** due to the risk of dangerous infections in individuals with compromised immune systems, asthma, and chronic illnesses.

BIO 6.6 Loops

1. Wire loops used for transferring bacteria cultures should be flamed until the *entire* wire is **red** hot before and after each transfer is made.
   a. Inoculating loops must be used with care.
   b. A hot loop inserted into a liquid may cause spattering. Loops should be allowed to cool before insertion into liquids.

c. The procedure may require the use of more than one loop so that as one is being used, others are cooling.

2. When a contaminated loop is inserted into a flame for sterilization, an aerosol may be generated by the boiling and volatilization of the material before the flame can kill all pathogenic microorganisms.

BIO 6.7 Bunsen Burner Safety Guidelines

Bunsen burners present fire hazards. They produce an open flame and burn at a high temperature, and as a result, there is potential for an accident to occur. For the safety and convenience of everyone working in a laboratory, it is important that the following guidelines be observed.

1. Remove all papers, notebooks, combustible materials and excess chemicals from the area.
2. Tie-back any long hair, dangling jewelry, or loose clothing.
3. Inspect hose for cracks, holes, pinch points or any defect and ensure that the hose fits securely on the gas valve and the burner. Replace all hoses found to have a defect before using.
4. Notify others in the laboratory that the burner will be in use.
5. Have the sparker/lighter available before turning on the gas.
6. Utilize a sparker/lighter with extended nozzle to ignite the burner. Never use a match to ignite a burner.
7. Adjust the flame by turning the collar to regulate air flow and produce an appropriate flame for the experiment (typically a medium blue flame).
8. Do not leave open flames unattended and never leave the laboratory while the burner is on.
9. Shut off gas when its use is complete.
10. Allow the burner to cool before handling. Ensure that the main gas valve is off before leaving the laboratory.

BIO 6.8 Spills & First Aid

1. **A spill kit should be prepared prior to starting microbiology labs. It should include all items required to clean up a spill, including disinfectant, paper towel, gloves and plastic bags and containers for disposal.**
2. Students must report all spills to the teacher.
3. Only the teacher or laboratory safety manager should be allowed to clean up such spills.

4. When cleaning spills disposable gloves must be worn.

BIO 6.8.1 Liquid Spills

1. Small (less than 1.0L) liquid spills should be covered with paper towels soaked in disinfectant (e.g. Sodium Hypochlorite with 1 % available chlorine) for at least 20 minutes.
    a. The area should be cleaned with fresh paper towels soaked in disinfectant.
    b. ALL paper towels should then be placed in a biohazard bag for disposal
    c. Make sure the bag is sealed and labeled.
    d. Notify Facilities and Maintenance for pick-up and disposal. Document the date pick-up was requested and the date it occurred**.**
2. In the event of a large spill (1.0L or more):
    a. Evacuate the room immediately.
    b. Contact an administrator and then contact Facilities and Maintenance and provide the following information
        - Your name
        - The name of the school
        - Location of spill
        - Content of spill
        - Amount of the spill
        - Time of spill

BIO 6.8.2 Spills on the Body

1. The teacher must be informed immediately.
2. Contaminated clothing should be removed and the affected area washed vigorously with soap and water.
3. Medical attention may be sought if required.
4. The incident must be documented in the first aid &/or OHSW records.
5. Contaminated clothing must be disinfected before washing.

BIO 6.9 Contaminated Broken Glassware

1. Contaminated broken glassware should never be picked up directly with the hands.
2. It should be cleaned up using aids such as brush and dustpan, forceps or cotton wool swabs.
3. Follow the procedure for liquid spills.
4. All aids must be disinfected following use

BIO 6.10 Sterilization

1. Broth cultures should be sterilized by either autoclaving or adding a suitable disinfectant (SEE SECTION BIO **8.1 Disinfectants).** Once sterilized it may be poured down the sink.
2. If you are trying to sterilize soil samples or large volumes of culture, continue with the following procedure:
   a. Wait one day for any resistant spores to leave the resting stage and begin to grow.
   b. Sterilize a second time. Wait one day. Sterilize a third time.
   c. **All resistant spores should by now be killed. The plate may be safely opened for cleaning or discarded in the regular trash.**

BIO 6.11 Used Petri Dishes

1. **Note: Most plastic containers and equipment, such as plastic petri dishes, are not autoclavable.**
2. Autoclaves, heat sterilizers, and pressure cookers should be run only by the teacher or professional aide or by the student aide if he/she is under the direct supervision of the teacher or professional aide. The teacher, professional aide, and student aide should be knowledgeable about the operating instructions of the pressure cooker, autoclave, or heat sterilizer.
3. Prior to disposal the Petri dishes should be taped shut with masking tape (**do not use duct tape**). Once the Petri dishes have been taped shut, they should not be opened again.
4. To sterilize plates before cleaning or disposal, follow these steps:

a. The teacher should carefully read instructions before using an autoclave or pressure cooker.
    - If using a pressure cooker, make sure the safety valve is in good working order.
    - Materials may be sterilized by using 15 pounds of pressure at 121°C for 20 minutes.
    - Use approved eye protection and allow the pressure to return to zero before removing the cover or door.
    - Open the stop cock on a pressure cooker and wait until the hissing stops before releasing any clamps. Lift the lid so it is tilted away from the teacher to protect from heat and steam.
b. Autoclave the unopened plates in the usual manner. Usually, steaming under pressure of 15 pounds per square inch for 15 to 20 minutes kills the majority of microbes.
c. If autoclaves or pressure cookers are not available or large enough an alternative is to bleach the plates.
    - Saturate the plates with a 20% or "1 in 5" household bleach solution (in other words, 1 part bleach and 4 parts water). Let them sit and soak overnight in the bleach solution before disposing of them.
d. **Please note that the bleach solution is corrosive and needs to be properly disposed of after use.**
    - The bleach solution should be stored in a Hazardous Waste Container that is appropriately labeled.
    - Facilities and maintenance should be contacted for pick-up and disposal. Document the date pick-up was requested and the date it occurred.

5. After the plates are removed, they should be sealed in a hazardous waste bag, labeled and dated.
6. Facilities and maintenance should be contacted for pick-up and disposal. Document the date pick-up was requested and the date it occurred.

BIO 7: DNA Study

Work with deoxyribonucleic acid (DNA) is at the core of many of the hands-on activities in molecular biology and biotechnology that have been introduced into the high school biology laboratory. The study of the chemical and physical properties of DNA often involves the spooling, isolation, enzymatic digestion, gel electrophoresis, and manipulation of bacterial cells to introduce new genetic information. Many such laboratory activities can be purchased as complete kits that provide documentation and guidelines helpful to both students and teachers. These kits are especially recommended for teachers who are not familiar with standard procedures in research laboratories. Safety, as always, is a crucial part of any molecular biology experience. **Research requiring containment is prohibited by federal law.**

BIO 7.1 Electrophoresis

Electrophoresis, a technique which separates molecules based on their electrical charge, is frequently used in today's laboratories. **Be aware that ALL components of an electrophoresis gel require an MSDS and that students and other individuals must be informed of all risks prior to use.**

BIO 7.1.1 Handling Electrophoresis Chambers

Precautions to prevent electrical shock and using electrophoresis apparatus safely include:

1. Turn the power off before connecting the electrical leads.
2. Connect one lead at a time using one hand only.
3. Insure that your hands are dry while connecting the leads.
4. Keep the apparatus away from sinks or other water sources.
5. Turn off power before opening lid or reaching inside chamber.
6. Don't override safety devices.
7. Don't run electrophoresis equipment unattended.

BIO 7.2 Electrophoresis Gels and Additives

Many of the commonly used electrophoresis gels are harmless, but the additives can be extremely hazardous.

BIO 7.2.1 Ethidium bromide

Ethidium bromide is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis. It is a mutagen and should be handled with caution, even when mixed in the gel.

BIO 7.2.2. Formamide

Formamide is also used as an RNA stabilizer in gel electrophoresis by deionizing RNA. In capillary electrophoresis, it is used for stabilizing (single) strands of denatured DNA. Also known as **methanamide**, is an amide derived from formic acid. It is a clear liquid which is miscible with water and has an ammonia-like odor. Formamide is highly corrosive on contact with skin or eyes and may be deadly if ingested. Inhalation of large amounts of formamide vapor may require medical attention. It is also a teratogen.http://en.wikipedia.org/wiki/Formamide Formamide should never be handled without proper safety attire including gloves and goggles. There is a small risk of decomposition into hydrogen cyanide and water.

BIO 7.2.3 Acrylamide

In recent years polyacrylamide gels have been prepared in some school laboratories to achieve the isolation of specific molecules by electrophoretic techniques. Schools are cautioned to cease this practice because acrylamide poses a potentially serious health hazard as a neurotoxin. This substance has been classified as 2B (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC).

Because there is a serious risk of inhalation exposure during the weighing of acrylamide powder for the preparation of gels, schools should purchase only pre-poured polyacrylamide gels from laboratory supply houses. The pre-poured gel presents less

health risk because the acrylamide has chemically reacted to form a solid gel. Once the gel has solidified and been rinsed, very little of the raw acrylamide remains. Gloves should be worn at all times to prevent dermal exposure to any residual acrylamide found on the gels. To avoid the hazard altogether, schools can purchase pre-poured gels made with acrylamide substitutes.

BIO 7.2.4 TAE buffer

TAE buffer is a buffer solution containing a mixture of Tris base, acetic acid and EDTA. In molecular biology EDTA is used in agarose electrophoresis typically for the separation of nucleic acids such as DNA and RNA. It is made up of Tris-acetate buffer, usually at pH 8.0, and EDTA, which sequesters divalent cations. It is extremely irritating to the skin, eyes, and the upper respiratory tract. It is easily absorbed through the skin and is a mutagen. It is harmful if swallowed or inhaled.

BIO 7.2.5 Stains

Bromophenol blue, Orange G, xylene cyanol, and Coomassie Brilliant Blue are just 4 of the many stains commonly used in electrophoresis. ALL stains have hazards associated with them; carefully read the MSDS for each stain you use and make sure that you and all other individuals take the necessary precautions when using them.

BIO 7.3 Electrophoresis Waste Disposal Guidance

The following three types of wastes are commonly generated from electrophoresis methods in RCSS labs:

BIO 7.3.1 Stock Solutions

1. Collect all electrophoresis stock solutions in an appropriately sized container. The container cap should be closed securely when the container is not in use.
2. Label all electrophoresis stock solutions. Indicate on the label that it is a liquid waste, and check off the appropriate constituent box on the label.
3. Contact Facilities and Maintenance for pick-up and disposal. Document the date pick-up was requested and the date it occurred.

BIO 7.3.2 Electrophoresis Gels and Contaminated Non-Sharp Debris, (i.e. gloves, tips, paper towels, etc.)

1. Collect electrophoresis gels and contaminated non-sharp debris screw-top pails with a clear plastic liner. The container lid should be closed securely when the container is not in use.
2. Label electrophoresis gels and contaminated non-sharp debris. Indicate on the label that it is a solid waste, and check off the appropriate constituent box on the label.
3. Contact Facilities and Maintenance for pick-up and disposal. Document the date pick-up was requested and the date it occurred.

BIO 7.3.3 Electrophoresis Buffer Solutions and Filtration

Buffer solutions containing must be collected for disposal by following the directions above for disposal of stock solutions.

BIO 73.4 Electrophoresis wastes containing acrylamide or polyacrylamide

Electrophoresis wastes containing acrylamide or polyacrylamide must be managed as hazardous waste.

BIO 7.3.4.1 Disposal of Hazardous Waste

1. **The use of sinks for the disposal of chemicals is strictly prohibited!**
    a. When rinsing glassware that contained chemical, discard the first rinse volume into the appropriate waste container.
    b. Subsequent rinses can be discarded to the sink.
2. Water/air reactive wastes are restricted by waste disposal companies and must be deactivated prior to disposal.
    a. This is particularly true of materials which ignite or release gases on contact with air or water.
3. Dispose of chemically contaminated paper and disposable clothing in approved solid waste containers.
4. Do not treat hazardous waste on-site.

5. Contact Facilities and Maintenance for pick-up and disposal. Document the date pick-up was requested and the date it occurred.

BIO 7.3.4.2 Record Keeping

1. Reassigned samples must be re-labeled with the new custodian's name and the date the waste was generated and stored.
2. Contact Facilities and Maintenance for pick-up and disposal. Document the date pick-up was requested and the date it occurred.

BIO 8: Disposal and Cleanup

BIO 8.1 Disinfectants

Disinfectants and antiseptics (disinfectants for use on living surfaces e.g. skin) vary in their ability to kill bacteria, viruses, fungi, spores and protozoa. Disinfectants should always be diluted and used according to the manufacturer's instructions. The Material Safety Data Sheet should also be consulted for specific protective equipment and ventilation requirements. The following types of disinfectants are suitable for use in schools.

BIO 8.1.1 Alcohols

Alcohols have good activity on bacteria, and fungi but less on viruses and poor activity on spores. 70% ethanol is rapid acting and dries quickly. 90% ethanol is good for viruses. **100% ethanol is NOT an effective disinfectant**. 60-70% Isopropyl Alcohol (Propan-2-ol) is also effective.

BIO 8.1.2 Chlorhexidine

Chlorhexidine has good activity on gram-positive bacteria but less activity on gram negative bacteria, viruses and fungi and poor activity on spores. It has low toxicity and irritancy and so is a good antiseptic. 0.5% for face - 4% for other skin. It is often combined with alcohol, which may dry the skin.

BIO 8.1.3 Sodium Hypochlorite

Household bleach has good activity on bacteria, fungi and viruses, but less activity on spores. Varying amounts of available chlorine in hypochlorite solutions are required for different purposes. They must be prepared fresh daily from the concentrated stock solution to ensure the correct level of available chlorine. 1% for spills, 0.25% for discard jars, 0.1% for cleaning benches and 0.05-0.1% for equipment and instruments.

BIO 8.1.4 Providone-Iodine

Tincture of Iodine as 10% aqueous or alcoholic solutions is also suitable as skin disinfectant but it stains.

BIO 8.1.5 Other

DO NOT USE quaternary Ammonia compounds as they are not effective disinfectants against many bacteria and viruses. Peracetic acid, aldehydes and phenolic disinfectants are considered too hazardous for use in schools.

BIO 8.2 General Cleanup

All contaminated items should be decontaminated prior to reuse or disposal. Items for reuse should be immediately placed in disinfectant and soaked according to the manufacturer's instructions, prior to washing. (e.g. 25% Sodium Hypochlorite solution, soaked overnight)

BIO 8.2.1 Glassware and Sharps

1. All biohazardous dissecting pins, scalpel blades, or other such items must be disposed of in the **Red Biohazard Sharps Container**, NOT in the regular trash.
2. All bio hazardous disposable glass items (i.e., slides, cover slips, Pasteur pipets, etc.) must be disposed of properly in the **Red Biohazard Sharps Container.**

BIO 8.2.3 Broth Cultures

1. Broth cultures should be sterilized by either autoclaving or addition of a suitable disinfectant. (e.g. enough sodium hypochlorite to bring the culture to 1% solution, left overnight).

2. Once sterilized it may be poured down the sink.

BIO 8.2.4 Incubator

Following use the incubator must be thoroughly cleaned and disinfected with an appropriate disinfectant. (e.g. 0.1% sodium hypochlorite)

BIO 9: Chromatography

1. Chemical splash safety goggles and aprons should be worn.
2. Only water baths or hot plates with water baths (and not open-flame fires) should be used for chlorophyll extraction. Extraction may also be accomplished by leaving the plant material in the solvents overnight at room temperature.
3. Only Pyrex or comparable glass tubes should be used.
4. Dissolving and developing solvents give off toxic vapors. They must be stored in closed containers and the room
5. Solvents are highly flammable and must not be used near an open flame.
6. Avoid skin contact when spraying the developing solvents.
7. Use a fume hood when appropriate.

BIO 10: Radiation/Radioisotopes

Activities involving radioactive material of any type is forbidden in RCSS laboratories.

BIO 11: Water Chemistry

Water chemistry kits contain a variety of hazardous chemicals. All necessary precautions must be taken in order to avoid injury.

1. Students must wear goggles, gloves, and closed toes shoes when performing water chemistry.
2. Students should be closely supervised to make sure they are following instructions.
3. Students should be provided with a hazardous waste container in which to place rinse water and analyzed samples.
    a. This container should the labeled with the name of the teacher, the contents, the approximate amount of solution, and the date the waste was generated.

b. Facilities and Maintenance should be contacted for pick-up and disposal. Document the date the request was made and the date on which the waste was picked up.

BIO 12: Animals in the Classroom

BIO 12.1 Introduction

The use of live animals in the classroom can help students understand and appreciate life processes. Before bringing animals into the classroom, teachers should check the school or school system policy. It is important to select animals that are appropriate to the instructional needs and are practical to maintain. Good safety procedures should be established for the protection of students from the hazards of classroom animals as well as to ensure the humane treatment of animals.

The humane treatment of animals in research and teaching is a sensitive issue. The Council of State Science Supervisors, the National Association of Biology Teachers, the National Science Teachers Association, the Humane Society of the United States, the Animal Welfare Institute, and the National Society for Medical Research all have established guidelines and position papers supporting the safe and humane treatment of animals used for the cause of science.

The following websites offer more information on this topic:

www.enc.org/csss/index.html - Eisenhower National Clearing House

www.nabt.org - National Association of Biology Teachers

www.nsta.org - National Science Teachers Association

www.hsus.org/programs/research/animals_education.html

www.animalwelfare.com - Animal Welfare Institute

BIO 12.2 Before You Bring Animals into the Classroom

BIO 12.2.1 Permission to Keep Live Animals on Campus

You must complete a **Richmond County School System Permission to Keep Live Animals on Campus (**Appendix A) and submit it to the Curriculum and Instruction Department attn. Science Curriculum Department. It will help you think through some necessary planning measures such as animal enclosure options, how the enclosure will be cleaned, and weekend, holiday and summer care arrangements.

BIO 12.2.2 Parental Notification

You must obtain **Parental Notification Forms** (Appendix A)**.** It is not recommended that students be permitted to handle any animal(s) or be given caring or cleaning duties without prior parental/legal guardian consent.

BIO 12.2.3 Hand Washing Education

You must educate all students, paraprofessionals, and adult volunteers on proper hand washing. If anyone does handle an animal, they should wash their hands with hot soapy water for at least 60 seconds (instant hand sanitizers should only be used in addition to proper hand washing, NOT IN LIEU OF).

BIO 12.2.4 Educational Purposes

Animals in the classroom must have an educational purpose. Classroom animals should be limited to animals that are bred in captivity, and necessary to achieve the learning objectives. Wild animals can be a source of infectious agents, parasites, and are likely to bite.

BIO 12.2.5 Healthy Animals

Make sure all animals are healthy**.** All potential classroom animals should be examined by a veterinarian prior to being introduced to a classroom. The animals should be up to date on all vaccinations recommended by the veterinarian, and follow all of the veterinarian's guidance on proper handling, habitat, feeding, care, and other conditions for the particular type of animal(s).

BIO 12.2.6 Allergies

Be aware of allergic reactions. Allergies and sensitivities of students should be considered before bringing any animal into the classroom, and students should be observed for signs that they are becoming sensitive to an animal (allergies can develop at any time). Please communicate with parents to determine what allergies and sensitivities are known.

BIO 12.2.7 Special Permits

Avoid animals requiring special permits. Some animals require a written permission from the local health department, the Georgia Department of Natural Resources, and/or the United States Department of Agriculture to be kept in a classroom setting. These include venomous and nonvenomous snakes, wild turtles, certain species of frogs, wild newts and salamanders, hogs, deer, cattle, alligators, crocodiles, caimans, wild fowl, and all domestic fowl. DO NOT attempt to keep any of the animals mentioned above.

BIO 12.2.8 Animals Not Allowed in Richmond County Schools

1. Farm animals excrete *E. coli* O157:H7, *Salmonella*, *Campylobacter*, and *Cryptosporidium* intermittently and in substantial numbers; therefore these animals are not appropriate unless meticulous attention to personal hygiene can be assured.
2. Mammals at high risk for transmitting rabies (e.g., bats, raccoons, skunks, foxes, and coyotes) are not appropriate as residents in the classroom.
3. Nonpsittacine birds (any bird other than parrots, parakeets, and cockatiels).
4. Inherently dangerous animals (e.g., lions, tigers, cougars, and bears).
5. Nonhuman primates (e.g., monkeys and apes).
6. Mammals at higher risk for transmitting rabies (e.g., bats, raccoons, skunks, foxes, and coyotes).
7. Aggressive or unpredictable animals, wild or domestic.
8. Stray animals with unknown health and vaccination history.
9. Venomous or toxin-producing spiders, insects, reptiles, and amphibians.

BIO 12.3 Housing and Caring for Your Classroom Animal(s)

BIO 12.3.1 Habitats

Ensure that a proper habitat can be kept for the animal(s) (free of drafts and harsh sunlight). Also consider what type of care the animal will receive over weekends, and during school breaks (paying close attention to building heat and air conditioning status during times when school is not in session).

BIO 12.3.2 Food

Store all animal food in rigid containers with tight fitting lids to prevent access to food by pests. Also, some animals require fresh foods that may require refrigeration, or live foods. Should this be the case, make sure you have necessary equipment before bringing the animal in to the classroom. Food and water bowls should be thoroughly scrubbed and rinsed with hot soapy water.

BIO 12.3.3 Enclosures

Animals should be housed in an enclosure constructed from a nonporous material that is easily cleanable. Cleaning of animal(s) enclosures should be done as often as necessary to keep the animal healthy, prevent odors from building up, and eliminate any unsanitary conditions. It should be noted that cleaning and disinfection may be necessary as often as daily, however it should be done weekly at a minimum.

BIO 12.3.4 Sanitation

Enclosures should be sanitized after each cleaning with a fresh bleach solution (4oz of 5.25% unscented chlorine bleach to one gallon of water) OR a quaternary ammonia solution at a dilution suggested by the manufacturer for food service uses (NEVER MIX CHEMICALS!!!). As animals can be sensitive to sanitizers, care should be taken in adequately rinsing and drying the enclosure before putting the animal back in the enclosure. Some pathogens will not be killed by the sanitizers, but may be removed by rinsing thoroughly with water (this will also remove residual amounts of sanitizers). An animal's sensitivity is not an adequate reason to avoid the use of sanitizers.

Animal enclosures must never be cleaned in plumbing fixtures used for food service, drinking water, or hand washing purposes. After cleaning the enclosure, the fixtures used to clean the enclosure should also be cleaned and sanitized.

BIO 12.3.5 Security

All animal(s) enclosures should be securely covered and locked if possible. This will help protect the students and animals from one another by discouraging unsupervised handling and reducing potential of escape.

BIO 12.3.6 Aggressive Animals

It should be noted that any animal may behave aggressively, naturally aggressive species, and animals that are unusually aggressive or those displaying odd or uncharacteristic behaviors for their species should be removed immediately. Animals capable of causing substantial injury through aggressive or defensive reflexes should also be avoided (i.e. snapping turtles, venomous snakes, poisonous frogs, large birds).

BIO 12.3.7 Injured and Sick Animals

Animals that are injured or in poor health should be removed from the classroom immediately and given proper care. It should be noted, however that even animals that are or appear to be in good health can still shed potential pathogens.

BIO 12.3.8 Animal-Specific Guidelines

BIO 12.3.8.1 Invertebrates

1. Invertebrate animals are often used for observation and learning activities.
    a. Teachers should obtain manuals available from biological suppliers.
    b. These manuals are inexpensive and serve as a complete guide to maintaining and studying the organisms in the classroom.
2. If experiments are done with fruit flies, take care in quieting them and/or killing them.
3. **ETHER AND/OR TRIETHYLAMINE $(C_2H_5)_3N$ MAY NOT BE USED TO ANESTHESIZE OR EUTHANIZE ANY ORGANISM IN AN RCSS LABORATORY!**

4. Place the fruit flies in a Petri dish, gently covering them with cotton, and then invert the dish for examination under the dissecting microscope.
    a. Refrigerate culture jars and place “chilled” flies on a Petri dish over ice.
    b. Anesthetizing kits also may be used.
        - FlyNap® kits containing relatively harmless components may be purchased from biological supply companies.
    c. Any anesthetic should be used in a properly ventilated room according to the supplier.

BIO 12.3.8.2 Vertebrates (Nonhuman)

1. Do not take vertebrates from the natural environment.
    a. Most municipalities prohibit the removal of vertebrates from the natural environment because doing so upsets nature’s balance and may introduce unwanted microorganisms or diseased animals into the classroom.
2. Obtain animals from a certified disease-free source such as a biological supply house or a certified breeder.
3. When studying developing chicken embryos, do not use any embryos that are more than 18 days old.
    a. Do not work with virus-infected eggs.
    b. Dispose of dead embryos, which may carry pathogenic bacteria. Follow appropriate hazardous waste guidelines.
4. Do not give away or sell any animals, including baby chicks.
5. Do not release animals that are not indigenous to the area into the environment.
    a. Release of indigenous animals must be approved by the State Department of Natural Resources.

BIO 13: Plants in the School

Plants can be used effectively to provide a living laboratory for high school science instruction. By providing experiential learning opportunities, science educators can help students to develop the kind of reasoned thinking that will result in responsible decision-making regarding human/ecosystem interaction. However, certain plants can trigger severe allergic reactions in the form of skin rashes and breathing difficulties in susceptible children. The following guidelines will help teachers determine how to best use plants as effective teaching tools.

BIO 13.1 Poisonous Plants and Plants with Spines

1. Teachers should confine their lesson on poisonous plants (poison ivy, poison oak or poison sumac) to pictures.
2. Cacti and other plants with spines should not be kept in the classroom. Spines can become embedded under the skin and become infected if not removed correctly.
3. When using an outdoor learning area, examine the site for the presence of poisonous plants. When visiting these sites, carefully monitor the children to keep them away from the poisonous plants.
4. Children should not put any plants or plant parts in their mouths.

BIO 13.2 Plants in the Classroom

1. Only plants that are not hazardous to children and with which you are familiar should be used.
2. Breathing spores or pollen can cause reactions in some students. Provide face masks to susceptible students as needed.
3. When using commercial seeds treat them with care because they may have been treated with toxic fungicides.
    a. Have students wear gloves when handling them.
    b. Alternatively, you may obtain untreated seeds from local farm equipment stores or online at www.seedsavers.org/
4. Make sure potted plants are placed on sturdy surfaces in order to prevent pots from tipping over.
5. Do not allow students to move large potted plants.

6. Supervise children closely and
    a. make sure that they never place any plant or part of a plant in the mouth.
    b. make sure that they do not touch any part of their face; even 'safe' plants can have hairs, oils, and other compounds that can irritate the skin.
7. Make hand washing routine procedure after any laboratory activity even when working with plants.

BIO 13.3 School Gardens

Before you begin your school garden program, you will need to ensure that the soil, water, and working environment are safe for the students. Test the soil for contaminants, know what is in soil amendments, the water and plants, and develop rules for working in the garden. Talk with the students about these important issues, and let them help develop the rules to be used in the garden.

BIO 13.3.1 Preparation

BIO 13.3.1.1 Manure

1. Do not use fresh or unsterilized manure. All animal manure is potentially hazardous and may contain E. coli as well as other disease-causing pathogens.
2. Use only sterilized or fully composted manure.
3. Aged manure is **not** the same as composted, and can contain disease-causing organisms.
4. For more information, contact your local county health department or cooperative extension office.

BIO 13.3.1.2. Lead Contamination

1. Lead is naturally present in all soils, generally in low levels, but pollution can increase lead to harmful levels.
2. If you plan to plant an edible garden in an area that may have lead-contaminated soil, first test a soil sample for lead to determine if the soil is safe.
3. This is a critical issue for schools. Areas at risk for lead contamination include those with a history of construction before 1978, where lead may have leached

into the soil from paint or other materials, or a history of heavy exposure to traffic that at one time used fuel-containing lead.

4. To be on the safe side, it is **always** a good idea to test the soil for lead before beginning an edible garden project.
5. For information about lead testing, contact your local county health department or cooperative extension office.

BIO 13.3.2 Underground Pipes

1. Prior to developing the garden space, check with the school district or local utilities to determine if there are any underground pipes or cables that may be a potential problem.
2. If digging begins without getting an "all clear," the chance exists of running into electrical cables, water pipes, or a gas main.

BIO 13.3.3 Water

1. Make sure all water used in the garden – for watering plants, washing produce, and washing hands – is potable (drinkable) water.
2. Water for washing hands and produce should be **running** water to prevent recontamination.
    a. Some newly developed school grounds may have two separate water systems – one for potable water and one for recycled water (used for irrigation).
    b. Check with district administrators to determine if this is an issue at the school site.
    c. Then make sure only potable water is used in the school garden.
    d. For more information on recycled water, contact the **WateReuse Association.**

BIO 13.3.4 Building Materials

1. Do not use railroad ties, treated lumber, or old tires for garden boundaries, raised beds or anywhere in the garden.
    a. These items contain toxic chemicals that can leach into the soil and be absorbed by the plants.

b. Old railroad ties contain creosote, a carcinogen; treated lumber contains cyanide, a potent poison; and tires can leach petroleum products into the soil.

2. Contact your county cooperative extension office for more information.

BIO 13.4 Harmful Plants

Some plants and plant parts are poisonous. Others, such as poison ivy or stinging nettle, can irritate the skin. Teach children never to taste a plant unless an expert says it is all right to eat. Refer to the List of Hazardous Plants below for more information or contact the local poison control center.

BIO 13.4.1 Hay Fever Plants

Grasses	Flowering trees, especially Alnus
Ragweeds	(Alder) and Quercus (Oaks)

BIO 13.4.2 Dermatitis & Skin Rashes

Buttercup	Fennel	Poinsettia
Cactus-like	Gas Plant	Poison Hemlock
Euphorbias	Iris	Poison Ivy
Carrots	Jimson Weed	Poison Oak
Crown of Thorns	Lady’s-slippers	Poison Sumac
Datura	Nettles	Rock Poppy
Dill	Parsnips	Snow-on-the-Mountain

BIO 13.4.3 Plants That Are Harmful When Eaten

Amaryllis	Bleeding Heart	Castor Bean
Autumn Crocus	Bloodroot	Celastrus
Azalea	Boxwood	Cherry, Jerusalem
Baneberry	Burning Bush	Cherry, Wild Black
Belladonna	Euonymus	Chokecherry
Bittersweet	Buttercup	Crocus, Autum
Black Locust	Caladium	Daffodil

Daphne	Jimson Weed	Oleander
Datura	Laburnum	Peyote
Delphinium	Lantana	Philodendron
Dieffenbachia	Larkspur	Poinsettia
(Dumb Cane)	Lily-of-the-valley	Poison Hemlock
Digitalis	Lupine	Pokeberry
(Foxglove)	Marijuana	Potato Vines,
English Ivy	Marsh Marigold	Sprouts from
Euonymus	Mayapple	Tubers, Green
False Hellebore	Mistletoe	Tubers
Glory Lily	Monkshood	Privet
Golden Chain Tree	Morning Glory	Rhododendron
Holly	Mountain Laurel	Rhubarb Leaves
Hyacinth	Mushrooms, Death	Skunk Cabbage
Jack-in-the-pulpit	Angel (Amanita)	Taxus
Jequirity Pea	Narcissus	Water Hemlock
Jerusalem Cherry	Nightshade	Wisteria

BIO 13.5 Disposal

1. Exotic plants should never be released into the environment where they may compete with local plants
    a. Such a release can result in an imbalance to the natural flora.
2. Native plants normally do not present a problem for the local environment.
    a. Such plants should be discarded in a manner consistent with school policy and local ordinances.

BIO 14: Greenhouse Maintenance and Operation

For schools that have greenhouses available for biology and environmental classes, the following guidelines are intended to aid in their smooth maintenance and operation. These guidelines, which supplement applicable school regulations, apply to any individual working in the greenhouse area, student or teacher.

BIO 14.1 Guidelines

The following guidelines are designed to ensure that all greenhouse components are functioning at an adequate level for optimum plant growth and at a safe level for student use.

1. Check water lines, heating system, fans, and temperature control. These are usually routine procedures that can be checked by the school maintenance staff.
2. Make sure all automatic equipment is functional and accurate.
3. Clean tools after use and store them appropriately.
4. Instruct students in the proper use of, and conduct in, the greenhouse area. It is recommended that students be required to obtain the teacher's permission to enter the greenhouse.
5. Rules which apply to the greenhouse must be clearly stated and explained to students. It is important that students understand that the rules are for the safety of both the organisms in the greenhouse and the students.
6. Students and teachers should be cautioned to handle fertilizer carefully to avoid inhaling the dust.
7. Wash fruits and vegetables before studying. Eating fruits or vegetables that have been cultivated in the greenhouse is not recommended unless special care has been maintained in the growth of such plants.
8. Inspect the greenhouse periodically to prevent the cultivation of unlawful plants such as marijuana.
9. Maintain all equipment so as not to impede the safe movement into and about the greenhouse. For example, hose lines should be properly mounted and stored to keep the floor clear.
10. Wash hands thoroughly after working in the greenhouse.
11. Make sure to maintain adequate ventilation.

BIO 14.2 Pesticides

1. Use organic methods of pest control when possible.
2. Make sure to maintain adequate ventilation. Ventilation is especially important when using pesticides.
3. Use the least toxic pesticides. Note signal words found on pesticide labels:
    - Danger = highly toxic.
    - Warning = moderately toxic.
    - Caution = slightly toxic.
    - No caution or warning = relatively non-toxic.
4. The safest insecticides contain pyrethrins.

BIO 14.3 Using Pesticides

1. Pesticides are toxic and should be used only according to instructions on container labels.
2. Pesticides can enter the body through the skin, mouth or nose. Before using pesticides, cover up exposed skin with water-repellent clothes and boots.
3. Wear a wide-brimmed hat and a full-face shield.
4. Use unlined, natural rubber gauntlet gloves.
5. Use exhaust hoods and ventilation systems when spraying.
6. Do not touch the mouth or face with hands, forearms or clothing.
7. Do not expose a drink or food container to pesticides.
8. Wash hands and face immediately after applying pesticides.

BIO 15: Special Concerns

BIO 15.1 Thermometers

1. **MERCURY FILLED THERMOMETERS ARE NOT ALLOWED IN RCSS SCHOOLS.**
2. Alcohol laboratory thermometers should be used in general laboratory activities.
3. For more advanced applications, a digital laboratory thermometer may be used.
   a. Care should be taken to choose a digital thermometer that contains a changeable battery; some are not changeable.
   b. The battery is a button cell battery and may contain 5-50 mg of mercury; it should be recycled through a battery collection program.

BIO 15.2 Using Microtomes

Microtomes are commonly instruments used in laboratories to section tissues. These devices pose potential hazards to users during sectioning and/or the cleaning process. It is strongly recommended that teachers use prepared slides whenever possible.

BIO 15.2.1 Training

1. Training must be documented and provided by a knowledgeable and responsible person within the laboratory before any work is completed.
2. Standard Operation Procedures should be made available to all users and posted near the point of operation.

BIO 15.2.2 Appropriate PPE

A lab apron, chemical splash goggles, and nitrile gloves must be worn while handling tissues to be sectioned.

BIO 15.2.3 Sharpness of Blade

1. A microtome blade is extremely sharp and must be handled carefully.
2. The rotary handle of the microtome must always be set in the locked position when changing a paraffin block or the blade.
3. A new blade should be placed in the blade holder and clamped before the rotary wheel lock is released.

4. Wrist guards should be added where possible.
5. Once the blade is seated and secured the rotary wheel lock can be released and the knife and holder advanced to the specimen block.
6. If adjustments need to be made to the specimen, remove the blade from the housing.

BIO 15.2.4 Removal of the blade

1. Disposable blades must always be removed using forceps or a similar instrument.
2. Do not remove the blade holder from the microtome with a blade present or transport the housing with the blade present. .
3. Dispose of used microtome blades in the Biohazard Sharps Container.
4. The Biological sharps container must be kept adjacent to the microtome to reduce the distance that a blade would be moved
5. For microtomes with reusable blades cut resistant gloves must be used when removing and sharpening the blade.

BIO 15.2.5 Microtome cleaning

1. Before the microtome is cleaned, the rotary wheel must be locked and the blade removed from the blade holder.
2. Use caution other components of the microtome may also have sharp edges.
3. Cut resistant gloves must be worn under nitrile gloves when a microtome is being cleaned.
4. Follow manufacturer's recommendations for cleaning the microtome.
5. All use cleaning materials and solutions should be treated as hazardous waste and secured in the appropriate containers.
6. Contact Facilities and Maintenance for pick-up and disposal. Document the date the pick-up was requested and the date the pick-up occurred.

BIO 15.3 Using Centrifuges

BIO 15.3.1 Operating Procedures

1. Check tubes for cracks/chips.
2. Use matched sets of tubes, buckets, etc.
3. Tightly seal all tubes and safety cups.
4. Ensure that rotor is locked to spindle and bucket seated.
5. Close lid during operation.
6. Allow to come to complete stop before opening.

BIO 15.3.2 Safe Operation

1. Use safety cups whenever possible.
2. Disinfect weekly and after all spills or breakages.
3. Lubricate O-rings and rotor threads weekly.
4. Do not operate the centrifuge without the rotor properly balanced.
5. Do not use rotors that have been dropped.
6. Contact your centrifuge rep for specific information.
7. If you suspect leakage occurred from the centrifuge, leave the area, do not open the centrifuge for at least 30 minutes to allow aerosols to settle, then access situation while wearing appropriate PPE and taking necessary precautions.

BIO 15.4 Human Studies

1. **Any lab activity involving the use of bodily fluids or tissues collected from students is forbidden in RCSS laboratories.**
2. Non-invasive, non-stressful laboratory activities using students as experimental organisms are encouraged.
   a. These include physiological measurements such as, pulse, heart rate, breathing rate, hearing, sight, etc.
   b. These activities need to be closely supervised by the teacher due the risk of physical injury, heart palpitations, shortness of breath, overheating, fainting, and death

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BIO 16: Chemical Safety in the Biology Laboratory

All teachers should be familiar with the RCSS Chemical Management policy that addresses how chemicals should be properly stored, labeled, and secured, as well as who should have access to these chemicals and chemical storage locations. The following guidelines are provided for teachers in order to reduce the risk of chemical accidents and ensure that chemicals and products in their schools are stored and handled safely.

BIO 16..1 Procurement of Chemicals

1. Prior to ordering, determine whether the chemical is in stock.
2. Order only quantities that are necessary for the project. Remember: "**Less is better**".
3. Upon receipt of the chemical, make sure the date received and the owner's initials are on the label.

BIO 16.2 Labeling of Chemical Containers

1. No unlabeled substance should be present in the laboratory at any time!
2. Use labels with good adhesive.
3. Use a permanent marker (waterproof and fade resistant) or laser (not inkjet) printer.
4. Print clearly and visibly.
5. Replace damaged, faded, or semi-attached labels.

BIO 16.2.1 Commercially Packaged Chemicals

1. Verify that the label contains the following information:
    a. Chemical name (as it appears on the MSDS)
    b. Name of chemical manufacturer
    c. Necessary handling and hazard information
2. Add:
    a. Date received
    b. Date first opened
    c. Expiration or —use by date (if one is not present)

BIO 16.2.2 Secondary Containers and Prepared Solutions

1. When a material is transferred from the original manufacturer's container to other vessels, these vessels are referred to as ―secondary containers.
2. Label all containers used for storage with the following:
   a. Chemical name (as it appears on the MSDS)
   b. Name of the chemical manufacturer or person who prepared the solution
   c. Necessary handling and hazard information
   d. Concentration or purity
   e. Date prepared
   f. Expiration or ―use by date

BIO 16.2.3 Containers in Immediate Use

1. These chemicals are to be used within a work shift or laboratory session.
2. Label all containers in immediate use with the following:
3. Chemical name (as it appears on the MSDS)
4. Necessary handling and hazard information

BIO 16.2.4 Chemical Waste

All containers used for chemical waste should be labeled with the following:

1. HAZARDOUS WASTE
2. Chemical name (as it appears on the MSDS)
3. Accumulation start date
4. Hazard(s) associated with the chemical waste
5. Date generated
6. ***Complete the Waste Disposal Form for removal of all waste (chemicals and broken glass) at the end of each semester. The Science/STEM Coordinator (Dr. Chaundra Creekmur; creekch@boe.richmond.k12.ga.us) will facilitate removal.***

BIO 16.3 Material Safety Data Sheets (MSDS)

1. There must be an MSDS on file for every chemical compound in use in the lab.
2. At a minimum, MSDS information should be located in all chemical storage rooms and cabinets and in a central place within the school (away from the chemicals), as well as a central location for the school district.

3. A copy must be kept in an area that is accessible to all individuals during periods of building operations.
4. If no MSDS is available for a product because 1) the manufacturer no longer exists; or 2) the manufacturer cannot be identified from the label that material should be considered hazardous waste and disposed of in a manner consistent with federal and state regulations.

BIO 16.4 Proper Chemical Storage

Guidelines for chemical storage must follow **O.C.G.A 45-22-2, O.C.G.A. 25-2, OSHA Standard 29 CFR 1910, and NFPA 45: Standard on Fire Protection for Laboratories Using Chemicals** and **NFPA 30: Flammable and Combustible Liquids Code.**

1. Hazardous chemicals in schools should be stored in accordance with MSDS specifications
2. Chemicals should not be stored in areas that are occupied by or accessible to students, such as classrooms or restrooms; they should preferably be stored in a central, secure location.
3. Organize chemicals first by **COMPATIBILITY**—not alphabetic succession (refer to section entitled Shelf Storage Pattern). Store alphabetically within compatible groups.

BIO 16.5 Proper Storage and Disposal of Chemical Waste

The following guidelines are provided to schools and administrators and should be used for storing and disposing of hazardous waste:

BIO 16.5.1 Chemical Waste Labeling

1. Clearly and permanently label each container as to its contents and label as hazardous waste.
2. All containers used for chemical waste should be labeled with the following:
    a. HAZARDOUS WASTE
    b. Chemical name (as it appears on the MSDS)
    c. Accumulation start date
    d. Hazard(s) associated with the chemical waste
    e. Approximate amount
    f. Date generated

BIO 16.5.2 Segregation and Storage of Waste

1. Separate waste containers are required to properly segregate waste for disposal. The following waste categories should be used:

a. Chlorinated Solvents
b. Cyanides
c. Hexavalent Chrome
d. High pH Alkaline Solutions
e. Hydrofluoric Acid
f. Low pH Acidic Solutions
g. Nitric Acid
h. Non-Chlorinated Solvents
i. Oxidizers
j. Palladium
k. Reducing Agents
l. Sulfides

BIO 16.5.3 Storage Guidelines

1. Chemicals that are stored for disposal off-site should be placed in suitable closed containers and should be clearly marked with the contents. If the chemicals are a RCRA hazardous waste, the school must ensure that they are transported offsite for proper disposal.
2. Store all waste in containers that are in good condition and are compatible with their contents. Avoid using metal containers; certain chemicals can cause the metal to corrode and the container to leak.
3. Store waste in a designated area away from normal laboratory operations and to prevent unauthorized access. Store waste bottles away from sinks and floor drains.
4. Do not completely fill waste bottles; leave several inches of space at the top of each waste container. Securely cap all waste bottles.

BIO 16.5.4 Disposal of Hazardous Waste

1. **THE USE OF SINKS FOR THE DISPOSAL OF CHEMICALS IS STRICTLY PROHIBITED!**
    a. When rinsing glassware that contained chemical, discard the first rinse volume into the appropriate waste container.
    b. Subsequent rinses can be discarded to the sink.

2. Water/air reactive wastes are restricted by waste disposal companies and must be deactivated prior to disposal.
    a. This is particularly true of materials which ignite or release gases on contact with air or water.
3. Dispose of chemically contaminated paper and disposable clothing in approved solid waste containers.
4. Do not treat hazardous waste on-site. Exception: Acids may be neutralized with sodium bicarbonate in a 50-50 ratio by weight.
5. Contact Facilities and Maintenance for pick-up and disposal. Document when pick-up was requested and when it occurred.

BIO 16.5.6 Record Keeping

1. Reassigned samples must be re-labeled with the new custodian's name and the date the waste was generated and stored.
2. A waste management log must be maintained and should indicate how and when the waste was generated, how and when it was isolated and stored, by whom it was generated and stored, and date and method in which it was disposed.

BIO 16.6 Drug-Related Items

1. **THE FOLLOWING SUBSTANCES ARE NOT ALLOWED IN RCSS LABS!**

a. **Acetaldehyde**	f. **Histamine**
b. **Adrenalin**	g. **Nicotine**
c. **Colchicine**	h. **Testosterone**
d. **Caffeine**	i. **Thiourea**
e. **Ethyl Alcohol (grain)**	j. **Tobacco**

BIO 17: Fire Hazards

Fire is a real danger in any laboratory setting, and all teachers need to be aware of how to prevent fires. In the event a fire does occur, teachers need to know how to respond appropriately. The following information is provided as guidance in preventing or combatting fires in the science laboratory.

BIO 17.1 Preventing Burns and Fires

BIO 17.1.1 When planning to heat materials or use open flames

1. instruct students on STOP DROP AND ROLL in the event clothing catches fire
2. make sure students know how to evacuate the classroom in the event of a large fire

3. know the location of the nearest fire extinguisher and make sure you know how to use it.
4. have a bucket of sand or a fire blanket nearby in the event that the nearest fire extinguisher too far outside of the classroom.

BIO 17.1.2 When heating materials

1. **DO NOT USE ALCOHOL BURNERS! T**hey are extremely hazardous. Safer alternatives to alcohol burners include candles and hot plates.
2. **DO NOT USE STERNO HEATERS!**
3. make sure that the area surrounding a heat source is clean and has no combustible materials nearby.
4. do not allow students to work with hot materials, such as very hot water.
5. do not use household glass. Use only borosilicate laboratory glassware, such as Kimax™ or Pyrex™ when heating substances.
6. do not heat common household liquids, such as alcohol or oil; these are flammable and should not be heated. Heat only water or water solutions.
7. handle all hot materials using the appropriate type of tongs or heat resistant gloves (those made of asbestos or thick silicon rubber).

BIO 17.1.3 When using Hot Plates

1. do not use hotplates designed for use in home kitchens. Use only laboratory type hot plates. These are sealed against minor spills.
2. do not place the hot plate on paper or wooden surfaces.
3. place the hot plate in a location where a student cannot pull it off the worktop or trip over the power cord.
4. never leave the room while the hot plate is plugged in, whether or not it is in use.
5. keep students away from hot plates that are in use or still hot, unless you are right beside the students and have given them specific instructions.

6. make sure that the hotplate is both unplugged and cool before handling a hotplate. You can check to see if a hot plate is still too hot by placing a few drops of water on the surface. If the water does not evaporate, it should be cool enough to touch.

BIO 17.1.4 When using open flames

1. use only safety matches. Make sure the matches are stored in a secure place between uses.
2. closely supervise students when they use matches. Make sure students are dressed properly (baggy clothes are tucked in, long sleeves are rolled up, smocks/aprons are properly tied) and have long hair/braids tied up.
3. closely supervise students when they use candles. Make sure students are dressed properly (baggy clothes are tucked in, long sleeves are rolled up, smocks/aprons are properly tied) and have long hair/braids tied up.
4. use tea candles that are short and wide, and cannot be knocked over in normal use.
5. place all candles in a "drip pan," such as an aluminum pie plate, that is large enough to contain the candle if it is knocked over.
6. never leave the room while a flame is lit or other heat source is in use.

BIO 17.2 In the event of a large, uncontainable fire

1. evacuate the classroom immediately.
2. locate and pull the nearest fire alarm.
3. notify public safety and/or administration about the fire. Make sure you include the location and source (chemical, paper, petroleum) of the fire.

BIO 17.3 In the event of a small, containable fire

1. identify the type of fire. The table below lists the four classes of fires and methods for extinguishing them:

Class	To Fight Fires Involving	Method to Extinguish
A	wood, paper, cloth	Use water or dry chemical extinguisher.
B	gasoline, alcohol, paint, oil, or other flammable liquids	Smother by using carbon dioxide or dry chemical extinguisher.
C	fires in live electrical equipment	Cut off power to electrical equipment. Use ABC or carbon dioxide fire extinguisher.
D	metals (Na, K, Mg, etc.)	Scoop dry sand onto fire.

2. Use the appropriate method to extinguish the fire.
3. File an incident report.

BIO 17.4 In the event a student's clothes catch fire

1. Roll the child on the floor to smother the fire.
2. Use a fire blanket if one is available.
3. Do not direct a carbon dioxide (CO_2) fire extinguisher at an individual because such extinguishers produce dry ice that can cause frostbite.
4. **DO NOT ATTEMPT TO ADMINISTER FIRST AID TO ANY BURNS THE CHILD MAY HAVE SUSTAINED!** Immediately notify the school administrator, school nurse, and public safety.

BIO 18: Electrical Hazards

BIO 18.1 Burns and Shock

1. Many electrical devices become quite hot while in use.
    a. In addition, "shorted" dry cells and batteries can produce very high temperatures.
    b. Students should never grasp a recently operated device or wiring without first checking for excess heat.
2. Students must be warned of the high death potential present even when the voltage is low.
    a. The severity of an electrical shock depends primarily on the amount of current to which a person is exposed.
    b. Since the current is related to the resistance and voltage, these two factors, as well as the part of the body involved and the duration of the contact, determine the extent of injuries to the victim.
    c. If the skin is wet or the surface broken, the resistance drops off rapidly, permitting the current to flow readily through the bloodstream and body tissues.

BIO 18.2 Electrical Apparatus

BIO 18.2.1 Batteries.

1. A battery is an unregulated source of current capable of producing large currents when resistance is low.
    a. When short-circuited, connecting wires can become very hot, raising the risk of burns. Short-circuited mercury batteries may even explode.
    b. Chemical leakage from batteries is a potential hazard, especially in the case of wet cells that contain caustic chemicals such as sulfuric acid.
2. Certain types of batteries are rechargeable while others are not.
    a. Carbon-zinc and nickel-cadmium type batteries can be recharged.
    b. Do not, however, attempt to recharge a completely dead carbon-zinc battery, a leaking or corroded battery, or any battery that carries a warning against recharging.

c. Such batteries can cause damage to the charger and may explode, causing personal injury. Lead-acid batteries can be recharged but produce explosive hydrogen gas during the process.
d. They should only be recharged in a well-ventilated area with an appropriate charger.

3. Do not discard any battery in the trash.
4. Contact Facilities and Maintenance for pick-up and disposal. Document the date of the request and the date the pick-up occurred.

BIO 18.2.2 Circuit Loads

1. Most school laboratory electrical circuits have a maximum power rating of 1,500 watts (if fuses are 15 amp) or 2,000 watts (if fuses are 20 amp).
2. The total power load on a circuit should not exceed these values.
3. The total load is the sum of the power ratings of all apparatus plugged into that circuit.
4. The individual power rating is usually found printed on a plate somewhere on the apparatus.

BIO 18.2.3 Extension Cords.

1. Use extension cords only when there is no convenient way to connect equipment directly to a receptacle.
2. If an extension cord must be used, it should be checked for damage, proper grounding, and electrical capacity.
3. An extension cord should be marked with its capacity in amperes and watts and the total load should not exceed these values.
4. If the cord is unmarked, assume that it is 9 amperes or 1,125 watts.
5. If an extension cord becomes very warm to the touch, it should be disconnected and checked for proper size.
6. In general, science laboratories should be equipped with sufficient receptacles to minimize extension cord use.

BIO 18.2.4 Fuses/Circuit Breakers.

1. Replace blown equipment fuses with fuses of the same amperage.
2. Replace fuses with the equipment unplugged.
3. Failure to use the correct fuse can cause damage to equipment and overheating.
4. Frequent blowing of circuit fuses or tripping of circuit breakers usually indicates that the circuit is overloaded or a short exists.
5. Circuit breakers and fuses that are tripped or blown should be turned on or replaced only after the cause of the short or overload is removed from the circuit.

BIO 18.2.5 Grounding

1. Use grounded 3-prong plugs when available.
2. If the outlet is 2-prong, use an adapter and secure the ground wire to the cover-plate screw on the outlet.
3. Any apparatus with a metallic case or exposed metal parts should be checked to make sure that the case is grounded.
4. Such ungrounded appliances should be retrofitted with a ground wire and three-pronged plug.
5. The use of ground-fault interrupters should be considered.

BIO 18.2.6 Power Cords.

1. Any power cord should be inspected periodically and replaced immediately if frayed or damaged.
2. Apparatus should be located to keep power cords away from student traffic paths.
3. When removing the cord from an outlet, the plug should be pulled, not the power cord.
4. Wet hands and floors present a hazard when connecting or disconnecting electrical apparatus.