WELCOME TO THE BIOLOGY DEPARTMENT'S LABORATORY SAFETY COURSE

JODIE DESHLER AND ALICIA MUELLING

This course is created for students and employees of the biology department to assist in the general knowledge of basic safety procedures in the laboratory.

This course will introduce several safety concerns including **hazard** codes, protective equipment, chemical useage and compatibilities, microbial and biohazard materials, and emergency procedures. This knowledge will enable you to become familiar with and comfortable in your laboratory surroundings.

Upon completion of the course you may be required to pass the final quiz posted on WebCT. For further instructions regarding the quiz please see your instructor or supervisor.

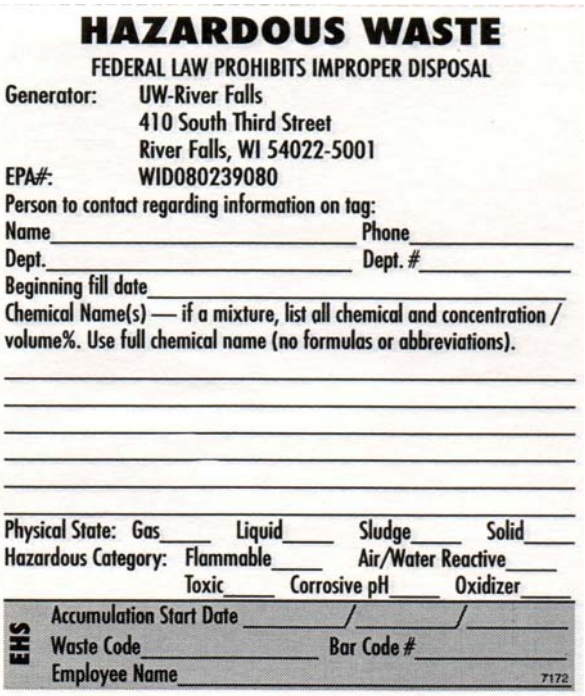
For optimal viewing, maximize your screen to 800 X 600 True Color (24 bit). This will allow you to see the entire page without difficulty. The bold, blue underlined words are linked to their definition in the glossary. If you need to clarify a word, click on it, and resume the course by clicking the back button.

HAZARD CODES

Hazard codes are very important in a laboratory setting; they reinforce safety standards and **protocols**. The specific colors and codes on the labels indicate what the particular hazard is. This gives high visibility to the chemical or solution being used and ensures safety. You must understand the significance of codes on container labels.

There are several labels with these colored codes in the laboratory. These labels include the **hazardous waste** label, the **National Fire Protection Association (NFPA)** labels, and original labels on chemical containers. These labels detail the level of protection needed for a specific **chemical** or **solution**. These labels provide safety information on **health** (H), **fire** (F), **reactivity** (R), and **personal protective equipment** (PE) required to work with chemicals or solutions.

The Hazardous Waste label used for Disposal



HAZARDOUS WASTE

FEDERAL LAW PROHIBITS IMPROPER DISPOSAL

Generator: UW-River Falls
410 South Third Street
River Falls, WI 54022-5001

EPA#: WID080239080

Person to contact regarding information on tag:

Name_____ Phone_____

Dept._____ Dept. #_____

Beginning fill date_____

Chemical Name(s) — if a mixture, list all chemical and concentration / volume%. Use full chemical name (no formulas or abbreviations).

Physical State: Gas____ Liquid____ Sludge____ Solid____

Hazardous Category: Flammable____ Air/Water Reactive____
Toxic____ Corrosive pH____ Oxidizer____

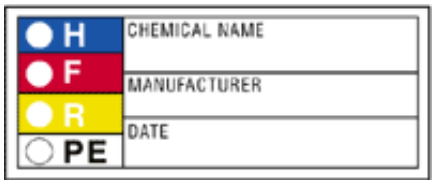
EHS

Accumulation Start Date ____/____/____

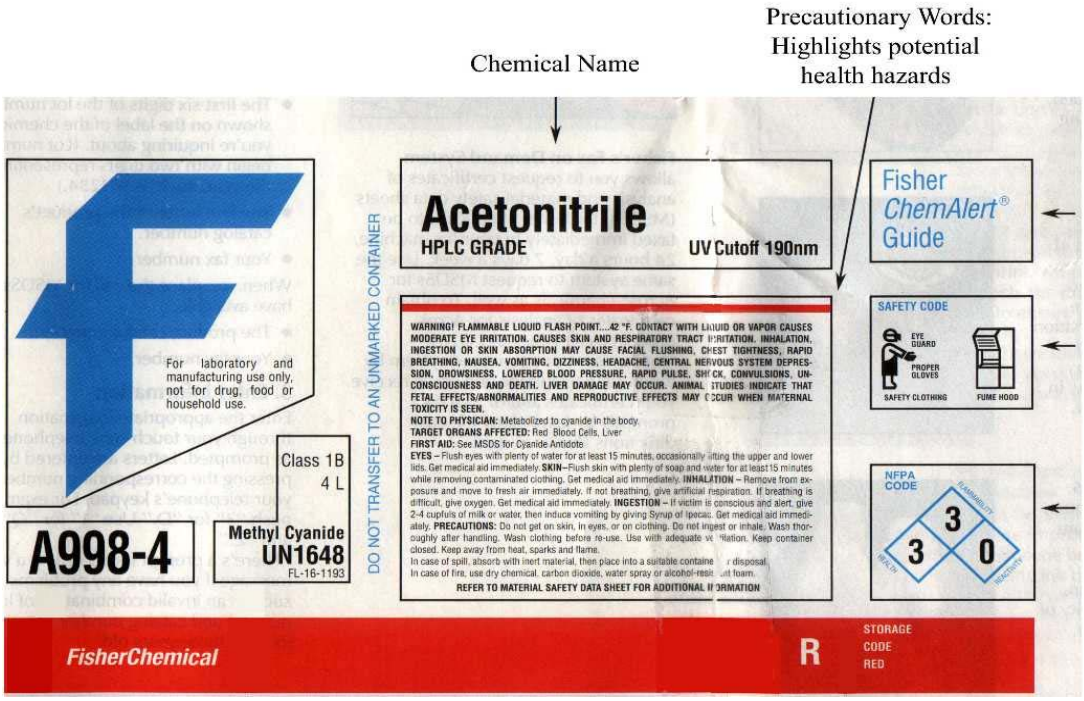
Waste Code_____ Bar Code #_____

Employee Name_____ 7172

National Fire Protection Association (NFPA) Label

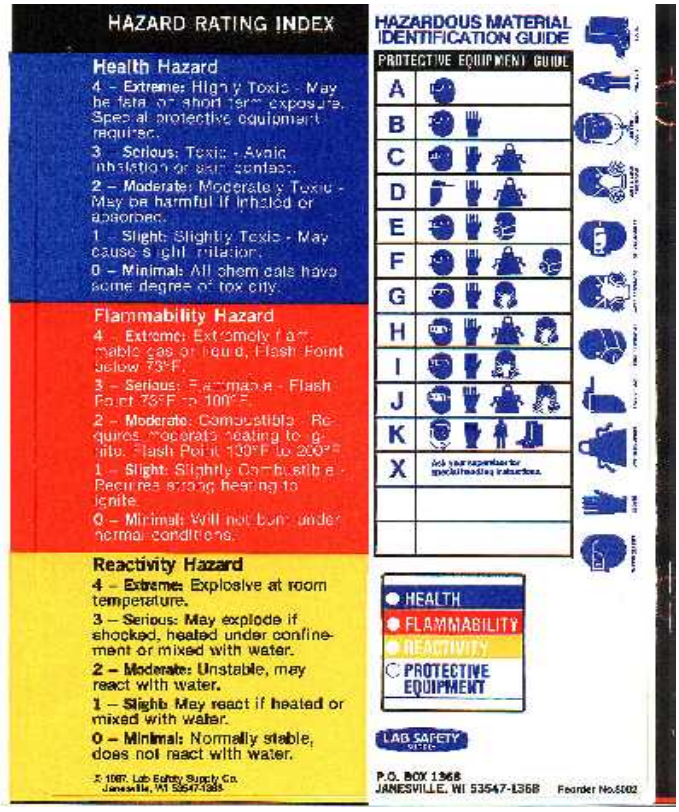


Original Container Label



All hazard categories listed on each type of label are indexed according to health, flammability, and reactivity levels. These are rated on a 0 to 4 scale, with 0 the least hazardous and 4 the most hazardous. Protective equipment (PE) has its own graphics or letter codes to explain the personal protective equipment needed. If you do not know these systems of safety, you are taking an unnecessary risk. Memorize the rating scale so that you have, at a glance, a very good indication of the hazards of that substance. All of these ratings are available, for your reference, on the hazard rating index chart posted in each laboratory. Know where to find this chart in every laboratory you work in.

Hazard Rating Index Chart



Material Safety Data Sheets (MSDS)

MSDS sheets include information on every chemical used in the department. These sheets can be found in the main biology office, room 414. They can also be found at Fisher Scientific MSDS on-line. If you need additional information on any chemical you can reference these sheets.

PROTECTIVE EQUIPMENT

Graphics, letters, or symbols are used to represent the type of safety equipment that is required for working with a specific chemical or solution. These symbols are found on the original container labels and NFPA labels (previous section). Understanding when and how to use protective equipment is also very important in the defense against exposure to hazardous materials.

The personal protective equipment used in the laboratory is critical for your safety. Protective equipment usage differs and depends on what chemical or solution you are using. Protective equipment is one of the best defenses

against injury or infection. You must understand what equipment to wear or use, how to use that equipment, and when you should use that equipment. There are five major ways to protect yourself in the laboratory: aprons, gloves, goggles, masks, and fume hoods.

A. APRON OR LAB COAT

This protects against splatters and spills from any material used in the laboratory. You must wear an apron when working with the variety of different chemicals and solutions so knowledge of when to wear it is fundamental. This will protect you against direct contact with or **residual** **contamination** from the substance. Aprons and lab coats can be found in room 420 of the biology department. Before leaving the lab, always remove your lab coat or apron to prevent transfer of a substance outside of the laboratory.

B. GLOVES

Gloves are made out of latex, nitrile, or other forms of non-latex rubber. They fit skin- tight to allow free movement and dexterity of fingers. It is **recommended** that you wear gloves whenever working in the laboratory, but it is **required** that you wear gloves when working with hazardous materials. Most of the gloves in the department are powder-free, vinyl gloves that are hypoallergenic. If you know you have a laytex allergy, pay close attention to the type of glove you use. Gloves are most directly involved with safety because hands are most directly involved with chemicals and solutions. Gloves are located in every laboratory and are easily accessible.

If you are not sure of the hazard level of the material, put gloves on!

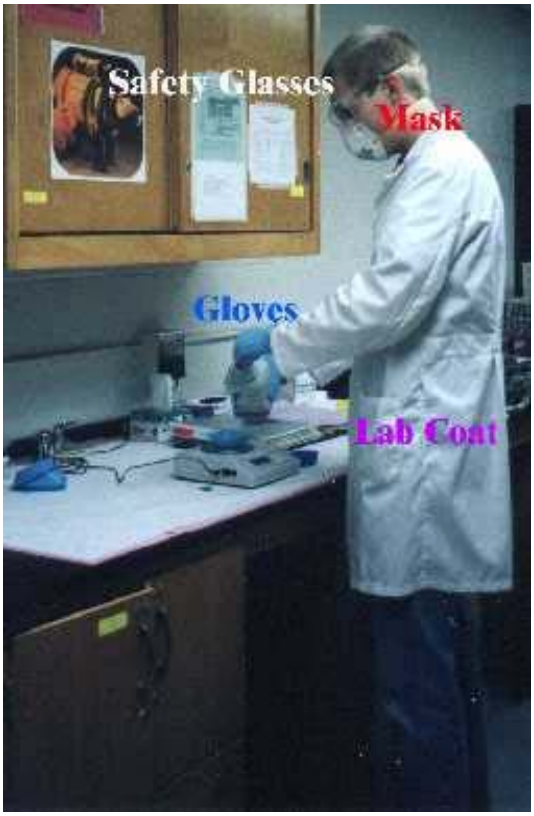
C. GOGGLES

These are protective eyewear. Eyewear can also include eyeglasses, but it is **recommended** that you wear goggles over eyeglasses to prevent splash accidents. You are **required** to wear protective goggles when working in the laboratory for certain experiments. Eyewear protects against contamination from substances that injure or infect the eyeball and cause vision loss. Goggles also protect against absorption of materials into the body via the eyeball. Contact lens wearers BEWARE. Contact lenses make it difficult to wash away substances accidentally introduced to the eye. They can also be ruined by chemicals and vapors. Contact lenses should not be worn in the laboratory. Protective glasses are available in most labs. If you have eyewear concerns, feel free to bring your own protective glasses.

D. MASKS

Masks are fitted coverings that are placed over the mouth and nose. They are used to filter out small chemical particles and other reagents in the laboratory. Masks filter out hazardous materials that enter the body via the respiratory tract and lungs.

Protective Equipment



E. FUME HOOD

A fume hood consists of a vacuum and a hood. This sequesters hazardous vapors inside the fume hood and draws them into a filter. The vapors are filtered and then disposed of properly. This is very important when working with solutions and chemicals that need to be handled carefully. When using the fume hood the vertically sliding glass door should be pulled down as far as possible allowing only enough space for you to be able to work. The fume hood is found in the back of room 421 C in the biology department and should be ON AT ALL TIMES.

Fume Hood

F. PERSONAL HYGIENE

When working in the laboratory it is important to keep yourself and your work area clean to avoid accidents and residual contamination. Clear your workspace, leaving only what you need to complete your experiment. Keeping the clutter down will reduce the chance of spills and breakage of equipment. You may unknowingly come in contact with a chemical or evaporated solution on counter-tops or other surfaces, or put someone else in danger of contamination. If there is a residual chemical or solution on the your hands, you will be in danger of ingesting or introducing the material into your body by other means (eyes or mucous membranes). You must ALWAYS thoroughly wash and scrub your hands before you leave the laboratory. There are sinks with liquid hand soap and paper towels in every laboratory. Wash your hands, dry them with a paper towel and then turn the faucet off using the paper towel to avoid re-contaminating your hands.

The safety regulations for working in a lab include no eating, no drinking, and no smoking at any time. Any food or beverages must be kept outside of the door of the laboratory. There are tables and waste baskets outside of the lab for your use. Please DO NOT discard any food or beverage items in the trash cans found in the laboratory. These standards protect you from ingesting a hazardous substance.

CHEMICALS AND COMPATIBILITY

A. HANDLING

Chemicals can enter your body through **absorption** by the skin and **mucous membranes**, **inhalation**, **ingestion**, and contact with your eyes. You will run across such chemicals in your laboratory experiences and need to know what your risks of exposure are. You must pay attention to your instructor's directions, warnings from your lab protocol, and hazard codes on the chemical's labels. Be sure to use all of the protective equipment required.

Some of the hazardous effects of chemicals found in thelaboratory include strong irritants, toxins, **carcinogens** and allergens. The following table highlights some of these chemicals, but there are also many more that you should be aware of in the laboratory.

CHEMICAL	TARGET ORGANS	HAZARDOUS EFFECTS
Acid and Alkaline solutions e.g. Sodium Hydroxide	Skin, membranes of eye, nose, throat, trachea, bronchi, and alveoli	Etches Tissue - sometimes leading to irreversible damage to tissue
Aliphatic Halogenated Hydrocarbons e.g. Chloroform	Liver and Kidneys	**Necrotic** cell death-- Redness, swelling, inflammation, CNS effects, PAIN!
Metals e.g. Lead and	Bones	**Sequestered** for extended periods of time-- Long term toxic effects - enzyme inhibition

Silver		
Amines e.g. Diphenylamine	Toxic to all tissues and adversely affect a number of organs	Strong irritant, **corrosive**, and carcinogenic
Amides e.g. Formamide	Central Nervous System, liver, kidneys	Very toxic-- Liver and Kidney toxicity CNS depression
Aldehydes e.g. Formaldehyde	Skin, eyes, mucous membranes	Severe irritant at just a few **ppm** as well as a strong **sensitizer** leading to-- Skin rashes, Puffy eyes, Headaches, Respiratory problems. Allergenic properties are worse than its potential carcinogenic properties.

Preventative measures:

1. Always open chemical and solution bottles with the mouth of the bottle aimed away from your body and others.

2. Replace covers after using the reagent to prevent spills and evaporation.

3. There are two options for transporting chemicals or solutions.
A) Use a cart when there are a large number of bottles.
B) Carry one at a time and use both of your hands. Position one hand on the bottom and one hand on the mouth of the bottle or handle if there is one. Never pick up a bottle by its cover!

4. Always place chemical or solution bottles away from the edge of the lab bench to prevent accidents.

B. DISPOSAL

Some of the chemicals you will be working with will require proper disposal. Follow all directions and locate the bottle for your waste labeled with the UW-RF hazardous waste label as pictured above. Any bottle labeled as hazardous waste should always be in **secondary containment** and have a secure screw-cap cover. Always replace the cover when you are finished disposing of your waste, even if someone is waiting to use the bottle. If you are not absolutely sure of the proper disposal methods first look around the lab for a container labeled with the Hazardous Waste label and read what its contents are. If you are unable to find a waste container, ask your instructor. Never pour anything down the drain if you are at all unsure of a solution's hazardous waste status.

C. STORAGE

There are three major areas in the department where chemicals are stored, cabinets, refrigerators, and freezers. Room 421 houses molecular biology's chemicals, 422A has general use chemicals and **microbiological media**, and 426 (stockroom) houses the **acids** and **flammables**, each in their own cabinets. These places are organized by **compatibility** and by the storage codes found on their labels. The materials in each cabinet are are alphabetized. Chemicals used in the laboratory can react violently when mixed. To avoid any dangerous reactions chemicals are stored in specific places. What you need to remember is, simply, TO PUT THINGS BACK EXACTLY WHERE YOU FOUND THEM.

Acid Cabinet: Room 426 & Flammable Cabinet: Room 426

Chemical Storage: Room 422A

MICROBIOLOGICAL MATERIALS

Microbial cultures and samples are a large part of the Biology Department at the University of Wisconsin-River Falls. **Microorganisms** are a hazardous material in the laboratory because if they are ingested or introduced into

the body or the outside environment, they may cause a harmful effect.

A. HANDLING

Always handle a microbial culture as if it is an infectious material, or **pathogen**. Always wash your hands before leaving the laboratory.

B. DISPOSAL

All microbial cultures must be discarded on the Microbiology Discard Cart found in room 422 A.

Microbiology Discard Cart

Autoclaving -- discard rules/regulations:

Autoclaving is a sterilization technique that uses extreme vacuum pressure, 15-17 pounds per square inch, and extreme steam heat, 121 degrees Celsius, to disinfect bacterial or fungal samples. The **autoclave** is used to sterilize media. This kills bacterial and fungal samples, allowing them to be discarded in the garbage. To discard cultures and media:

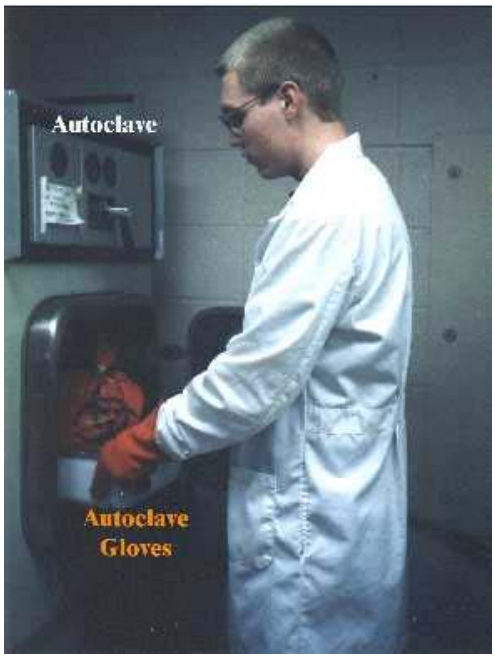
1. Remove all tape or labels from your sample.

2. Liquid and solid media must be separated on the Microbiology Discard Cart. Their respective sides of the cart are labeled.

3. Agar plates must be discarded in the orange **biohazard** bag. When the bag is 3/4 full, loosely tape the bag shut and place it on the bottom shelf of the cart. New bags are usually on the bottom shelf of the cart as well.

If you are unaware of the autoclaving procedure, you may endanger yourself and others by misusing the autoclave or not correctly sterilizing bacterial or fungal samples. Never use the autoclave without training from a biology staff member.

Autoclave



EMERGENCY RESPONSE

A. MICROBIAL SPILL RESPONSE

If a bacterial or fungal sample spill occurs, you should immediately pour a disinfectant, 5% Lysol ®, over the spill, let it disinfect for approximately ten minutes, and then wipe up spill with paper towels and throw in the Biohazard bag on the Microbiology Discard Cart. For large spills, follow with covering the spill area with 20% bleach, allow to disinfect for approximately ten minutes, and then wipe the area completely dry. Throw any contaminated articles into the Biohazard bag on Discard Cart. Pre-diluted Lysol is found on each bench in room 422 and 422 A and the bleach solution is found under the sink in 422 A.

B. FIRE

Bunsen burners are common in the laboratory setting. To prevent a fire, clear the space around your Bunsen burner of anything flammable (i.e. lab notebooks and paper towels). Avoid passing your arms or hands directly over the flame. Your hair will easily ignite. Don't lean over the flame and keep your hair tied back. Person's with long hair should always tie it back during lab. In the event of any fire, if possible, cover it to deplete the oxygen supply. If covering the flame isn't possible, evacuate the area, and if possible try to extinguish with a fire extinguisher found on the wall of every laboratory. Notify proper authorities as soon as possible. The fire alarms are located by each stair well of the fourth floor. If you catch on fire:

C. CHEMICAL EXPOSURE

1. Eyes

If any substance is introduced into your eyes IMMEDIATELY get assistance and go to the eyewash station which is located in the prep room of every laboratory. Holding your eyes open, flush for at least 15 minutes.

Get medical attention as soon as possible. You will be instructed on how to use the emergency eyewash station in the introduction to your lab class. There are also instructions posted next to the station. If it is not covered in class, don't be afraid to ask.

2. Skin

If you get a substance on your hands or arms flush with cold water, over the sink, for 15 minutes. If there is a large amount of a substance spilled on you, IMMEDIATELY get assistance, remove any contaminated clothing, and use the emergency eyewash as a shower. Rinse yourself for at least 15 minutes. Get medical attention as soon as possible.

Eyewash Station & First Aid Kit: Room 422A

D. CUTS AND PUNCTURES

Handling glassware and pipets in the laboratory can be quite dangerous. Much of the laboratory glassware is delicate and easily broken, which contributes to cuts and punctures. Many glass cuts occur when attempting to force a pipet into a pipetor or force open a lid on a bottle or jar. Avoid the use of unnecessary force. Inspect glassware for chips and cracks which will weaken the glass. Discard the damaged glass in the broken glass box and ask for new glassware. Pay close attention to your instructor's demonstration on how to handle these items. If you are washing glassware always use the brushes to clean the inside. Avoid putting your hands inside beakers, graduated cylinders and other glass items. Always wear rubber gloves for additional protection. In the event of an accident there are first aid kits found in every prep room for your use. Control any bleeding and seek medical attention in case of an accident.

Sharps include razor blades, scalpels, and needles. Always remember to cut AWAY from your body. In the event of any injury, follow the instructions above.

E. DISPOSAL OF BROKEN GLASS AND SHARPS

If you break any type of glassware, take care of yourself first, then CLEAN UP the broken glass to prevent others from cutting themselves. A broom and dustpan are located in each laboratory. All broken glass is

deposited in the blue and white broken glass box in the lab. Sharps are disposed of in a separate box. This box is red plastic and labeled for sharps disposal. It is usually located near the broken glass box in the lab.

Sharps & Glass Disposal Boxes

Always notify your instructor in the event of any accident or injury.

IMPORTANT SAFETY MESSAGES

1. Think safety first. Be prepared for lab. Determine what protective equipment you need; remember preventing and accident is easier than dealing with the consequences of one.

2. Know what you are working with. Read the container labels, reference the hazard rating index chart or the MSDS. Know where to dispose of that substance when you are done using it.

3. Follow all safety procedures. Carefully follow the protocol in your lab manual and pay close attention to your instructor's directions.

4. Practice good housekeeping and personal hygiene. Keep your area clean and uncluttered. Wipe up spills and use the trash cans! Wash your hands frequently.

5. Report dangerous activities and situations. Pay attention to the way other people in the lab are conducting themselves; someone else's mistakes or carelessness can hurt you. Be aware of your surroundings at all times.

6. Know emergency responses and exit routes. Look around the lab and locate fire extinguishers, eyewash

stations, and first aid kits. These items are there for your use in the event of an accident. Report all injuries and accidents to your instructor or supervisor.

7. No clowning around in lab. Use all equipment only for its intended use.

8. Notify your instructor of any allergies or health related concerns especially if you are pregnant, expecting to become pregnant, or nursing.

9. If you don't know....ASK!

ACKNOWLEDGMENTS

We thank the University of Wisconsin-River Falls Grants Office, Nan Jordahl and Bill Campbell, for their assistance with our grant. Funding for this WebCT project was provided by a UW-RF Instructional Technology Mini-Grant. We also thank Torey Kauth for the professional scientific modeling which he eagerly volunteered for and Karen Klyczek for her assistance and support with WebCT. We thank E. Katherine Miller for mini-grant authorship and editorial contributions. Finally, we thank Amanda S. Holder for her fabulous and continued help in the UW-RF IDe3A Center.

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GLOSSARY

absorption -- the movement of a chemical from the site of initial contact with the biologic system, across a biologic barrier, and into either the bloodstream or the lymphatic system

acid -- a substance which dissolves in water and releases hydrogen ions (H+); a proton donor or an electron acceptor; acids cause irritation, burns, or more serious damage to tissue, depending on the strength of the acid, which is measured by the pH, 1 strongest to 6 weakest

autoclave -- an airtight chamber that can be filled with steam under pressure that is used for sterilizing requiring moist or dry temperatures above 100 degrees Celsius (212 degrees Fahrenheit) without boiling

biohazard -- biological or infectious material

Bunsen burner -- a burner used in the laboratory that consists of a straight tube four to five inches long with a gas orifice and holes near the bottom for admission of air, the mixture of gas and air forms burning at the top, with a feebly luminous but intensely hot flame

carcinogen -- a chemical or physical agent capable of causing cancer. Such an agent is often described as carcinogenic. The ability to cause cancer is termed carcinogenicity. Words with similar meaning include onogenic and tumorigenic

chemical -- a substance characterized by a definite molecular composition

compatibility -- how well reagents react to one another

contamination -- the result of materials, chemicals, substances, or life forms located where they are not wanted

corrosive -- a liquid or a solid that causes visible destruction or irreversible alterations in human skin tissue at the place where it touches the skin

fire -- or flammability. The range of gas or vapor concentrations (percent by volume in air) that will burn or explode if an ignition source is present.

0 - Material that will not burn

1 - Material that must be preheated before ignition can occur.

2 - Material that must be moderately heated or exposed to relatively high ambient temperatures before ignition can occur.

3 - A liquid or solid that can be ignited under almost all ambient temperature conditions.

4 - Material that will rapidly or completely vaporize at atmospheric pressure and normal ambient temperature, or is readily dispersed in air and will burn readily.

flammable -- catches on fire easily and burns rapidly. The National Fire Protection Agency and the U.S. Department of Transportation define a flammable liquid as having a flashpoint below 37.78 degrees Celsius (100 degrees Fahrenheit)

hazard -- that which can be hazardous to life upon exposure; typically toxic, flammable, explosive, and corrosive materials or unsafe living or working conditions

hazardous waste -- waste materials that are classified as toxic, corrosive, flammable, explosive, radioactive, or biological/infectious wastes

health -- or toxicity. The quality or degree of being poisonous or harmful to plant, animal, or human life; the degree of danger posed by a substance to life

> 0 - Material that on exposure under fire conditions would offer no hazard beyond that of ordinary combustible material.
>
> 1 - Material that on exposure would cause irritation but only minor residual injury.
>
> 2 - Material that on intense or continued but not chronic exposure could cause temporary incapacitation or possible residual injury.
>
> 3 - Material that on very short exposure could cause death or major residual injury.
>
> 4 - Material that on very short exposure could cause death or residual injury.

ingestion -- taking in and swallowing a substance through the mouth

inhalation -- breathing in a substance

Material Safety Data Sheets (MSDS) -- a document produced by chemical manufacturers that describes physical and chemical properties of chemicals, first aid information, personal protective equipment to use, and suggested means of disposal

microbiological media -- any nutrient system for the artificial cultivation of a bacteria or other organism or cells that is sometimes a simple substance but more commonly a complex of inorganic and organic materials in a fluid base or one rendered more or less solid by coagulation or by the addition of gelatin or agar

microorganisms -- any organism of microscopic size e.g. bacteria, fungi, protozoa, or virus

mucous membranes -- the moist, soft lining of the nose, mouth, throat, bronchus, and eyes

National Fire Protection Association (NFPA) -- organization that has developed a scale for rating the severity of fire, reactivity, and health hazards of substances. References to the ratings appear on chemical labeling and MSDS's.

necrosis -- the death of tissue, usually as individual cells, groups of cells, or in small localized areas

pathogen -- any disease causing agent, usually applied to living agents

personal protective equipment -- eye, hand, and body protection as well as fume hood use requirement

ppm -- parts per million, a measure of concentrations, such as parts of a substance per million parts of air or water.

protocols -- the rules and outline of an experiment

reactivity -- the ability of a substance to undergo a chemical reaction (such as combining with another substance). Substances with high reactivity are often quite hazardous

> 0 - Material that in itself is normally stable, even under fire exposure conditions and is not reactive with water.
>
> 1 - Material that in itself is normally stable, but can become unstable at elevated temperatures and pressures.
>
> 2 - Material that is unstable and readily undergoes violent chemical change at elevated temperatures and pressures or which reacts violently with water or may form explosive mixtures with water.
>
> 3 - Material that in itself is capable of detonation or explosive reaction but requires a strong initiating source; or which must be heated under confinement before initiation; or may react explosively with water.
>
> 4 - Material that in itself is readily capable of detonation or of explosive decomposition or reaction at normal temperatures and pressures.

residual -- a residue of a substance left on surfaces even after the substance has been cleaned away

secondary containment -- a plastic container such as a tray or washtub that the waste bottle is placed into to prevent leakage or spills

sensitizer -- sensitization, a condition of being made sensitive to a specific substance such as protein or pollen, antigen or hapten, usually as a result of repeated exposure; activation of an immune response

sequestered -- storage of a substance in tissues for extended periods of time

solution -- a mixture in which the components are uniformly dispersed. All solutions consist of some kind of solvent (such as water or other liquid) which dissolves another substance, usually a solid

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