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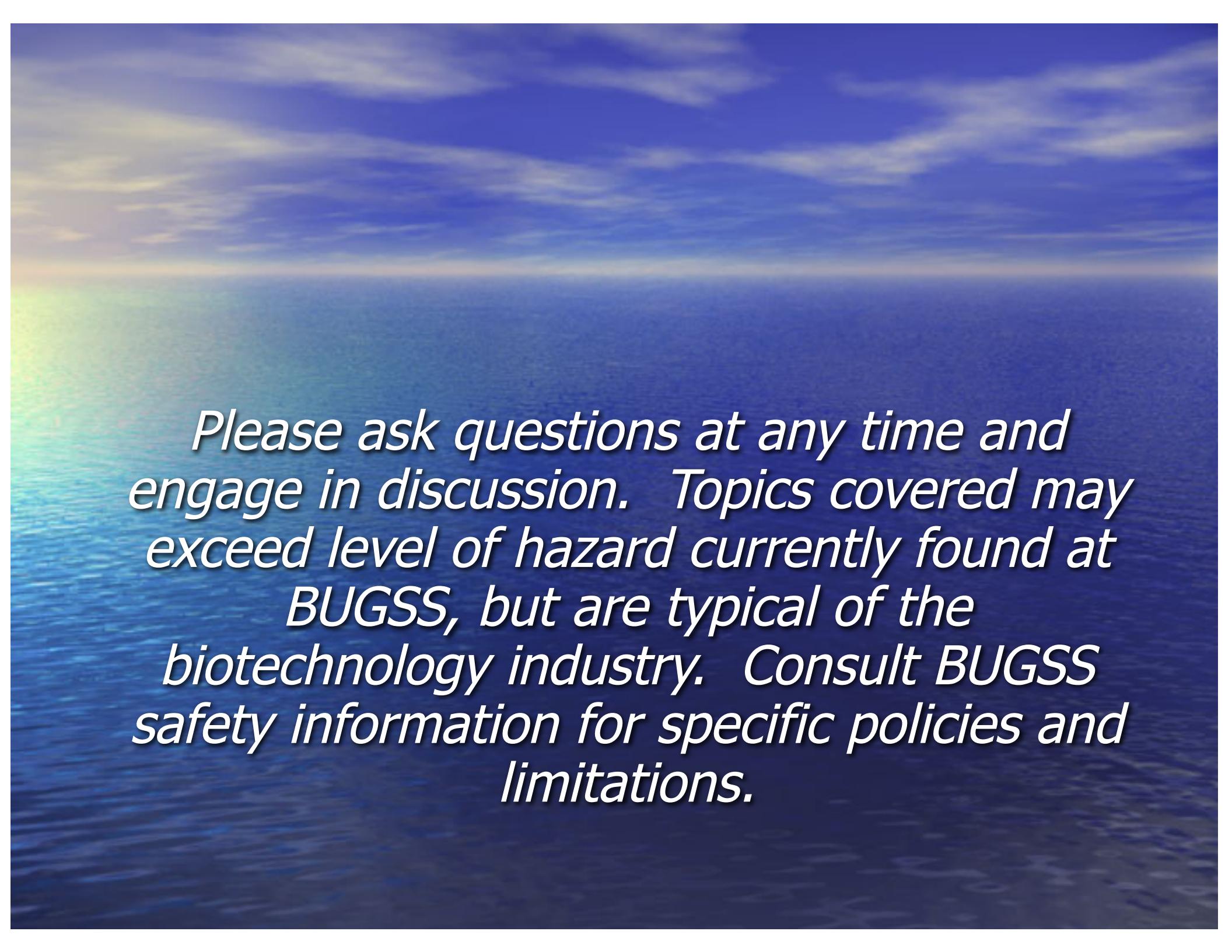
# DIY Biotech

*Issues for Safety and Security*  
February 26, 2012

# Instructor Information

- Ed Wonilowicz, CSP  
Clym Environmental Services, LLC
- Experience
  - B.S. Biology, 1982
  - M.S. Applied Molecular Biology, 1988
  - Research Associate, Genetic Therapy Inc.
  - Certified Safety Professional, 1997
- Member, American Biological Safety Association
- Safety Manager for 3 Biotech Companies
- Consultant in Biotech since 1999
- Please call with your Environmental, Health and Safety Questions





*Please ask questions at any time and engage in discussion. Topics covered may exceed level of hazard currently found at BUGSS, but are typical of the biotechnology industry. Consult BUGSS safety information for specific policies and limitations.*

# Why are we here?

- Do safety and environmental laws apply?
  - OSHA/EPA/DOT/CDC/NIH
    - Apply to Industry
    - Your work may have similar hazards/impacts
      - Voluntary compliance recommended
  - Contractual Obligations?
  - Common Sense and Ethics
    - Scrutiny of DIY Biotech

# Today's Scope

- General Lab Topics
- Chemical Safety
- Biological Safety
- Biological Security

# Lab Safety Fundamentals

- Applicable in All Labs
  - Universal Lab Rules
  - Personal Protective Equipment
  - Waste Handling and Disposal
  - Transporting Materials
  - Injuries, Exposures and Exposure Evaluation



# Starting Principles

- Avoid underestimation of risk
- Avoid exposure by all routes
- Use proper control measures
  - Administrative
  - Engineering
  - PPE
- Exposure reporting, assessment, follow up



# Routes of Exposure



INHALATION



INGESTIO  
N



ABSORPTIO  
N



“INJECTION”

# Universal Lab Practices

- No food or drink in labs
  - Or just outside labs!
- Hand Washing
  - Soap, water, paper towels
  - Hand Creams
- Dress codes
  - Open-toed shoes “forbidden”
  - Shorts/skirts/dresses not recommended
- Proper storage and use precautions
- Proper disposal
- Emergencies
  - Evacuation, Spill, Exposure

# Transportation of Hazardous Materials

- Requires U.S. DOT HAZMAT Employee Training
  - This training is not sufficient
  - Rules for transportation off site?
  - Mail may not be appropriate
  - Your car may not be appropriate
- Do not sign paperwork for waste or other shipments
- Consult a trained person
- Arrangements can be made to ship



# Environmental Protection

- Impacts
  - Possible pollution of air, water, ground
  - Environmental Laws
    - Govern INDUSTRY
      - Your compliance is voluntary
      - Your behavior represents the DIY biotech community
- What can I put in the trash?
- What can I put down the drain?

# Sink Disposal “No-Nos”

- Regulatory
  - Any “hazardous waste”
  - Any “regulated medical waste”
    - Exception – Liquids after disinfection
  - Materials of pH<6 or pH>12
    - Exception – acids and bases after neutralization
  - Provided no other hazardous wastes are generated
- Advisory
  - Any toxic chemical
  - Any mutagen or carcinogen
  - Flammable or combustible liquids
  - Dyes – concentrated/large quantities
  - Insoluble materials
  - ANYTHING, unless you’re sure



# What is “Hazardous Waste”

- CHEMICAL waste that is...
  - Flammable/Combustible
  - Corrosive
  - pH < 2 or > 12.5
    - For sink disposal, must be between 6 and 12 in Baltimore!!
  - Reactive
  - Explosive, spontaneously combustible, etc.
  - “Toxic”
  - Many exceptions
  - Judge conservatively
- BUGSS not permitted to generate hazardous waste at this time.

# What is Regulated Medical Waste?

- Does not have to be from medical source
  - U.S. Department of Transportation
    - Known infectious material waste
  - Maryland Department of the Environment
    - Infectious materials
    - Also ANY human or animal materials
      - Including cell culture
  - Centers for Disease Control and NIH
  - Render ALL materials non-viable before disposal
  - Conservative Approach Recommended
    - If it looks like “lab stuff,” dispose in a controlled way

# Personal Protective Equipment

- PPE
  - Anything worn on body for protection
    - Lab Coats
    - Safety Glasses and Face Shields
    - Gloves
    - Safety Shoes
    - Respirators
    - Etc.



# Typical Biology Lab Hazards

- Biohazard/Infection
  - Latex/nitrile gloves
  - Eye protection for splashes
  - Lab Coats
- Chemical Exposure
  - Toxic, corrosive, irritating
- Temperature
  - Wet heat
    - Water impervious gloves
    - Heat-protective gloves
  - Dry Heat
  - Heat-protective gloves
  - Cold
    - “Cryogloves”



# Other Hazards...

- Compressed Gases??
  - Safety glasses while making connections
- Liquid Nitrogen, Liquid Carbon Dioxide??
  - Increased splash protection
    - Face shields worn WITH safety glasses
- Ultraviolet Light???
  - UV-protective face shields
    - Liquid nitrogen also present?
      - ALL faceshields provide UV protection
        - Do not want to mix them up!!

# OSHA PPE Rules

- For advisory purposes...
  - Hazard Assessment
    - Identify and document hazards and PPE
  - Train Employees
  - PPE available and in good condition
  - Avoid “optional” PPE policies
  - Respirators
    - Should not be needed
    - VERY significant additional requirements

# **THERE IS NO STRICT OSHA REQUIREMENT FOR PPE IN LABS**

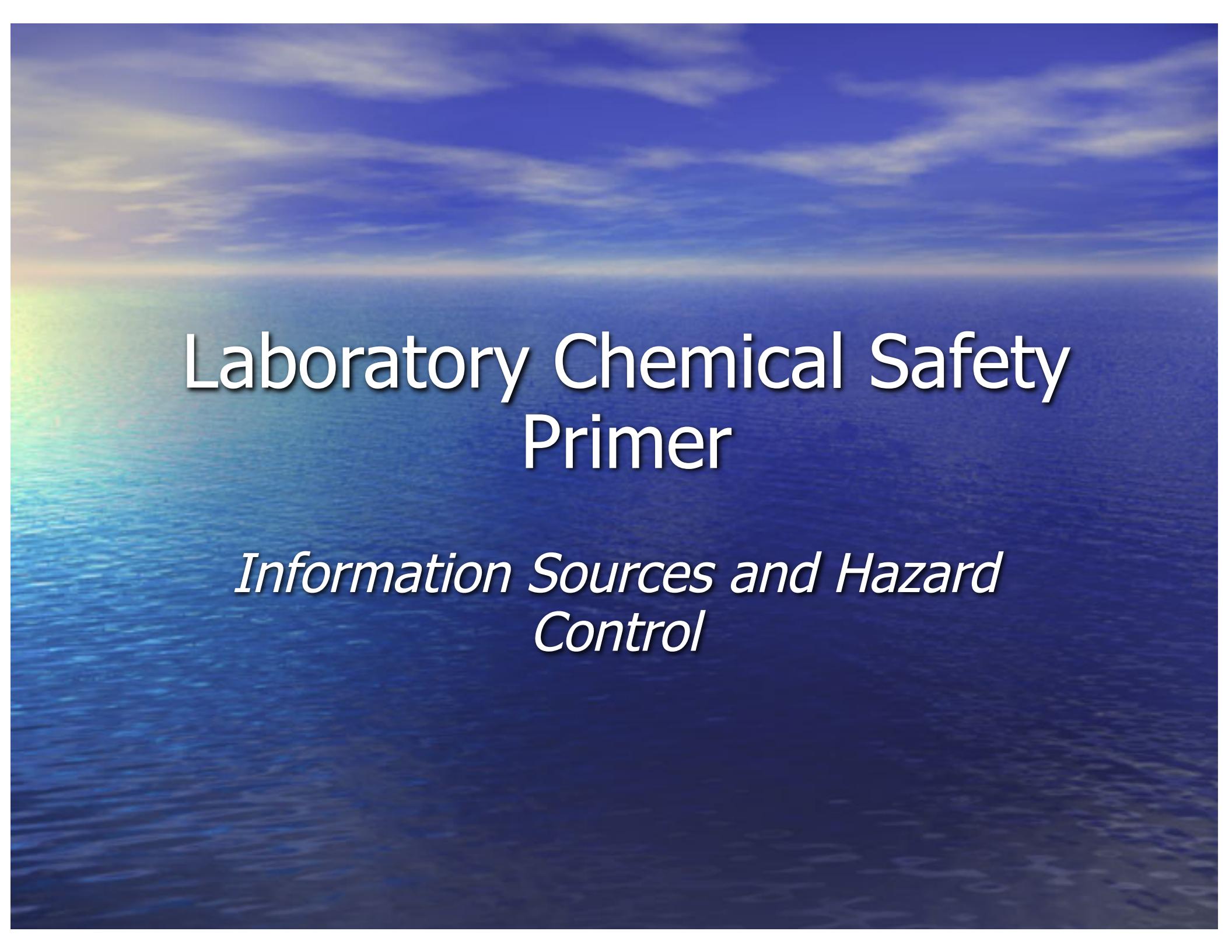
*It is up to each institution to  
determine hazards and decide if  
PPE is necessary*

# Current BUGSS PPE Policy

- Labcoat, eye protection, gloves
  - Corrosives
  - Other “Hazardous Chemicals”
  - Potentially infectious biologicals
  - Policy applies to members and guests

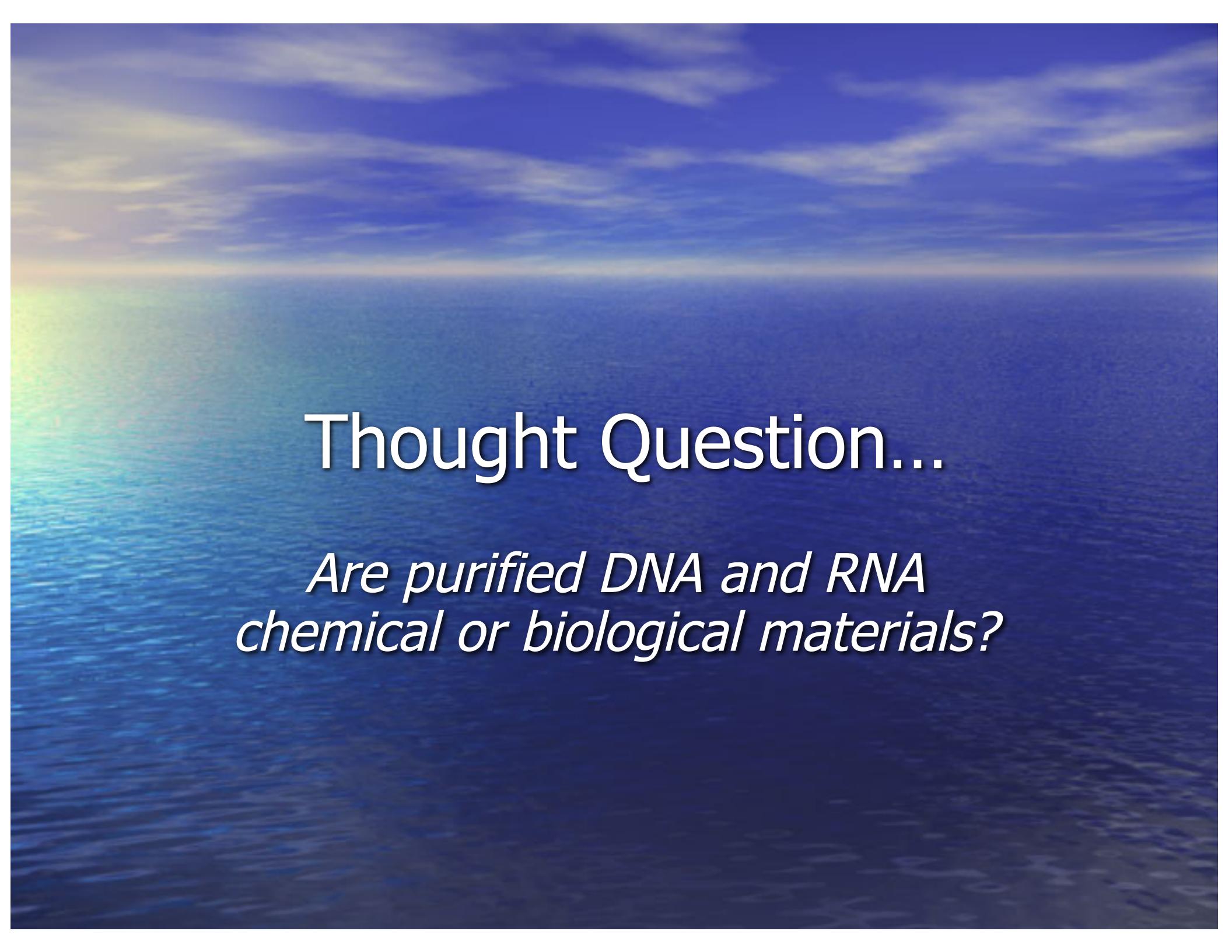
# Other Safety Information...

- Importance of injury/exposure reporting
  - Medical care necessary
- General Facility Safety
  - Evacuation Procedures
    - Where do you go?
  - Spill Response
    - Can you clean it up yourself?????
    - Should you evacuate?
  - Fire safety
    - Are you trained to use a fire extinguisher?
      - Should you use one?

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# Laboratory Chemical Safety Primer

*Information Sources and Hazard  
Control*

The background of the slide features a wide-angle photograph of a vast ocean meeting a horizon under a blue sky. The sky is filled with soft, white, wispy clouds. In the upper left corner, there is a subtle gradient of yellow and green light, possibly from the sun or a sunset/sunrise. The water in the foreground is a deep blue with gentle ripples.

# Thought Question...

*Are purified DNA and RNA  
chemical or biological materials?*

Answer...DNA and RNA are  
CHEMICALS  
(Usually nonhazardous ones)

Exception: Biological if molecule  
encodes a fully-infectious viral  
genome such as HIV

# Chemical Safety Maxims

- Know hazards BEFORE ordering
- Control Inventory
  - Approval based on need and available hazard controls
  - “White List” of acceptable chemicals
- Store according to hazard type
  - Compatibility is most important consideration
- Use smallest possible quantities
- Use proper controls and protection
- Never deliberately touch, smell, taste chemicals
- Substitute nonhazardous for hazardous
- Know how to deal with waste

# Current Chemical Inventory

- “White List”
  - Chemicals of low or no hazard
  - Small quantities dilute acids and bases
  - Small quantities of alcohol
    - Flammable liquid storage
      - 500 ml limit in lab

# Communication of Hazards

- Manufacturers must provide hazard info
  - Labels and hazard documents
- OSHA is changing requirements
  - New requirements being phased in
  - Chemical users may see old and new systems
    - Until 2016
- New system
  - Standardized, consistent information
  - Standard definition of terms used
  - Better hazard communication

# Commercial Chemical Labeling

- Current Labels
  - Manufacturer's Labels
    - Name of Chemical
    - Hazard Information (non-standardized)
    - Manufacturer Contact Information
- New Labels
  - Signal Word
    - “Danger” or “Warning”
      - “Danger” = More Hazardous
  - Hazard Statement (Standardized)
  - Hazard Pictogram

# Standard Hazard Pictograms

## GHS – Hazard Pictograms and correlated exemplary Hazard Classes

### Physical Hazards



Explosives



Flammable Liquids



Oxidizing Liquids



Compressed Gases



Corrosive to Metals

### Health Hazards



Acute  
Toxicity



Skin Corrosion



Skin Irritation



CMR<sup>1)</sup>, STOT<sup>2)</sup>,  
Aspiration Hazard



Hazardous to the  
Aquatic Environment

1) carcinogenic, germ cell mutagenic, toxic to reproduction / 2) specific target organ toxicity

# Chemical Safety Data Sheets

- Material Safety Data Sheets
  - Old Name
- Safety Data Sheets
  - New Name
- Brief documents summarizing hazards
  - Old format not standardized
  - New format standardized
  - Should be available at all time for review

# Material Safety Data Sheets

- Old system...
  - / NO Required Format: must contain...
    - / Chemical and common names
      - / > 1% for health hazards
      - / > 0.1% for carcinogens
    - / Physical/Chemical Characteristics
    - / Physical Hazards
    - / Health Hazards
    - / Published Exposure Limits
    - / Carcinogen??
    - / Safe Handling Precautions
    - / Emergency/First Aid Procedures
    - / Date of Preparation
    - / Manufacturer Contact Information

# Safety Data Sheets: 16-part standard format

- 1: Product/Company ID
- 2: Standard hazard information
- 3: Composition/Ingredients
- 4: First Aid Measures
- 5: Fire Fighting Measures
- 6: Accidental Release Measures
- 7: Handling and Storage
- 8: Exposure Controls and Personal Protection
- 9: Physical/Chemical Properties
- 10: Stability/Reactivity
- 11: Toxicological Information
- 12: Ecological Information
- 13: Disposal Considerations
- 14: Transport Information
- 15: Regulatory Information
- 16: Other Information

# Chemical Storage

- Always by compatibility first
  - Segregation
  - Acid/Base, Oxidizer/Flammable
  - Use alphabetical storage only after segregation
  - Bins, etc. when separate cabinets not feasible
  - Posting of Storage Locations
- Chemical Distribution
  - Secondary Containment



# Types of Hazardous Chemicals

- May fit **one or more** of the following:
  - Acute Toxins
  - "Poisons"
    - Act quickly
  - Chronic Toxins
  - Cause cancer, mutation, organ damage
  - Act slowly
  - Solvents/Flammables
  - Reactive Chemicals
  - Corrosives
  - Irritants
  - Allergens and Sensitizers
  - Compressed Gases, Cryogenic Liquids, Cryogenic solids

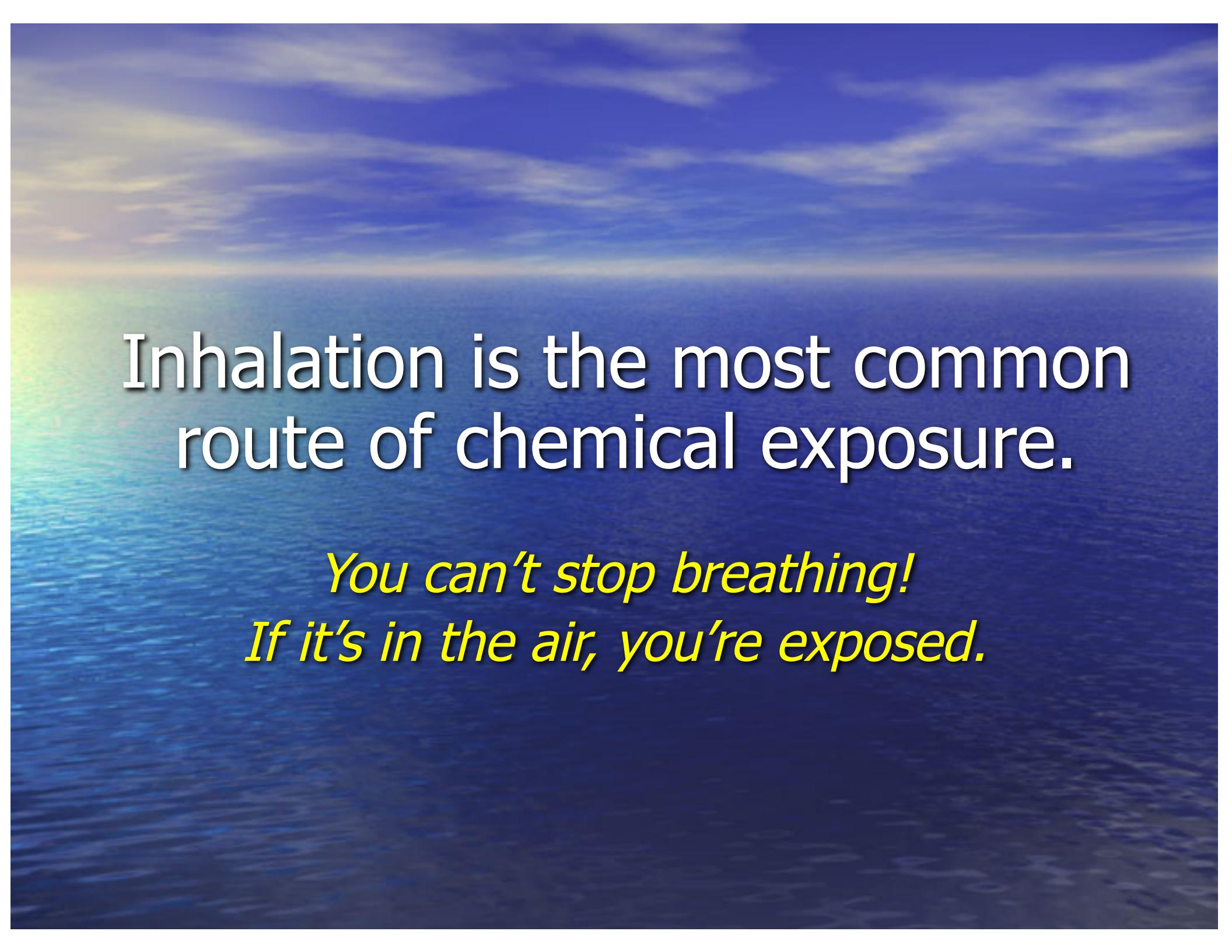
# Avoiding Chemical Exposure

## Route of Exposure

- Inhalation
- Ingestion
- Absorption
- “Injection”

## Control Method

- Exhaust Ventilation
- No food/drink
- PPE
- Avoid touching face, mouth
- PPE
- Avoid skin/eye/mucous membrane contact
- Avoid sharps use
- Do not pick up broken glass
- Cover cuts/rashes

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Inhalation is the most common route of chemical exposure.

*You can't stop breathing!  
If it's in the air, you're exposed.*

# Consider the difference...

- Both of these compounds are highly toxic
  - Sodium Azide (by ingestion)
  - Hydrogen cyanide (by inhalation)
- Which would pose the greater hazard?

# What's in a Word?

- Highly Toxic: Causes death or serious injury at very low doses
- Toxic: Causes death or serious injury at higher doses
- Harmful: May cause death or serious injury, but at very high doses

# Spills and Exposures

- Do not risk harmful exposure
  - Clean up only low hazard materials
  - Consider inhalation hazard
  - Consider fire risk
  - Consider damage to skin and eyes
  - Consider irritating or allergenic properties
- Evacuate Area
- Report the spill
- Exposures to hazardous substances
  - Medical evaluation and followup

# Exposure Signs and Symptoms

- Consult labels and MSDS
  - Toxicity
    - Headache, nausea, narcosis, anesthesia, neurological effects
    - “Target Organ” Effects
  - Physical
    - Burning, itching, irritation, full thickness destruction
    - Eyes, nose, throat, skin
- Other chemical-specific effects
- Other sources of same symptoms
  - REPORT ALL SYMPTOMS

# Chemical Exposures

- Detection methods
  - Senses
  - Monitoring/Measurement
- OSHA Exposure Limits
- “Safe” limits for inhalation exposure
- Usually represented in parts per million
  - Example Chloroform = 10 ppm
  - Example Ethanol = 1000 ppm
- Availability of Exposure Limits
  - 29 CFR 1910.1001, “Z-Tables”
  - MSDS

The background of the image is a wide-angle photograph of a calm ocean. The water is a deep blue, with some lighter reflections on the left side. Above the horizon, the sky is filled with wispy clouds, colored in shades of yellow, orange, and blue, suggesting either a sunrise or sunset. The overall atmosphere is serene and expansive.

# Biosafety and Biosecurity

# Major Biosafety Guidelines

- All available free on-line:
  - “Biosafety in Microbiological and Biomedical Laboratories” 5th Edition, January 2007 (BMBL)
  - NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), April 2002
  - OSHA Bloodborne Pathogens Standard
  - WHO Laboratory Biosafety Manual, 3rd Edition
- Additional Resource similar to BMBL

# Universal Precautions

- General Concept from Medical Field
  - All human materials treated as infectious
    - Regardless of testing and other knowledge
    - Roughly translates to Biosafety Level 2
  - No human materials currently approved
    - Except autologous cells
    - But what about other people in the lab?
    - No culture allowed

# The Hepatitis B Vaccine

- Recommendation for human materials work
- Highly Effective
- Three injections
- Younger people vaccinated at birth
- Thought to be effective for life
- Consult your physician

# Biosafety Level 1: General

- Well-characterized agents
- No CONSISTENT disease in healthy adults
- Minimal potential hazard
- Open bench work
- Limited access optional
- Personnel trained in lab procedures
- Qualified supervision
  - Training in microbiology or “related science”

# Standard Microbiological Practices

- Biosafety Level 1
  - Access policies as determined necessary
  - Hand washing required
  - No eating, drinking, smoking, cosmetics, lip balm, food storage
    - Hand cream is NOT a cosmetic
  - No mouth pipetting
  - Sharps policies and engineering required
  - Minimize splashes/aerosols
  - Decontamination of surfaces at least daily and after spills
  - Decontamination of cultures/stocks before disposal
  - Biohazard sign not generally required
  - Integrated Pest Management
    - Some organisms travel through insects and rodents
  - Adequate Training

# Biosafety Level 2: General

- Not currently allowed at BUGSS
  - Agents of moderate potential hazard
  - Specific training in handling pathogens
  - Restricted access to labs
  - Extreme sharps precautions
  - Aerosol-producing processes in BSCs
  - Non-aerosol allowed on open bench
- What about your own cells??

# Biosafety Level Comparison

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices only	None required	Open bench and sink required
2	<ul style="list-style-type: none"><li>oAgents associated with human disease</li><li>oRoutes of transmission include percutaneous injury, ingestion, mucous membrane exposure</li></ul>	BSL-1 practices plus: <ul style="list-style-type: none"><li>oLimited access</li><li>oBiohazard warning signs</li><li>o“Sharps” precautions</li><li>oBiosafety Manual defining any needed waste decontamination or medical surveillance policies</li></ul>	Primary barriers: <ul style="list-style-type: none"><li>oClass I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials</li></ul> PPE: <ul style="list-style-type: none"><li>oLaboratory coats; gloves; face protection as needed</li></ul>	BSL-1 plus: <ul style="list-style-type: none"><li>oAutoclave available</li></ul>

# Biosafety Summary

- Biosafety Level 2
  - Currently NOT approved at BUGSS
    - Examples
      - Human-source materials
        - ❖ Not infectious???
        - ❖ Universal Precautions/Biosafety Level 2
      - Non-Human Primate Materials
      - Some environmental organisms
        - ❖ *Salmonella* sp.
        - ❖ *Staphylococcus aureus*
  - Biosafety Level 1
    - Molecular Biology E.Coli (K-12 and derivatives)
      - Not enterolytic/pathogenic
    - Most animal and plant cells
    - *Saccharomyces cerevisiae*
      - Baker's and Brewer's Yeast

# Hygiene and Sanitation

-- *Hand and Skin Washing Policy*

- When to wash hands and skin
  - After removing gloves
  - Before leaving the lab
  - When contaminated
- What soaps, etc. are to be used?
- Where must washing facilities be located?
  - Can alternative means be used?

# Sharps and Sharps Precautions

- Sharps are needles, razor blades, scalpels, etc.
- Sharps ARE NOT serological pipettes and Pipetman® tips  
Glass Pasteur pipettes considered sharps
- Avoid use whenever possible
  - Don't pipette with needles
  - Don't cut tubing, etc. with bare razor blades
  - Use alternate methods for macerating tissue
- Use basic precautions
  - Closed/protected storage
  - No recapping, bending, shearing, removal of needles
  - Never handle broken glass
- Use sharps containers for disposal
  - Commercially available/proper design
  - Upright at point of use
  - DO NOT OVERFILL
  - Close and dispose in burn box



# Signs and Labels

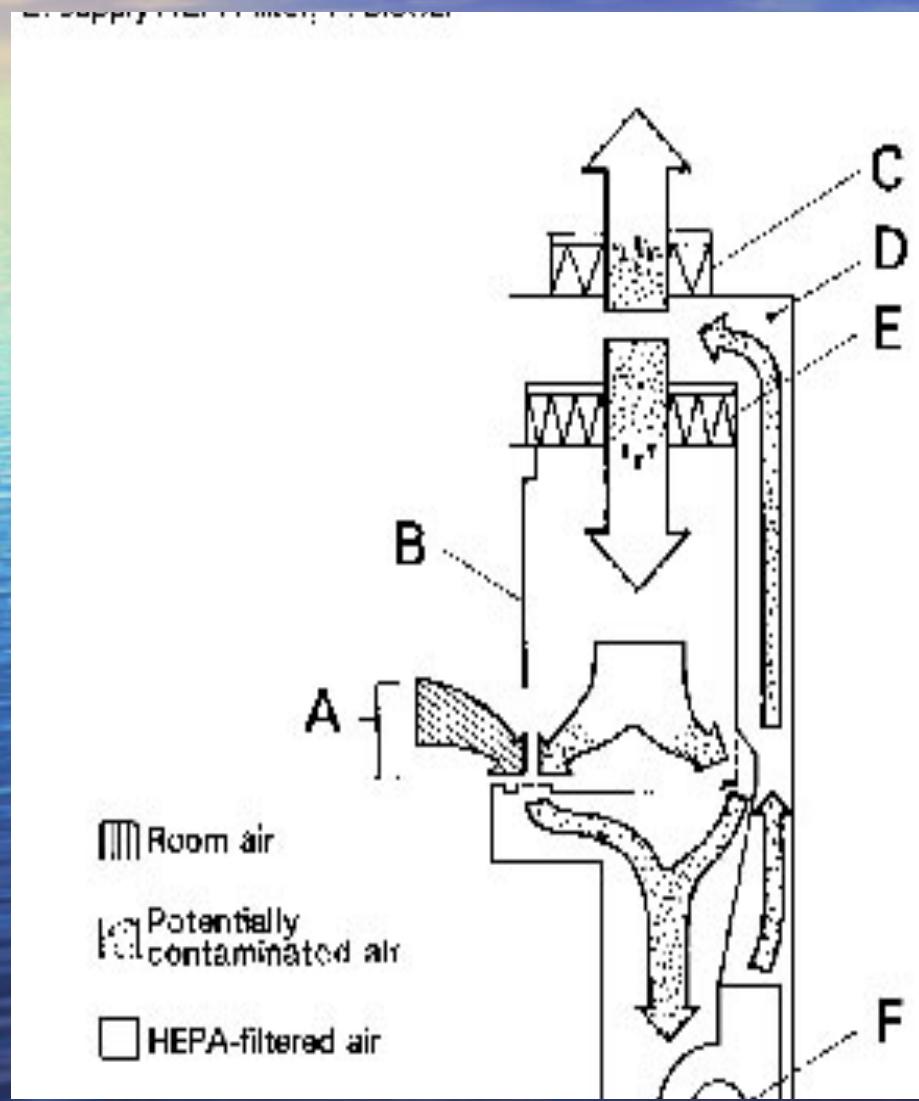
- **BSL2 and above**
- Biohazard Door Sign
  - Biosafety Level
  - Agent information
  - Responsible person
  - Contact Info
  - Entry/exit procedures
- Biohazard Labels
  - Equipment/Areas
  - Refrigerators/Freezers
  - BSCs
  - Sample Containers
  - Waste Containers



# Decontamination

- Interval
  - At least Daily
  - After any spill
  - Before servicing equipment
- Use an effective agent
  - Alcohol requires a contact time
  - Lysol
  - Wescodyne
  - Be wary of bleach!

# Biological Safety Cabinets



- Provide sterility  
Cell culture
- Provide biosafety
  - Required at BSL2 And higher
- Additional Training

# Biological Spills

- Put on PPE
  - If not already wearing
  - Lab Coat, Safety Glasses, Eye Protection
- Remove broken glass with tongs
- Dispose in sharps container
- Cover with paper towels
  - Drop from a height
- Add decontaminant around outside
  - Capillary action
- Wait 10 – 30 minutes
- Clean up (don't use hands if possible)
- Wipe/disinfect (careful of small glass shards)

# Exposures to Biological Materials

- Overt eye/mucous membrane, oral or inhalation exposure to potentially infectious agents
  - Report
  - Evaluate severity
  - Medical evaluation?
- Actions
  - Review by Laboratory Director/Safety
    - Changes to process, training, materials, etc. as needed
    - Review with healthcare professional for recommended action

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# Biosecurity

*Do loose lips sink ships?  
A discussion of potential for  
misuse of biotechnology*

# Rights, Benefits, and Issues

- Innovation comes from individuals
- You have the right to practice science
  - Even at home
- Public knowledge of science lacking
  - Ignorance breeds fear
  - Some fear justified??
    - 9-11 and anthrax
  - Knowledge is power
    - Recognize activities
    - Prevent misuse

# Concerns of Law Enforcement

- “Traditional” cloning methods
  - Imparting resistance to pathogens
  - “Runaway” organisms
    - Controls such as mutations in E. Coli K-12
- Synthetic Biology
  - PCR
  - Not even trained scientists can produce life
    - Can you?

# Law Enforcement Steps/Missteps

- Cooperative Relationship/Education
  - FBI
  - Local Law Enforcement
- Paranoia Exists
  - SUNY Buffalo case

# A shot in the foot??

- DIY Biotech Public Relations?
  - “Biohackers”
  - “Biopunks”
  - “Under Ground”
- A persons perception is their reality
  - Your behavior is critical
  - You set the example
  - Exemplary safety and environmental protection
  - How well do you know that person??



# Is the threat real?

- Will a terrorist join a community lab?
- Will a community lab provide WMD potential?
  - Under scrutiny
  - Above board
  - Not conducive to criminal activity
- What you know can provide safeguards
  - What if your neighbor has lab equipment?



I

# Is this art or mischief?

# Final Words and Opinions

- DIY Biotech will not go away
- DIY Biotech SHOULD not go away
- Law Enforcement is VERY interested
- Law Enforcement SHOULD BE interested
- Expect regulation
  - What is an acceptable cost for freedom?
  - What role do you play?

# Biosecurity Reference Links

- National Science Advisory Board for Biosecurity
- American Association for the Advancement of Science
- DC Bar: Regulation of DIY Science
- The Rest is Art Biotechnology Article
- Biotechnology Ethics: Santa Clara University