



**Lab  
Safety**



# Laboratory Biosafety In Molecular Biology and its levels

**16-17 October 2012**



Workshop “

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# Biohazard

By

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**Biosafety is** the measures employed to avoid infecting oneself, others or the environment when handling biohazard materials.



# Who needs this training?

Individuals who work with or have access to biohazardous materials.



A microscopic view of cells, likely bacteria or fungi, showing various shapes and sizes, some with internal structures visible. The image is overlaid with a semi-transparent text box.

# **What You Need to Know When Working With Biological Material**

**BIOHAZARD**

# What is a Biohazard?

- An agent of biological origin that has the capacity to produce deleterious effects on humans, i.e. microorganisms, toxins and allergens derived from those organisms.

## Examples:

- Micro organisms such as viruses, bacteria, fungi, and parasites.
- Blood and body fluids, as well as tissues from humans and animals.
- Transformed cell lines.



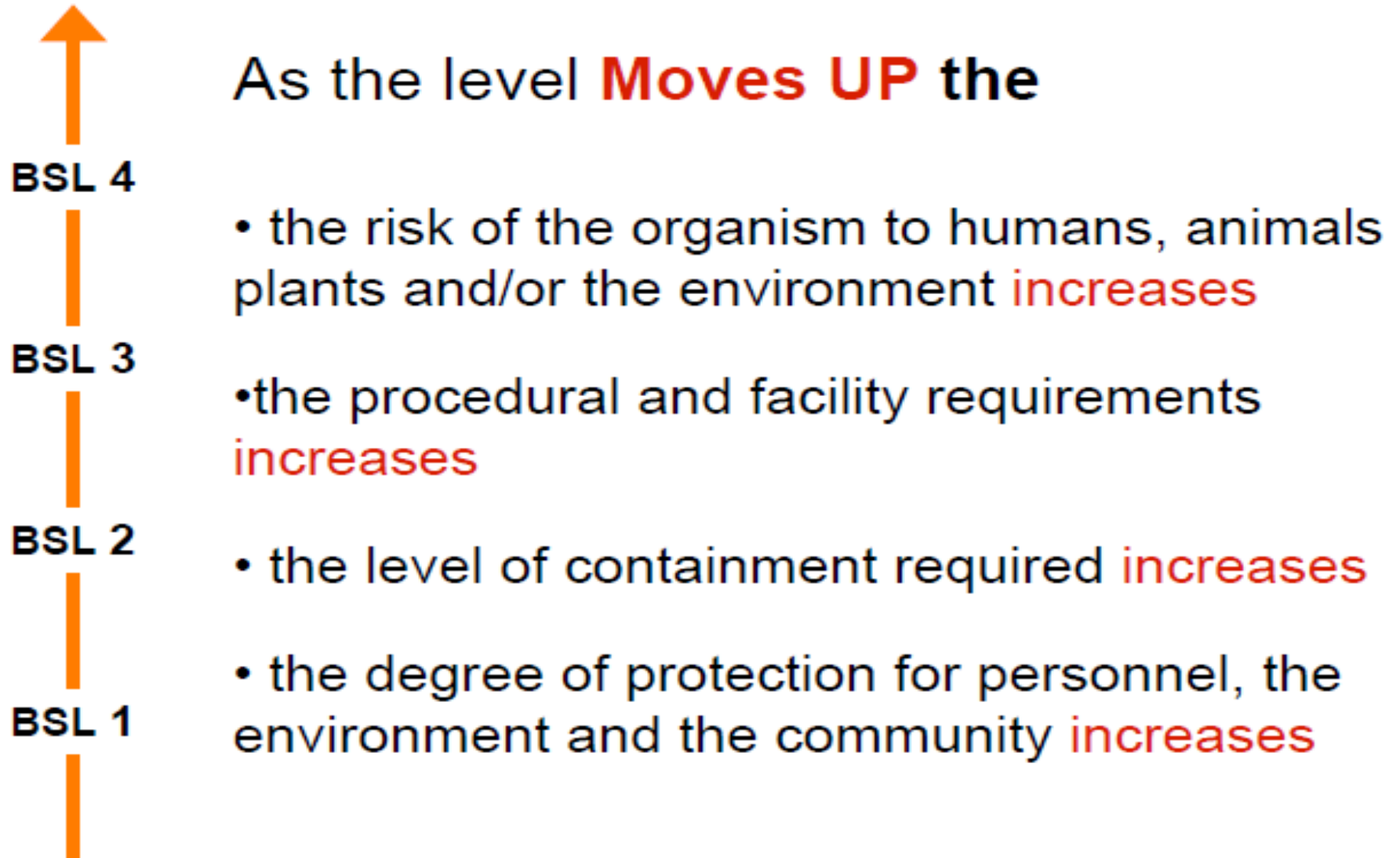




# Classification of Biohazards

## Classified into 4 major levels

As the level **Moves UP** the



# All Biosafety Levels Need

- A knowledgeable supervisor.
- Personnel aware of potential hazards
- Personnel proficient in practices/techniques
- A biosafety manual specific to the lab.







# BSL 1

**BSL 1 organisms are well-characterized agents not known to cause disease in healthy adult humans and are of minimal potential hazard to laboratory personnel and the environment**

**Examples:**

***E. coli (certain strains), Lactobacillus acidophilus***

# BSL1

## Microbiological Practices

- Standard Practices
  - Use Mechanical Pipetting devices
  - No Eating, Drinking, Smoking in Lab
  - Minimize splashes and aerosols
  - Decontaminate work surfaces
  - Safe handling of sharps
  - Wash Hands before leaving lab
- Use Personal Protective Equipment
  - Lab coat
  - Gloves
  - Eye protection (safety glasses)

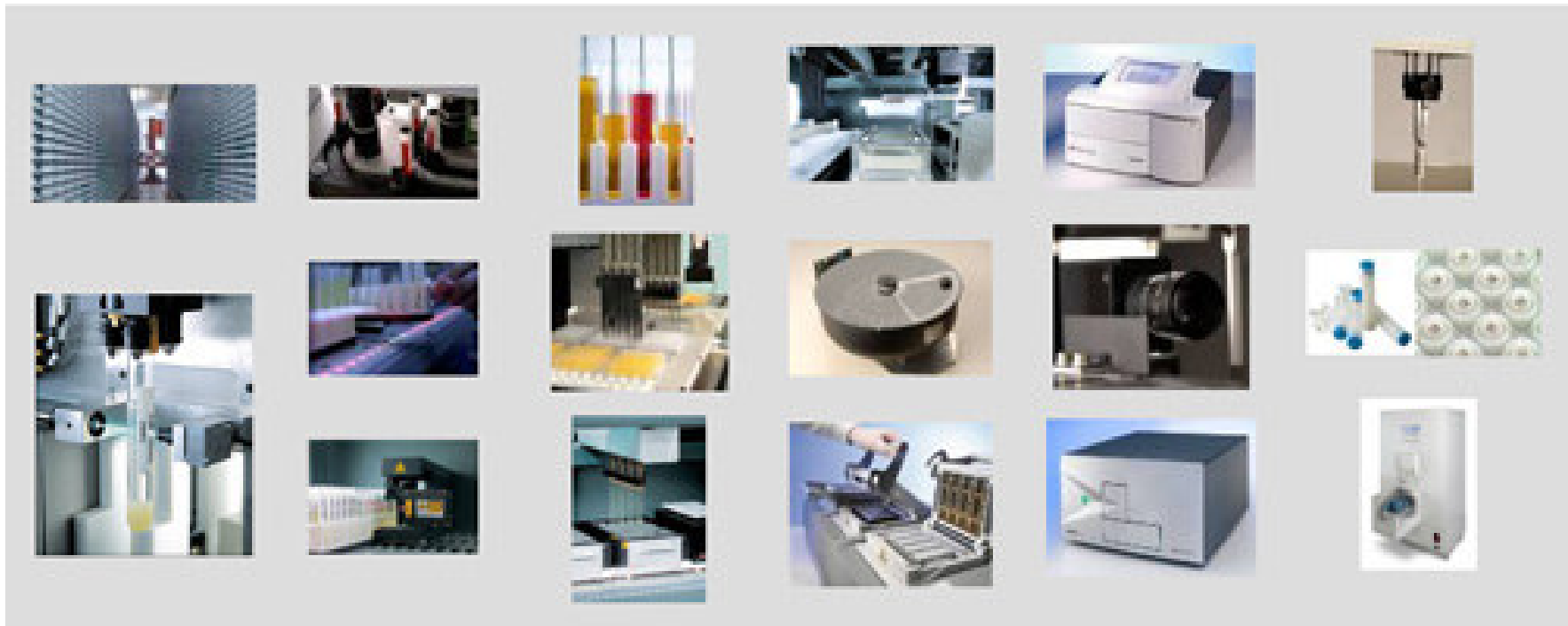


# BSL 2 Special Practices



- **Needles and Sharps Precautions**
- **Special policies and procedures for laboratory entry**
- **Post biohazard warning signs**
- **Use a biosafety manual specific to the lab**
- **Specific training provided to workers and must include annual updates**
- **Use leak-proof containers to transport infectious materials**
- **Immunize when possible**
- **Decontaminate work surfaces frequently**
- **Report to lab director spills and accidents that result in overt exposures of infectious materials**

# General Laboratory Safety Guidelines



# Biohazard work area

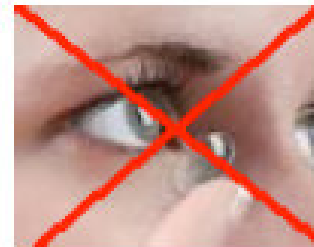


Mark the work  
area with  
the warning  
sign and contact  
information

# Laboratory Hygiene

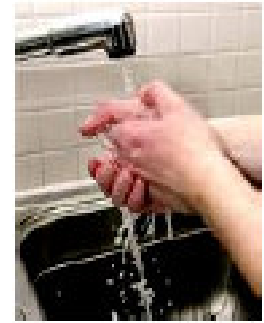
## DO NOT:

- Eat
- Drink
- Smoke
- Apply cosmetics (including lip balm)
- Handle contact lenses
- Store food or drink in lab refrigerators
- Wear open-toed shoes





# Hand Washing



- Wash hands immediately after removing PPE
- Use a soft soap
- A hand sanitizer can be used but wash with soap and water as soon as possible.

# Safe use of Centrifuges

- Before use please check
  - Overfilled? Balanced?
  - Caps or stoppers properly in place?
  - Run conditions achieved?
- Use sealable buckets (safety cups) or sealed rotors
- After run
  - Centrifuge completely stopped?
  - Spills or leaks?
  - Allow aerosols to settle (30 min) or open in a BSC.

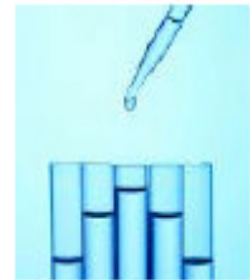


# Needles and Syringes

- **Avoid use whenever possible.**
- **Fill syringes carefully.**
- **Shield needles when withdrawing from stoppers.**
- **Do not bend, shear or recap needles.**
- **Dispose of all used needles and syringes in yellow sharps containers.**



# Pipettes



- Mouth pipetting is prohibited.
- All biohazard materials should be pipetted in BSC's.
- Never force fluids out, use 'to deliver' pipettes.
- To avoid splashes, allow discharge to run down dispense the receiving container wall.
- Never mix material by suction and expulsion.
- Reusable pipettes should be placed horizontally in a disinfectant filled pan. Autoclave before reuse.

# Blenders, Grinders, Sonicators and Lyophilizers

- Operate in a BSC whenever possible. Allow aerosols to settle for 5 minutes before opening.
- Safety Blender
  - Do not use glass blender jars
  - Decontaminate immediately after use
- Lyophilizers
  - Use glassware designed for vacuum work, ensure there is no damage before using.
  - All surfaces should be disinfected after use
  - Use vapour traps whenever possible.

# Inoculation Loops



- Sterilization in an open flame may create aerosols which may contain viable micro organisms.
- Use a shielded electric incinerator.
- Shorter handles minimize vibrations.
- Disposable plastic loops are good alternatives.



# Cryostats

- Wear gloves during preparation of frozen sections and heavy gloves when accessing the cryostat.
- Decontaminate frequently (70% ethanol)



# Personal Protective Equipment

- PPE can become an important line of defence (last line of defence).
- USE proper PPE





# Spills

- Spill response will vary depending on:
  - What was spilled?
  - How much was spilled?
  - Where was the spill?
  - What is the potential for release to the environment?
- Spills should be cleaned up immediately (unless an aerosol was generated), to ensure proper decontamination.
- All spills are to be reported ASAP to the lab supervisor and Safety office.
- Please refer to the safety manual for cleanup procedures for different spills



# Spills

- When cleaning up surfaces use 10% bleach solution or approved disinfectant (Mix bleach solution fresh each time.)
- Spray and allow it to stand for at least **ten minutes** before wiping up.
- Dispose of all wipes in biohazard containers.
- Decontaminate any materials used to clean up blood or OPIM (mops, sponges, buckets, etc.)
- PPE should be removed and disposed of in biohazard containers.



# Decontamination

- Generally for disinfection rather than sterilization
- Choice depends on;
  - Type of material to be disinfected
  - Organic load
  - Chemical characteristics
- Most common are chlorine compounds and alcohols (broad range)



# Disinfection: What to use for my organism?

## Bacteria

### Vegetative bacteria (E.coli.)

- 2% domestic bleach
- 75% Ethanol
- Quaternary ammonia
- 6% formulated Hydrogen peroxide\*

### Mycobacteria and fungi

- 10% domestic bleach
- 75% Ethanol
- Phenolic compounds
- 6% formulated Hydrogen peroxide\*

### Spore forming bacteria (Bacillus)

- 10% domestic bleach
- Gluteraldehyde
- Formaldehyde
- 6% formulated Hydrogen peroxide\*

## Viruses

### Enveloped (HIV, Herpes)

- 2% domestic bleach
- 75% Ethanol
- Quaternary ammonia
- 6% formulated Hydrogen peroxide\*

### Non enveloped (Hepatitis, Adenovirus)

- 10% domestic bleach
- 6% formulated Hydrogen peroxide\*
- Gluteraldehyde
- Formaldehyde





# Waste Management

## **Biomedical waste**

Discarded biological material from teaching, clinical and research laboratories and operations. Biomedical waste includes but is not limited to;

- Animal waste
- Biological laboratory waste
- Human anatomical waste
- Human blood and body fluid waste
- Sharps

- All biological waste should be decontaminated prior to disposal (including level 1 agents).
- Treated waste is no longer considered 'biomedical' (i.e. tissue culture, microbiological waste, animal bedding) and can be disposed in the regular waste stream.
- Any waste that cannot be treated (i.e. sharps, carcasses, tissues and body parts) remains biomedical waste and must be incinerated off site.

# Biohazardous Waste Containers



- Biohazardous waste containers shall be clearly marked with the universal biohazard symbol.



## Sharps Disposal



- Syringes and syringes without a needle attached go into a sharps container
- Contaminated micropipettes, pipette tips, and Pasteur pipettes are discarded in a puncture-resistant container or a sharps container for disposal.
- Don't place needles or sharps in office waste containers

# Transportation



## Transportation of Dangerous Goods

- packaging requirements (primary and secondary containers, dry ice etc)
- means and route of transportation (use of cart with guard rails, low traffic area etc.)
- regulatory requirements (classification, labelling, signing, documenting)

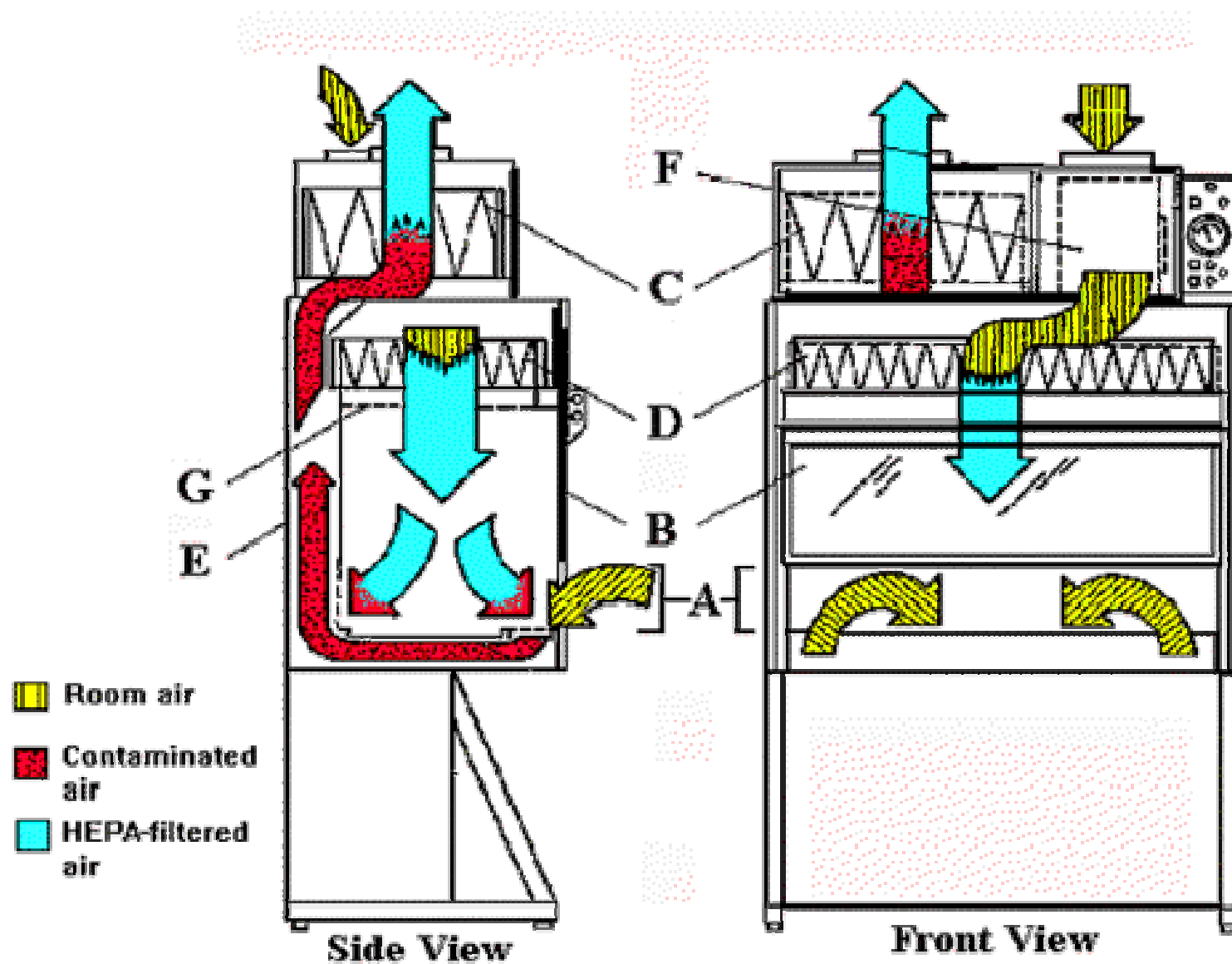
Comply, or assure compliance, with applicable U.S. Department of Transportation, EPA, and USDA criteria in the transportation (on campus) or shipping (off campus) of regulated potentially biohazardous materials or wastes.

**With proper knowledge, planning and care, a biological exposure is avoidable.**

**Let Us be Safe !!!!!!!!!!!**



# Biological Safety Cabinet II type 2



# Chemical safety



# Risk Factors

## Read the sign

### Intrinsic hazard associated with the substance

#### Severity of hazard



#### Low health hazard

*Substances designated as irritant (those that affect the skin and sometimes the eyes)*



#### Medium health hazard

*Substances designated as corrosive, irritant (particularly by the inhalation route) or harmful (particularly by the inhalation route)*



#### High health hazard

*Substances designated as toxic, very toxic, carcinogenic, mutagenic and those that may impair fertility or harm the unborn child*

# Chemical Categories



# Physical Hazard...

Hazards due to physical characteristics of a chemical

**Flammable** - catches fire easily and burns rapidly.

**Combustible** - will burn under most conditions.

**Explosive** - will explode / detonate releasing hot gases.

**Oxidizer** - yields oxygen to enhance combustion, may cause ignition of combustibles with no external source.

**Organic peroxide** – bivalent – O – O – structure, tends to be reactive and unstable

**Unstable** – tends to decompose during normal handling and storage

**Water reactive** – reacts with water to release flammable gas or present a health hazard

# Health Hazard...

**Produces acute or chronic effects in exposed workers**

**Carcinogen** – Cause cancer or suspected cancer causer.

**Toxic agent** – Poisonous / cause acute or chronic effects.

**Reproductive toxin** – Could have harmful effect on male or female reproductive system or on developing fetus

**Irritant** – Can cause inflammation of skin or eyes

**Corrosive** – Cause irreversible damage to living tissue

**Sensitizer** – Cause exposed persons to develop allergies to the substance

**Target organ-specific agents** – Hazardous to specific organs in body (e.g., lungs, liver, blood, kidneys, nervous system)

# Route of Entry...

For a chemical to have an effect on a worker, they must be exposed to it and some of it must get into their system

**Inhalation / breathing** – most common route, gases /vapors can pass to blood, solid particles inhaled into lungs.

**Absorption through the skin** – many solids, liquids, vapors and gases can be absorbed through the skin.

**Ingestion / swallowing** – while not intentional, failure to wash hands, eating in contaminated lab, etc.

**Injection** – accidents handling glass, sharps, etc.

**Eye contact** – either physical damage or absorption.

The route of entry dictates protective equipment

## Chemical Categories

### Bases





## Chemical Categories: Bases

*Some pointers for safe storage of strong bases:*

- **Store bases and acids separate from one another.**
- **Store solutions of inorganic hydroxides in polyethylene containers.**
- **Have spill control pillows or caustic neutralizers available for spills.**

## Chemical Categories: Bases

*Examples of strong bases:*

**Ammonium Hydroxide**

**Calcium Hydroxide**

**Bicarbonates**

**Potassium Hydroxide**

**Carbonates**

**Sodium Hydroxide**



## Chemical Categories

# Flammable Chemicals



## Chemical Categories: Flammable

*Some pointers for storage of flammable materials:*

- **Store in approved safety cans or cabinets**
- **Segregate from oxidizing acids and oxidizers.**
- **Keep away from any source of ignition: flames, heat or sparks.**
- **Know where fire fighting equipment is stored and how to use.**
- **If volatile flammable liquids are stored in a refrigerator it must be in an explosion-proof (lab-safe) refrigerator.**



## Chemical Categories

# Oxidizers



## Chemical Categories: Oxidizers

*Some examples of oxidizers:*

- **Store in a cool, dry place.**
- **Keep away from flammable and combustible materials, such as paper or wood.**
- **Keep away from reducing agents such as zinc, alkaline metals, formic acid.**

## Chemical Categories



## Chemical Categories: Pyrophoric Substances

*Safety pointers for pyrophoric substances:*

- **Store in a cool place.**
- **Store in containers that omit air.**
- **Beware of low humidity circumstances in which static electricity may be high.**



## Chemical Categories

# Carcinogens



## Chemical Categories: Carcinogens

*Safe storage pointers for carcinogens:*

- **Label all containers as Cancer Suspect Agents.**
- **Store according to hazardous nature of chemicals, e.g., flammable, corrosive.**
- **When necessary, store securely.**



## Chemical Categories: Carcinogens

*Some examples of carcinogens:*

**Antimony compounds**

**Acrylonitrile**

**Arsenic compounds**

**Benzene**

**Chloroform**

**Dimethyl sulfate**

**Dioxane**

**Vinyl chloride**



# Microscopy Risk Factors

- **Awkward and static posture of the lower back**
- **Wrist and palm contact pressure in the carpal tunnel area**
- **High repetition**
- **Eye strain and fatigue**
- **Awkward and static posture of the neck and head**



# Microscopy Tips

- Use a fully adjustable ergo-task chair or stool with built-in solid foot rest. Adjust the chair to fit you properly.
- For prolonged standing alternate between feet, wear low-heeled shoes with good cushioning or use anti-fatigue floor mats.
- Pull the microscope to the front edge of the work surface for upright posture and elevate if needed.
- Adjust the eyepieces and angle of observation to prevent neck strain. Use adjustable microscope stands.
- Provide armrests to support forearms during knob adjustments.

- **Do not rest forearms on the edge of workstation; use padding**
- **Take stretch and eye breaks and rotate tasks.**
- **Don't use a microscope for more than 5-hours a day.**
- **Maintain straight wrists and keep elbows close to the body.**
- **Ensure that sufficient knee and leg space is available.**



# Fume Hoods and Biological Safety Cabinets Risk Factors

- Constrained knee and leg space, especially in older BSCs
- Contact pressure on the forearms, wrists and knees, or legs
- Awkward and static posture of the neck, back, legs, arms and wrists
- Constrained body position, overloading muscles, tendons, and joints in asymmetrical manner
- Working with elbows winged
- Overreaching



# **Fume Hoods and Biological Safety Cabinets Tips**

- **Adjust the chair properly before you start work so it provides adequate back support. Remove the chair arms if they interfere with the ability to get close to your work. Sit back in the chair for lumbar support.**
- **Prevent extended reaching. Place materials as close as possible to you.**
- **Avoid resting arms on the sharp edges of the hoods or cabinets. If possible apply closed-cell foam padding to the front edge of the hood or cabinet.**
- **Remove drawers, supplies, refrigerators from under the cabinet or hood to provide leg room.**



- **If you must stand to work at the hood or cabinet alternate between feet, wear low-heeled shoes with good cushioning or use anti-fatigue floor mats.**
- **Take short breaks to relieve forearm and wrist pressure caused by leaning on front edge of hoods/BSCs.**
- **Maintain straight wrists and keep elbows close to the body.**

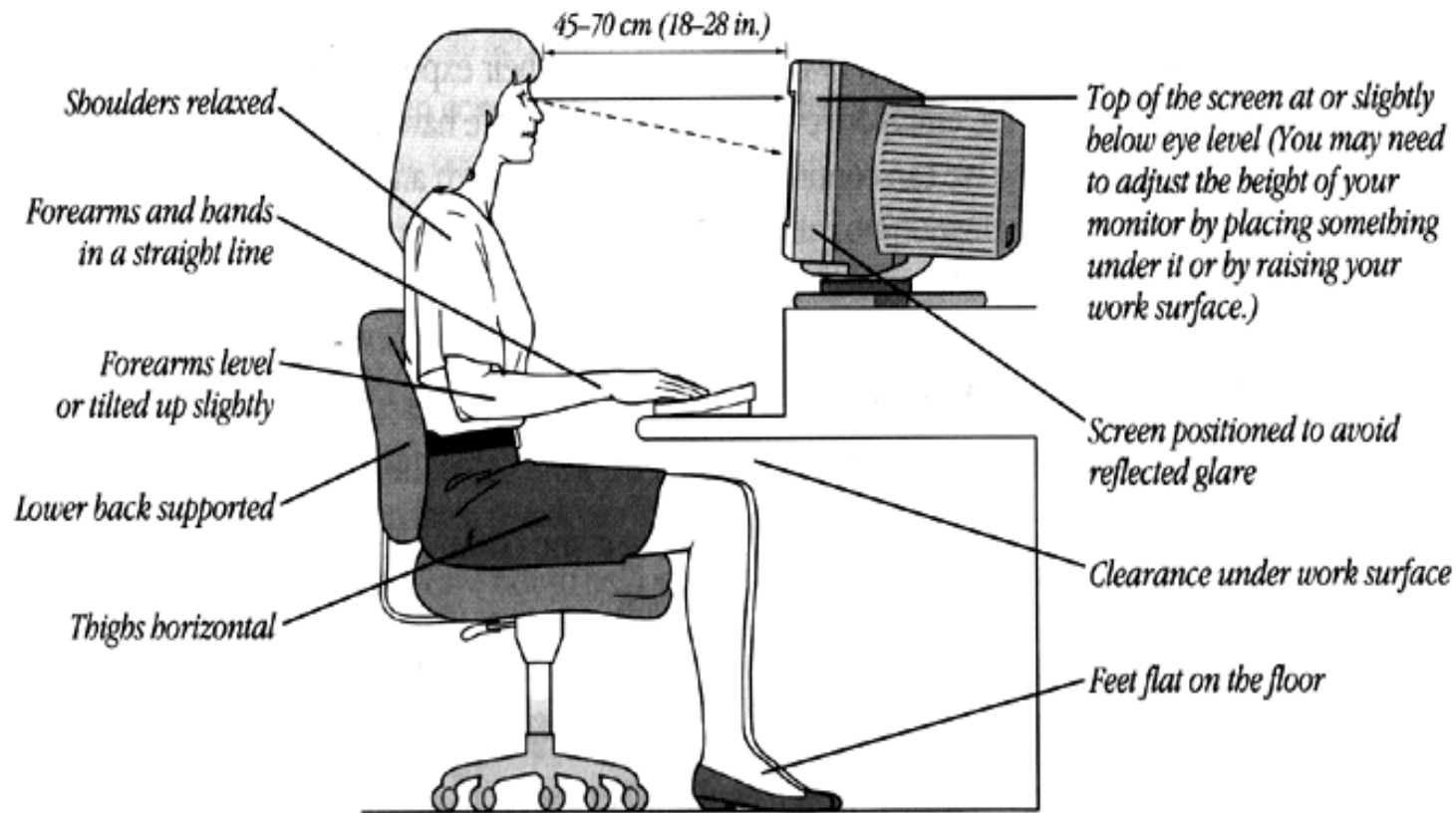


# Test Tube Handling Tips

- Avoid resting arms on the sharp edges of the workstation or lab bench.
- Remove drawers, supplies, refrigerators from under the cabinet or hood to provide leg room.
- If you must stand to work at the hood or cabinet alternate between feet, wear low-heeled shoes with good cushioning or use anti-fatigue floor mats.
- Take short breaks to relieve forearm and wrist pressure.
- Maintain straight wrists and keep elbows close to the body.



# Computer Ergonomic Risk Factors



**If your computer workstation is not set up ergonomically correct it will cause MSD risk factors to the eyes, neck, shoulders, upper and lower back, elbows, wrists, fingers and legs.**

- **Be aware of hazardous materials in your workplace.**
- **Know where to find information.**
- **Use safe practices and procedures.**
- **Ask questions before not after.....**
- **What happens can have a lasting effect! .....Be safe stay and safe.**

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