Safe Handling of Acutely Toxic Chemicals, Mutagens, Teratogens and Reproductive Toxins

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Hazards Classes for Chemicals

- Flammables
  - *Risk of ignition in air when in contact with common energy sources*

- Corrosives
  - *Generally destructive to materials and tissues*

- Energetic and Reactive Materials
  - *Sudden release of destructive energy possible* (e.g. *fire, heat, pressure)*

- Toxic Substances
  - *Interaction with cells and organs may lead to tissue damage*
  - *Effects are typically not general to all tissues, but targeted to specific ones*
  - *Examples:*
    - *Cancers*
    - *Organ diseases*
    - *Inflammation, skin rashes*
    - *Debilitation from long-term accumulation with delayed emergence*

Poison (ingestion)

Acute risk

Cancer, health or reproductive risk

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Toxic Substances Are All Around Us

- Pollutants
  - Cigarette smoke
  - Automotive exhaust
- Common Chemicals
  - Pesticides
  - Fluorescent lights (mercury)
  - Asbestos insulation
  - BPA (Bisphenol A used in some plastics)
- Natural toxins
  - Venoms (snakes, spiders, bees, etc.)
  - Poison ivy
  - Botulinum toxin
  - Ricin
  - Radon gas
  - Arsenic and heavy metals in ground water

Application at UNL

- Chemicals in Chemistry Labs
  - Chloroform
  - Formaldehyde
  - Acetonitrile
  - Benzene
  - Sodium azide
  - Osmium/arsenic/cadmium salts
- Chemicals in Biology Labs
  - Phenol
  - Ethidium bromide
  - Acrylamide
- Toxin-producing Microorganisms
  - Fungi
  - *Staphylococcus* species
  - Shiga-toxin from *E. coli*
- Select Agent Toxins (see register)
  - *Botulinum* neurotoxins
  - T-2 toxin
  - Tetrodotoxin
  - Diacetoxyscirpenol
- Radioactive Substances

  with toxic effects not related to radioactivity

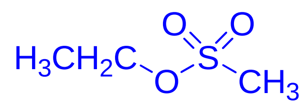
Scope of This Talk

- Subject: Laboratory Safety With Highly Toxic Materials
- Topics
  - **Definitions**
  - **Resources for Determining Toxicity**
    - Known substances

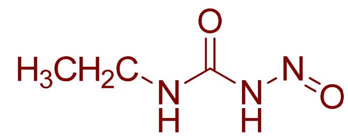
      Case History: 1997 dimethylmercury incident
    - Unknown substances
  - **Working With Toxic Materials**
    - Regulatory obligations
    - Biologically-derived toxins
    - PPE (respirators and gloves)
    - Lab hygiene
    - Waste disposal
  - **Case Study: Procedure Involving Reaction of a Carcinogen**

Definitions

- **Mutagen** a substance that is suspected to cause mutation in DNA



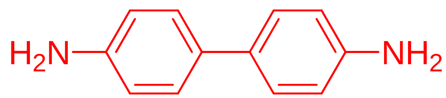
ethyl methanesulfonate
(EMS)



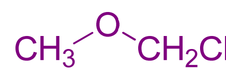
ethylnitrosourea
(ENU)

- **Carcinogen** a substance that is suspected to cause cancer

A common initiation of cancer is a mutation in DNA.



benzidine

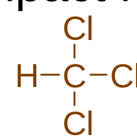


methyl chloromethyl ether

- **Teratogen** a substance that is suspected to impact fetal development

$CH_3-C\equiv N$

acetonitrile



chloroform

- **Reproductive Hazard**

a toxic substance that targets reproductive organs and cells

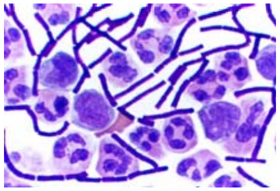
ethylene oxide

Pb

lead

More Definitions

- **Biotoxin** a toxin with a biological origin; source can be microorganisms, insects, reptiles, fish, and amphibians





- **Cytotoxic** a substance that is lethal to cells or limits their ability to divide

  Many anti-cancer drugs are cytotoxic because of their ability to target cancer cells and kill them or stop them from growing.

  Cytotoxic drugs will likely destroy cells indiscriminately and only be beneficial if targeted to tumors and if dosed very carefully.

- Other forms of toxicity—by targeted organs and cells

Neurotoxic	Hemotoxic	Myotoxic
Hepatotoxic	Cardiotoxic	etc.

Acute and Chronic Toxicity

- Duration and level of exposure are important elements in assessing a toxic risk
- Acute exposure risks
  - Substances that produce a toxic response almost immediately after exposure
  - Generally involves a tangible quantity of toxic agent to cause toxic response
  - Examples: snake bite, carbon monoxide poisoning
- Chronic exposure risks
  - Substances that accumulate over prolonged exposure to produce a delayed toxic response
  - Level of exposure at any time could be very small
  - Examples: asbestos, lead poisoning

Example: **Alcohol**
Acute risk—impairment, nausea, unconsciousness
Chronic risk—liver disease

Determining Toxicity for Known Substances

- Container label
- MSDS
  - Hazard identification
  - First aid measures
  - Personal protection
  - Toxicological summary
  - Regulatory information

- What to look for:
  - Health hazard placard
  - Acute toxic effect
  - Chronic toxic effect
  - Target organs
  - Exposure level for effect
  - Recommended precautions
  - Applicable standards and regulations
  - Antidotes or countermeasures

Precautionary Note: Toxicology data is only as good as the testing that has been done! "No data" does not mean it is non-toxic.

Toxicity Risks Are Not Always Obvious From the Documentation

- Read toxicity data in MSDS's and on labels carefully
- Look for "red flags"
  - Evidence of lethality by means other than ingestion
  - Substance listed or suspected as carcinogen, mutagen, teratogen, or "highly toxic"
  - Recommended use of unusual handling equipment or PPE
  - Specific and detailed regulated limitations and requirements
- Interpret what you read against your lab situation
- Don't take chances with your health

Put away the rose-colored glasses when evaluating toxicity risks.

Case History: Dimethylmercury Exposure

- 1997 Incident involving ***48-year-old university professor***
- Wearing disposable ***latex exam gloves***
- Transferred ***dimethylmercury*** by pipet
- Some drops fell on her gloved hand
- She removed the gloves immediately and washed her hands
- She experienced no symptoms for five months
- Then, she reported neurological problems (tingling, difficulty speaking, etc.)
- She was diagnosed with ***mercury poisoning*** (five months after exposure)
- In spite of aggressive chelation treatment, she died of encephalopathy *after 10 months*

$H_3C{-}Hg{-}CH_3$

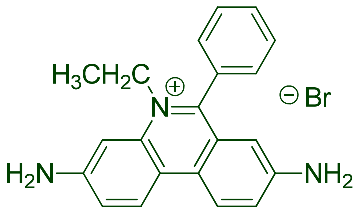
Dimethylmercury Incident: What It Teaches Us

- It can happen to ***anyone***, even the most experienced scientists
- Gloves are not always an adequate defense

  …and ***latex exam gloves offer no protection*** to chemicals at all
- Some substances can ***pass through your skin*** very quickly
- Some substances can ***cause great harm*** without obvious signs of exposure
- Toxicity effects may be significantly delayed
- Red flags that were missed
  - Heavy metal alkyl and liquid
  - "Highly toxic by skin absorption" (MSDS)
  - "Fatal if swallowed or in contact with skin." (MSDS)
  - "Latex, neoprene and butyl gloves do not provide suitable protection for direct contact with dimethylmercury." (Wikipedia)

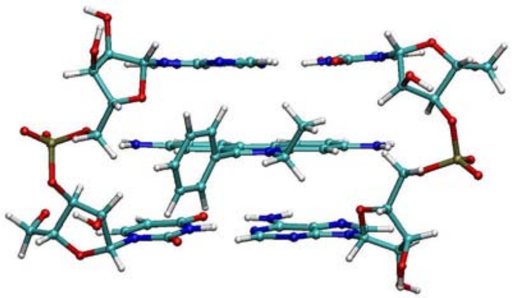
In 1997, this information may not have been available.

Evaluating Potential Toxicity for Untested and Unknown Substances



ethidium bromide

- Known mutagens disrupt DNA through…
  - Alkylation ex. Ethyl methanesulfonate, methyl iodide
  - Deamination ex. Nitrosoureas, nitrosamines
  - Base substitution ex. Bromodeoxyuridine
  - Intercalation ex. Cationic planar polyaromatics



Two A-T base pairs in DNA

- Other common sources of high toxicity…
  - Compounds of heavy metals, especially organometallics
  - Bio-active compounds (like antibiotics and other microorganism-derived compounds)
  - Analogs of known toxic materials (such as an active drug derivative)

- *Handle all uncharacterized materials with care.*
- *If the material contains any "red flag" characteristics of a highly toxic material, treat it as highly toxic.*

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Biologically-Derived Toxins

➢ Definition:

- Poisonous substances produced as by-products of microorganisms, plants or animals
- Not living organisms
- Not contagious

➢ Examples in use at UNL:

- **Cholera toxin** (secreted by *Vibrio cholerae* bacteria)
- **Mycotoxins** (derived from fungi) (e.g. aflatoxin, ochratoxin, fumonisins)
- **Pertussis toxin** (*Bordetella pertussis*)
- **LPS** (Lipopolysaccharide – from cell membrane of gram-negative bacteria)
- **Diptheria toxin** (Corynebacterium diphtheriae)

So You Need to Work With a Highly Toxic Material… What Next?

1. **Seek Alternatives**
   - *Do you really need to do this?*
2. **Are there regulations that apply?**
   - Occupational Safety and Health Administration, Nuclear Regulatory Commission, Department of Homeland Security, State regulations, UNL and Departmental policies
   - UNL Committee approval may be required if the work involves biotoxins, microorganisms, recombinant DNA, RAM?
     - For biologically-derived toxins (contact the UNL Biosafety Officer at 472-9554). Some are subject to strict Select Agent regulations and possession above a designated threshold requires registration.
     - An Institutional Biosafety Committee protocol may be required
   - Notification, monitoring, and reporting may be required
   - Area isolation and marking may be required
   - Strict procedures may be mandated

So You Need to Work With a Highly Toxic Material… What Next?

3. **Confirm availability of antidotes in case of emergency**
4. **Characterize the hazard and potential routes of entry**
   - Biological or chemical
   - Respiratory (gas, vapor, fume or dust)
   - Skin contact (especially liquids and solutions)
5. **Choose work area that best isolates hazard from other lab personnel**
6. **Choose PPE to best protect the handlers**
7. **Determine how you will deactivate/destroy residues and excesses**
8. **Buddy system—have a trained backup ready to assist**

Work With Biologically-Derived Toxins

- **Work Practices**
  - Most work with biological toxins should be conducted in compliance with BSL-2 containment guidelines

    http://ehs.unl.edu/sop/s-bio-containment_levels.pdf

    http://www.cdc.gov/biosafety/publications/bmbl5/index.htm
- **Exposure Routes**
  - Aerosol production (centrifuge, aspiration, etc)

    http://ehs.unl.edu/sop/s-bio-aerosol.PDF
  - Dermal exposure (e.g., T-2 mycotoxin)
  - Mucous membranes exposure
  - Ingestion
  - Parenteral (e.g., needlestick, bite)
  - Toxin work involving animals

Work With Biologically-Derived Toxins

- **Storage and Security**
  - Maintain an accurate inventory of purified toxins
  - Toxins should be secured whenever unattended (e.g., locked room, locked freezer, locked box)
  - Possession of Select Agent toxins requires specific security and storage requirements
- **Disposal/Inactivation**
  - Use an appropriate disinfectant for the toxin. Many toxins are susceptible to 10% bleach, 2N NaOH
    - Work surfaces
    - Equipment
  - Procedures for inactivation of dry biological toxins or solution containing biological toxin, see http://ehs.unl.edu/sop/s-bio-disinfectants.pdf
  - Treat with 2N NaOH for at least 1 hr
  - Other proven effective inactivating agents

PPE for Chemical Toxins

- Three primary considerations for selection of PPE
  - State of hazard (gas, liquid, solution, solid, fume or dust)
    - This tells you what kind of exposure you need to guard against (inhalation, skin, etc.)
  - Chemical compatibility and resistance to penetration
  - Severity of effect of exposure
- Gases, vapors, fumes and dusts
  - Inhalation hazard
  - Review MSDS for toxic effects of inhalation
  - Review MSDS for toxic effects of skin or eye contact
  - Consider respirator
  - May require full body isolation
    - Supplied breathing air
    - "Moon suit"

NOTE: Researchers should contact EHS **prior to** assigning work or working in atmospheres that may require respiratory protection. UNL has a Respiratory Protection Program for workers required to use respirators.

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PPE for Toxic Liquids and Solutions

- Liquids may be inhalation hazards
- Liquids also may be able to penetrate protective equipment material
- Some liquids can be absorbed through skin
- Review EHS guides for specific chemicals and PPE recommendations
- Review MSDS for toxic effects of inhalation
- Review MSDS for toxic effects of skin or eye contact
- Consider inhalation and full body protection
- Gloves
  - Review for breakthrough rate to liquid to be used (link)
    - It is just a matter of time before a liquid will penetrate
  - Consider potential for cuts, needle sticks, and abrasions
  - Multiple gloving can protect against multiple hazards, increase penetration time
    - Example: Silver shield inner gloves
    - Evaluate mobility, flexibility, dexterity

Z529559 (sm)
Z529567 (med)
Z529575 (lg)

Laboratory Hygiene and Control

- Trained persons only should be allowed to handle highly toxic materials
- Isolate hazard from co-workers
  - Regulated zone—no unauthorized personnel allowed while materials in use
- Handler must return all toxic materials to a stable, safe condition before leaving control zone
- Work area must be thoroughly decontaminated immediately after toxic materials are stowed
  - Decontamination is necessary even if no spill is obvious
  - All exposed equipment should be decontaminated in the regulated zone before being taken out for routine cleaning
  - Consider the appropriate decontamination procedure ***before*** handling the toxic material
    - Cleaning solvent or solution
    - Chemical destruction
    - Sterilization, etc.

Laboratory Hygiene: Quantities and Storage

- Highly toxic materials must be stored separately from routine-use chemicals to prevent unprepared or untrained persons from contact with them
- Preferably, highly toxic materials won't need to be stored at all
  - The best plan is to acquire just what is required
  - Use it all up immediately
  - Destroy or dispose any excesses following guidelines available within EHS Safe Operating Procedures or consult EHS
- Toxic materials retained for long periods may require reporting with regulatory bodies and UNL-Environmental Health & Safety.

NOTE: The Department of Homeland Security requires reporting of possession or intent to possess ANY "chemical of concern (COC)" above the established threshold quantity.

Waste Disposal

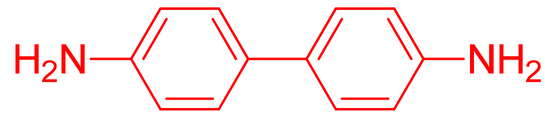
- Waste streams containing highly toxic agents should be separated from general waste
  - Protects lab workers from toxic exposure
  - Allows separate disposal protocol

    Ethidium Bromide *Disposal SOP*

    Disposal of ***Chloroform Contaminated Materials*** *SOP*
- Waste Disposal at UNL is handled by Environmental Health and Safety
  - EHS can address specific collection considerations.
- Waste containers must be clearly labeled and tagged following the protocol within the SOP ***Hazardous/Radioactive Materials Collection Procedures***
- Disposal of toxic waste is much more expensive than routine lab waste
  - It pays to keep volumes to a minimum and not mix with other wastes

Waste Disposal at UNL is handled by a third-party waste disposal contractor.

Case Study: Reaction Using Benzidine



benzidine

- Lab team decides to make a compound using benzidine as a starting material.
- Review of the product information and MSDS of benzidine
  - Carcinogen (known, human)
  - Regulated (OSHA, several states' registers)
- Handling of OSHA carcinogens is covered by 29 CFR 1910.1003, which mandates…
  - Full body PPE
  - Respirator (fit tested)
  - Cordoned "regulated zone"
  - Strict washing protocol
  - Ventilated work area
  - Medical surveillance
  - Warning signs
  - Formal, documented training

CANCER-SUSPECT AGENT
AUTHORIZED PERSONNEL ONLY

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Case Study: Preparation to Use Benzidine

- Reaction is thoroughly planned
  - Quantities
  - Procedural step by step
  - Wastes, hazard content, disposal plan (clean with solvent, water)
- Work area and equipment are identified
  - Secure containment of hazard
  - Safe for handlers (adherence to regulation)
- Operators are identified
  - Formal training provided in carcinogens and 29 CFR 1910.1003
  - Respirator fit test for all operators
  - PPE selected and acquired
  - Medical surveillance conducted (exam and blood test)
- Chemical is acquired
  - Minimum quantity to perform reaction

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Case Study: Set Up to Use Benzidine

- Set up apparatus according to plan
  - “Clean” period—no benzidine yet in area
  - Normal procedures
  - Bring all needed materials into area while it is unrestricted
  - Bring in materials required for waste handling (destruction and containment)
    - Large drum or bucket for used PPE and other large items
- Set up “regulated zone”
  - Put up zone limit boundaries and warning signs
  - Handler(s) suit up
  - Handler(s) enter regulated zone
  - Strict requirements in order to leave zone
    - Carcinogen must be sealed, stabilized
    - Handler must decontaminate
    - Decontaminated PPE must be taken off at zone boundary and left in regulated zone (for hazardous waste disposal)
    - Handler must immediately wash hands, forearms, face, and neck (after leaving zone)

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Case Study: Using Benzidine

- Aim: to complete all carcinogen handling in one sequence without leaving zone
  - Open container
  - Charge (weigh)
  - Close and decontaminate (dispose) spent container
  - Run reaction
  - Decontaminate used equipment
  - Verify everything is stable and contained
- The "regulated zone" must remain in force as long as the carcinogen is present above mandated levels outside of a permanent sealed container
  - The limit for benzidine is 0.1% by weight
  - Anything suspected of being above this level must be sealed before the regulated zone may be taken down
    - Sealed, labeled reagent bottle for excess unspent reagent
    - Sealed, labeled waste containers for items to be disposed

Case Study: Finishing the Benzidine Reaction

- When the reaction is complete and all benzidine-containing vessels are sealed and labeled
  - Remove sealed chemicals from zone
    - Reaction mixtures or products for work up (benzidine < 0.1%)
    - Excess reagents
    - Chemical wastes
  - Decontaminate (or dispose) used apparatus
  - Wash down work area
  - Collect washes for hazardous waste disposal
  - Remove everything (clean) not bolted down from zone
  - Remove PPE and dispose as hazardous waste
  - Take down zone boundaries and warning signs
  - Wash hands, forearms, face, and neck (or take a shower)
- Follow up
  - Medical surveillance (exam and blood test)

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Summary

- Toxic substances are common in…
  - the environment
  - consumer products
  - the laboratory (especially)
- Look for "red flags" that you may encountering a highly toxic material
  - Information resources (MSDS, websites)
  - Your own awareness (biological origin, chemical structure)
- First line of defense: ***seek alternatives***
- Educate yourself on applicable regulations
- Consult with EHS on what needs to be done
- Plan all aspects of handling in advance
  - Quantities needed
  - Area control
  - Labeling
  - PPE
  - Destruction of residues
  - Waste handling
  - Health monitoring
  - Washing
  - Antidotes/emergency actions

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Contacts

- **Environmental Health and Safety**
  402-472-4925   or ehs@unl.edu

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